BACTERIA STANDARDS OF
MERLUCCIUS CAPENSIS / PARADOXUS (CAPE HAKE)
AND OTHER DEEPSEA WHITEFISH PRODUCTS

I. H. VENNEMANN

BACTERIA STANDARDS OF
MERLUCCIUS CAPENSIS / PARADOXUS (CAPE HAKE)
AND OTHER DEEPSEA WHITEFISH PRODUCTS

by

INGO HEINRICH VENNEMANN

submitted in partial fulfillment of the requirements
for the degree of

PhD (Microbiology)

In the Faculty of Biological and Agricultural Sciences

Department of Microbiology and Plant Pathology
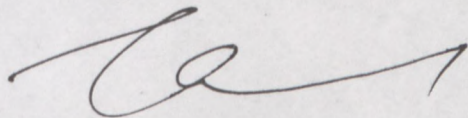University of Pretoria,
Pretoria, South Africa

April 1996

I declare that this dissertation hereby
submitted by me to the University of
Pretoria for the degree of PhD
(Microbiology) has not been submitted
for a degree to any other University.

....
INGO HEINRICH VENNEMANN
APRIL, 1996

To my loyal and supporting wife
ANNELIESE
and family

TABLE OF CONTENTS

BACTERIA STANDARDS OF

MERLUCCIUS CAPENSIS / PARADOXUS (CAPE HAKE)

AND OTHER DEEPSEA WHITEFISH PRODUCTS

by

INGO HEINRICH VENNEMANN

PROMOTOR   : PROFESSOR T E CLOETE
CO-PROMOTOR : PROFESSOR A VON HOLY
DEPARTMENT : MICROBIOLOGY AND PLANT PATHOLOGY
DEGREE   : PhD (MICROBIOLOGY)

SUMMARY

This study analysed the bacteria numbers of Hake products
manufactured at 6 different levels of processing complexity,
analyses were conducted at 20°C on Salt Water Agar (SWA) for a
48h incubation period. This enumeration method was suitable
for indicating time and temperature related abuse in the
processing system. This method was sensitive in indicating the
level of processing complexity. The aerobic bacteria
enumerations at 20°C (SWA) were compared to the current EU/FDA
(European Union/Federal Drug Administration USA) standard
procedure testing samples on Plate Count Agar, (PCA) incubated
at 30 - 35°C. The method using SWA incubated at 20°C provided
better and more accurate bacterial counts under "good" and
"poor" manufacturing practice.

The hurdle concept was applied, using gamma irradiation, temperature (Time), chemical preservatives and Vacuum packaging in an attempt to prolong the shelf life of fresh, uncooked Cape hake and Kingklip products. Radurization was effective only when the treated product was kept at refrigerated temperatures. Temperature was the most important control measure in extending the shelf life of the products. No shelf life extension was achieved with the use of chemical preservatives. Vacuum packaging was not effective in prolonging shelf life of the products and did not show any benefit when compared to oxygen permeable vacuum (microaerophilic) packaging.

Determination of the total aerobic viable bacteria numbers at 20°C SWA, were suitable for estimating the shelflife of deepsea whitefish products.

BAKTERIESE STANDAARDE VAN

MERLUCCIUS CAPENSIS / PARADOXUS (KAAPSE STOKVIS)

EN ANDER DIEPSEE WITVIS PRODUKTE

DEUR

INGO HEINRICH VENNEMANN

PROMOTOR : PROFESSOR T E CLOETE

MEDE-PROMOTOR : PROFESSOR A VON HOLY

DEPARTEMENT : MIKROBIOLOGIE AND PLANTPATOLOGIE

GRAAD : PhD (MIKROBIOLOGIE)

OPSOMMING

Die gebruik van totale bakterie tellings by 20°C op SWA (Sout Water Agar) was geskik en effektief vir die instel van mikrobiologiese standaarde vir Stokvis produkte. Die tellings was ook geskik vir die uitwys van tyd and temperatuur misbruike in die produksie proses. Die metode was sensitief genoeg om veranderings uit te wys met 'n toename in hantering. Bakterie tellings by 20°C op SWA was meer geskik as die huidige EG/FDA (Europese Gemeenskap/ "Federal Drug Administration" VSA) standaard prosedure 30 - 35°C inkubasietemperatuur op standard "PCA" ("Plate Count Agar") vir die bepaling van standaarde.

Die hindernis konsep, wat van gamma bestraling, temperatuur (Tyd), chemiese preserveermiddles en vakuum verpakking gebruik maak was benut in 'n poging om die rakleeftyd van vars, ongekookte Kaapse stokvis en Koningklip produkte te verleng. Bestraling was net effektief by verkoelde, temperatuurbeheerde berging. Temperatuur was die mees effektiewe metode om bederf van die produkte te vertraag. Rakleeftyd kon nie met behulp van chemiese preserveermiddels bewerkstellig word nie. Vakuumverpakking was ook nie effektief om rakleeftyd te verleng nie. Totaale seegebonde, koue verdraende bakterie-tellings by 20°C op SWA was geskik om rakleeftyd te bepaal.

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

A word of thanks to the many individuals who, without question, assisted in the successful completion of this task. The secretaries, the clerks and many more who supported me loyally through running endless photocopies, telephone calls, typing of labels and keys with strange sounding names and words, computer instructions and worksheet layouts. A special word of thanks to some major contributors:

To Professor Eugene Cloete, whose positive attitude and longdistance advice was instrumental in pushing this program to completion and ensured its contribution to the industry as a whole.

To Professor Alex von Holy, whose constructively critical review of submitted documentation and sound advice on structuring research programs made this work worthwhile.

To Engela Cloete (formerly Lamprecht) of The Fishing Industry Research Institute and Frank Loewenadler for running countless identifications on marine bacteria.

To Dr. Kobie Wessels of the Fishing Industry Research Institute for allowing me to use the excellent FIRI facilities and experience.

To the librarian of the Fishing Industry Research Institute for doing endless literature researches, copying and posting to a specific address in Saldanha. One of the backbones of this project.

To the Quality Assurance Department of Sea Harvest Corporation for their willingness to be turned into large scale research

assistants and for doing a great job of running endlessly
repetitive experiments, another backbone of this project.

To the management of Sea Harvest Corporation for making it all
possible.

And last but not least to my wife, Anneliese and family who
never failed to encourage me to forge ahead and complete this
work, when energy and motivational levels ran low.

CHAPTER 1

Bacteriological standards of
Merluccius capensis / paradoxus (Cape Hake)
and other Deepsea Whitefish Products

INTRODUCTION

The food industry in South Africa, as in most parts of the world, is experiencing a new global trend towards safer, longer lasting foods. As the demand for food increases with a rising world population, the risk of foodborne disease is increasing (WARD and HACKNEY, 1991; MILLER JONES, 1992; TAYLOR, 1994). The single largest hazard to shelf life and fresh food safety is bacterial contamination. The whitefish resource in South Africa is represented mainly by the Cape hakes (*Merluccius capensis* and *Merluccius paradoxus*) which are highly perishable due to their soft texture and high water activity (SIMMONDS and LAMPRECHT, 1985). Due to these characteristics, whitefish products are amongst those most in need of good manufacturing practice (GMP) and safety assurance. Food microbiology, and in particular the microbiology of whitefish, is therefore of interest to both manufacturer and customer alike (LORD, 1990; ANON., 1992). There is consequently an increasing demand for bacteriological standards, or legally enforceable minimum specifications.

Current standards for total aerobic bacteria on fresh or frozen uncooked food products, including deepsea whitefish products, allow a maximum of 10^6 cfu/g. These are based on counts on aerobic plate count agar (PCA) incubated at $35 \pm 2^\circ$C for 48h which increasingly changed to 30°C for 48h (SABS 1977; FDA 1986, 1994; EEC 1991, 1993; CODEX ALIMETARIUS, 1993). Currently the European Union is still revising bacteria standards for imported food products. Recently however, with the advent of value added processing, these and bacteriological pathogen standards were ineffective in protecting customers from premature spoilage and foodborne disease (BONNELL, 1994). For example, with the increasing concern about *Listeria monocytogenes*, as a major foodborne pathogen, this organism achieved the dubious honour of throwing the standardization authorities into disarray. Authorities in many countries went from a completeley exclusive standard or zero tolerance in all foods to a more realistic (*Listeria monocytogenes* are ubiquitiuos in nature), limited

number of organisms for uncooked raw foods, which require cooking by the consumer. The only zero tolerance remaining, is for processed, ready to eat foods (BONNELL, 1994). Another example of an ineffective standard is the use of "coliforms" as an indicator for the sanitary safety of a particular food item which has been shown to be largely irrelevant (MOSSEL and VAN NETTEN, 1991).

The above casts serious doubts on the relevance of "blanket" bacterial counts as standards for a large variety of foods. According to Mossel and van Netten (1991), bacteriological standards should be derived via a five step process which includes the assessment of the type and origin of the product and its microbiological ecology. This, in turn, will determine the relevant maximum permissible numbers of bacteria, as well as the method of analysis. Emphasis is therefore placed on the importance of constant adaptation and innovation regarding the setting of standards, taking cognisance of the ecology, origin and type of food substance, as well as processing environment and controls (MOSSEL and VAN NETTEN, 1991).

The current standard of total aerobic bacterial counts relies on incubating samples at 30 - 35°C on PCA which the EU (European Union) and the FDA (Federal Drug Administration / USA) apply to all foods regardless of type or origin. This might not be representative of bacteria which occur on fresh or frozen, uncooked deepsea whitefish products. These products are manufactured from fish caught and processed in marine, psychrotrophic environments (4 - 12°C). The current "blanket" standard might thus not be as applicable to fresh or frozen, uncooked deepsea whitefish products, as it would for products derived from a mesophilic environment such as pork, beef and poultry.

Instead a bacteriological standard is required for the deepsea whitefish industry in South Africa, relying on a test method

based on the assessment of the marine psychrotrophic environment to give a relevant and accurate measurement of potential shelf life. The primary objective of this study was therefore, to use the process of Mossel and van Netten (1991) to determine how meaningful bacterial counts are, when total aerobic bacterial counts are determined at 20°C on salt water agar (SWA). This method should also be evaluated for its suitability to monitor critical control points (CCP's) of fish processing. Samples were then split and counted on SWA incubated at 20°C as well as on PCA incubated at 35°C according to the existing standard (SABS 1977; EEC 1991; CODEX ALIMENTARIUS, 1993). The results from the monitoring of control points in fish processing were used to compare the two test methods directly for their suitability regarding shelf life or freshness of fishery products.

The secondary objective of this study was to show whether shelf life in terms of the internal standard (maximum 10^6 cfu/g on salt water agar, SWA, for 48h) could be extended using several preservation techniques (hurdles). The hurdles were specific measures to inhibit or reduce bacterial growth on various deepsea whitefish products such as irradiation, temperature control, chemical preservation and vacuum packaging, in order to extend the shelf life of these products stored under psychrotrophic (refrigerated or chilled) conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 **THE MICROBIOLOGICAL SAFETY OF MARINE FIN FISH PRODUCTS**

Seafood consumption in the world has increased in recent years.
Whilst quality and safety are important issues in the manufacture
of seafoods, microbiology is one of the principal sciences
associated with quality and safety and the ability of the
manufacturer to guarantee his products in these respects (WARD
and HACKNEY, 1991). In order to ensure quality and safety for
consumers many countries have adopted legally enforced minimum
specifications or standards regarding various bacterial
populations potentially present on these seafoods (FDA, 1986,
1994; EEC, 1991, 1993; CODEX ALIMENTARIUS, 1993).

The microbial ecology of seafood processing has direct
implications for the quality and safety of these products, given
the tendency of processing larger quantities of seafoods (GARRET
and HUDACK-ROOS, 1991; MILLER JONES, 1992). Indeed,
microbiological factors have been identified as being the major
"hazard" or risk category for the consumer compared to other
risks of physical or chemical nature (MILLER JONES, 1992).

Bacteria are the most common microbiological hazards and cause
many different foodborne diseases. With an increasing market for
prepared, precooked and ready to eat, chilled seafoods there is
an increasing tendency for foodborne diseases. In the USA, the
Centre for Disease Control (CDC) indicates that reported
incidences of foodborne disease are increasing (MILLER JONES,
1992). One half to one third of all diarrhea cases in the USA are
of foodborne origin (MILLER JONES, 1992). Reasons contributing to
the increase of foodborne disease are amongst others modern
lifestyles of increased travelling, less home cooking, eating
foods which are more processed and therefore carry a higher risk
of containing bacterial pathogens and ignorance regarding correct
food handling and preparation in catering establishments and the
home (MILLER JONES, 1992; TAYLOR, 1994).

Usually, the more common agents of foodborne disease are closely associated with the human environment. *Salmonella*, *Staphylococcus areus*, *Shigella* and more recently *Listeria monocytogenes* have been linked to poor sanitation habits during processing and coupled with unsafe food storage and preparation techniques and are common causes of seafoodborne disease (JAY, 1986; WARD and HACKNEY, 1991; MILLER JONES, 1992).

2.2 INTRINSIC SEAFOODBORNE MICROBIAL HAZARDS

Despite the fact that fishery products are of diverse origin, the composition of the naturally occurring microbial community is similar in most cases (ICMSF, 1986; VENNEMANN; 1991). The micro-organisms most commonly encountered mirror the communities found in seawater and sediment and rarely include mammalian pathogens. Hence fish caught in waters not polluted by human or animal wastes are free from intrinsic microbiological hazards when handled according to good manufacturing practice (GMP), (SHEWAN, 1961, 1977; SHEWAN and MURRAY, 1979; SHIPMAN and WYLER; 1989; LORD, 1990; GARRETT and HUDACK ROOS, 1990, 1991). Fish and other free-swimming marine animals do not usually carry organisms considered to be typical of the mammalian microbial community, including *Escherichia coli*, the "faecal coliforms" and enterococci. The presence of human enteric organisms on marine food products is evidence of contamination from a terrigenous source. The relative impact seafoods have in the greater part of foodborne illness is limited in the USA and Europe (ICMSF, 1986). However, there is a continuing incidence of bacterial borne disease from fish products in Japan and other Asian countries where fish is traditionally consumed raw or only partially prepared. In most cases the aetiologic agent are *Vibrio* spp. However, some botulism, due to improperly prepared products also occurs (ICMSF, 1986). Of particular note are several *Vibrio* species such as *V. cholerae, V. parahaemolyticus* and *Vibrio*

vulnificus. These are usually associated with warm (>25°C) waters and estuaries where the water may be contaminated with effluent from large concentrations of human habitation. *V. parahaemolyticus and V. vulnificus* are marine organisms mainly occuring in warm waters (>25°C), in proximity of the shore (inshore) (ICMSF, 1986; WARD and HACKNEY, 1991; MILLER JONES, 1992).

2.2.1 <u>Intrinsic hazards in certain species of fish</u>

A hazard caused by a natural marine bacterial community is scombroid fish poisoning (also known as histamine poisoning). This is largely associated with warm water fish of the Scombridae (mackerel, tuna, mahi-mahi) and the Carangidae (yellow tail) from tropical or subtropical regions, although pilchards, salmon and also snoek (*Thyrsites atun*) from southern african pelagic (surface) waters have also been implicated (SIMMONDS and LAMPRECHT, 1985; WARD and HACKNEY, 1991, MILLER JONES, 1992). A common factor is that these fish are known as "fatty" fish and contain naturally high levels of histidine. Histidine, under certain abusive conditions, such as high temperature storage after death, can rapidly be decarboxylated into histamine. Decarboxylation is brought about by the enzyme histidine decarboxylase which occurs in bacteria from the enteric region of the fish. Bacterial genera implicated are *Morganella* (formerly *Proteus*), *Hafnia* and *Klebsiella* spp (SIMMONDS and LAMPRECHT, 1985; STRATTON and TAYLOR, 1991; MILLER JONES, 1992). There are a variety of symptoms of this disease which include rash, urticaria, edema, nausea, vomiting, diarrhea and abdominal cramps. Also typical are burning or tingling sensations in the mouth, a metallic or peppery taste, palpitations and facial flushing. High levels of histamine exceeding 100 mg per 100g of fish, sometimes up to 430 mg per 100g, was detected from almost every fish implicated in an outbreak (NIVEN *et al.*, 1981;

STRATTON and TAYLOR, 1991). The toxin produced is heat stable and is therefore not destroyed by cooking. The phenomenon is probably the most common and widespread form of foodborne illness related to the ingestion of seafoods, and hs been reported from almost all parts of the world (STRATTON and TAYLOR, 1991).

2.2.2 External afflictions caused by fish handling

A form of skin irritation in workers handling and particularly gutting, fish with hihgh histamine levels has sometimes also been ascribed to histamine poisoning. It is possible, however, that this condition is caused by *Erysipelothrix* spp, which have been repeatedly described as infecting wounds or abrasions on the hands of fish handlers. It is not clear whether these organisms are part of the natural flora of newly caught fish, or introduced by contamination (SHEWAN 1961; SIMMONDS and LAMPRECHT, 1985).

2.2.3 Absence of intrinsic hazards from south african deepsea whitefish

South african hake (*Merluccius capensis* / *Merluccius paradoxus*) is mainly caught in the cold to temperate waters of the southern atlantic Benguela current ecosystem, in deep water from 200 to 600 meters. Hake is also not a "fatty" fish with high concentrations of histidine and therefore represents no hazard regarding histamine poisoning (SIMMONDS and LAMPRECHT, 1985; WARD and HACKNEY, 1991; MILLER JONES, 1992). The temperature in these fishing grounds ranges from 4 to about 8°C on average, but may rise to 15 or 16°C on occasion. At these temperatures and removed from the influence of human habitation few *Vibrio spp,* have been isolated (SIMMONDS and LAMPRECHT, 1980, 1985; VENNEMANN, 1991). The south-west african subcontinent is also a predominantly arid region with few rivers draining into the sea. It follows,

therefore, that the incidence of human bacterial pathogens is likely to be low on freshly caught Cape hake.

2.2.4 <u>Quality and safety attributes of deepsea whitefish to be considered when initiating regulatory bacteriological standards</u>

Seafoods are regarded as more perishable than other high protein foods, and several factors can be used to explain this. Marine fish contain high levels of osmoregulators in the form of nonprotein nitrogen, ie., amino acids, trimethylamine, urea and others which are readily available to bacteria as a source of nutrients (FIELDS and RICHMOND, 1968; NICKELSEN and FINNE, 1984). Most marine whitefish species are caught from deep, cold waters and psychrotrophic microbial growth is not as effectively slowed down by chilling or icing as the microbial population from warm blooded animals. Safety in seafood products with reference to microbial contamination is usually concerned with the possibility of food poisoning mainly caused by pathogenic bacteria introduced into seafood products during stages of unhygienic processing (GARRETT and HUDACK-ROOS, 1991).

In this context, it is important to note that regarding the Cape hakes and for most other deepsea whitefish from cold water the predominant microbial flora consists principally of *Moraxella, Pseudomonas, Alteromonas* (*Shewanella*), and some other less frequently encountered (<20%) Gram negative genera such as flavobacteria, and Vibrionaceae. Of the Gram positive bacteria the predominant groups are *Micrococcus* and Coryneforms with other less frequently encountered genera (<10%) such as *Bacillus* and *Clostridium* (SHEWAN, 1971; MARTIN *et al.*, 1978; HODGKISS, 1980; HOBBS and HODGKISS, 1982; HOBBS, 1987; VENNEMANN, 1991). Of these organisms, only *Vibrio parahaemolyticus, V. vulnificus* and the clostridia are capable of inducing food poisoning, since they

occur naturally on fish from warmer, inshore waters. However, since these genera comprise only less than 10% of bacterial community and require specific conditions for growth (anaerobic and >25°C), the fish normally spoils before becoming toxic in abusive (above 10°C) situations. It also follows that whitefish remains a safe or hazard free food product provided that during processing the natural bacterial community structure was not altered (NICKELSEN and FINNE, 1984; VENNEMANN, 1991). Furthermore, *Vibrio* and *Clostridium* have rarely or never been isolated from fresh south african hake (*Merluccius capensis* and *M. paradoxus*) (SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991). The above are important considerations when formulating bacteriological regulatory minimum specifications on quality and safety of deepsea whitefish such as Cape hake.

2.3 EXTRINSIC SEAFOODBORNE MICROBIAL HAZARDS

2.3.1 Deepsea whitefish flesh as substrate for pathogenic bacterial contamination

High water activities (0.995) and relatively neutral pH (6.6 - 7.0), as well as high concentrations of osmoregulators (VENNEMANN, 1991) make the Cape hakes as well as other whitefish ideal substrates for "exogenous" bacterial contamination. Typically this contamination would come from the human environment such as sewage polluted rivers, estuaries, bays and inshore waters. Poor sanitary conditions during processing, or other abusive situations, such as cross-contamination when stored in close proximity with foods originating from warm blooded, terrestial animals also contribute. Since most commercially caught whitefish, including Cape hake, is trawled from deeper, colder waters far from the shore, chances of finding typical human environment-pathogenic bacteria on the fish are remote on freshly caught fish (SHEWAN, 1961, 1971; ICMSF, 1986).

Contamination of fish with organisms of public health
significance remains primarily a problem of handling and
processing (CONNELL and SHEWAN, 1980; HOBBS and HODGKISS, 1982).

2.3.2 <u>Microbiological parameters to consider when formulating</u>
       <u>bacteriological standards for regulatory purposes</u>

Microbial foodborne disease has been known and studied since
1880. Subsequently, numerous cases of foodborne disease have been
recorded (JAY, 1986; MILLER JONES, 1992). Among the requirements
for foods to be of good sanitary quality, they must be shown to
be free of hazardous microorganisms, or those present should be
at a safe level. In general, it is not feasable to examine each
seafood product for the presence of all these microorganisms. The
practice that has been in effect for many years and continues to
be followed, is to determine the sanitary quality and safety of
foods by using indicator or index organisms. Typically, for Cape
hake these organisms are faecal coliforms, *E. coli*,
Staphylococcus aureus, *Shigella*, *Salmonella*, *Clostridium*, *Vibrio*,
and more recently *Listeria monocytogenes* (SABS, 1977, 1987).

The coliform group of organisms as indicators of food sanitary
conditions are being eliminated from most legislation due to the
large diversity of genera and species now belonging to this order
(ICMSF, 1986; MOSSEL and VAN NETTEN, 1991; WARD and HACKNEY,
1991). In cold water fish, total aerobic bacterial counts on PCA
incubated at 35°C, are also still used as an indicator of human
environment bacterial contamination, even though they have only
limited use as indicators of health risk (SABS, 1977; ICMSF,
1986; JAY, 1986; MOSSEL and VAN NETTEN, 1991; BONNELL, 1994). An
aerobic plate count (APC) is designed to measure composite
bacterial populations or the total number of microorganisms
capable of growth under aerobic conditions at a specified
incubation temperature. To be used as a quality (remaining shelf

life) index, an APC should provide at least two kinds of
information: the count should be indicative of the food's present
state of deterioration or freshness; and the count should allow
some prediction of future shelf life (MARTIN et al., 1978). The
accuracy of such deductions based on a total count determination,
is strictly limited to the specific application of especially the
incubation temperature. Since spoilage of Cape hake is mostly
brought about by Gram negative psychrotrophic bacteria, an APC at
20^oC, would be more meaningful to satisfy above conditions than
an APC at a less optimal growth temperature for marine
psychrotrophic organisms (SIMMONDS and LAMPRECHT, 1985; HOBBS,
1987; VENNEMANN, 1991). It follows therefore, that the currently
used standard, total viable bacterial counts on PCA incubated at
35^oC, might be in need of revision for fresh and uncooked frozen
deepsea whitefish.

2.4 MICROBIOLOGICAL STANDARDS FOR SEAFOOD PRODUCTS

In South Africa and indeed throughout the world, food safety
aspects have recently become of major importance. The concept of
Hazard Analysis Critical Control Point (HACCP) was first
introduced in the USA in 1959, when the Pillsbury Company was
asked to produce a food that could be used under zero gravity
conditions in the space capsules of the astronauts (PIERSON and
CORLETT, 1992). HACCP is the application of good manufacturing
practice (GMP) (FDA, 1986, 1994). Possible hazards are identified
and indicated on a "step by step" processing flow diagram;
appropriate preventive controls are designed and installed. The
controls are monitored and records are kept to assure that the
system is working properly. When problems do occur, they are
identified and promptly corrected (TAYLOR, 1994). The reason for
the recent re-emergence of HACCP in world food safety legislation
can be found in the fact that, although simple in its basic
concepts, it has features which make it a sophisticated and

powerful tool for meeting the industry's food safety responsibility for the following reasons (TAYLOR, 1994): (1) it is *science* based. HACCP takes advantage of what we know or have learned scientifically about a process to determine which potential hazards deserve focused attention. (2) HACCP is *preventive*. It is a systematic approach to preventing food safety hazards from becoming food safety problems. (3) HACCP *recognizes where the responsibility lies* for producing safe food. Each participant in the food production system that adopts an HACCP plan accepts responsibility for producing safe foods - for having in place a system that is *designed* to produce safe food. (4) HACCP provides an extraordinary opportunity to link the food industry's system for producing safe food with a government's system of regulatory oversight, by linking GMP and adherence to standards with minimal regulatory verification through reduced sampling (GARRETT and HUDACK-ROOS, 1990; TAYLOR, 1994; VAN SCHOTHORST, 1994).

In South Africa, the fishing industry and its production facilities are subject to the authority of the South African Bureau of Standards (SABS) which has also adopted the concepts of managing risk and quality (HACCP/ISO 9000). Whilst continuing to randomly sample seafood products for microbiological and physical analyses, there is a strong drive from this body to establish HACCP systems for the local industry acceptable to sophisticated consumers in all parts of the world. It is important to note that HACCP merely addresses the public need for food safety, thus setting minimum standards, whereas quality of the various products remains the choice of industry and consumer. Since it is impossible to test all products in sufficient quantities at all times, an HACCP system allows for random "monitoring" sampling. This system is designed with the benefit of reducing final sampling and inspection, especially for public health authorities (BROWN, 1991).

Regulatory oversight, control by the relevant authority, can only be achieved with the setting of standards, against which any sample's performance may be measured and assessed for risk or hazard (BROWN, 1991).

2.4.1   Sampling plans for seafood products

Included in most microbiological standards for seafoods today are sampling plans. These are statements of the criteria of acceptance applied to a lot or product batch based upon appropriate examinations of a required number of units by specified methods (JAY, 1986). The target value m is the lowest practically attainable level of bacterial counts (cfu/g) under conditions of GMP, above which a product would be acceptable only, if counts are not higher than a maximum permissible number (M). The value M is the acceptability limit defined as the maximum count permissible for a quality product (quality encompassing both safety and shelf life) (JAY, 1986; WARD and HACKNEY, 1991; BONNELL, 1994). Clearly, M remains below bacterial counts at which spoilage or illness upon ingestion could result. The number of samples for the analysis equals n and c represents the number of samples permitted to be between m and M in a typical three class plan (m = desirable standard, or process under control, m and c = tolerance, or process may be getting out of control, M = absolute permissible limit of cfu/g, or the process is out of control). If c is exceeded, or one sample exceeds M, the batch is rejected and the manufacturing process considered deficient (JAY, 1986; MOSSEL and VAN NETTEN, 1991; WARD and HACKNEY, 1991; BONNELL, 1994). The numerical difference between m and M is of concern. The acceptable size of this difference is determined by the limits within which the food processing system can be controlled to guarantee a quality product. Also included, should be the anticipated effect of post process conditions (transport, storage and distribution) and the

likely effect of home kitchen preparation on microbial numbers as well as on the bacterial community structure (therefore emphasizing the lowest, practically achievable bacterial counts). The effect of high counts of certain bacteria on the most sensitive group of consumers likely to eat the product, may also be taken into consideration (MOSSEL and VAN NETTEN, 1991; WARD and HACKNEY, 1991; VAN SCHOTHORST, 1994).

Another more simple version of a sampling plan, usually applied to large production batches, is the two class plan which only uses n, c, m. In its simplest form this plan can be used to accept or reject (thus the 2 classes: there is no tolerance in bacterial counts) a large batch of food, for example, testing for a pathogen in a presence/absence decision by a plan such as $n = 5$, $c = 0$, where $n = 5$ means that five individual units of the lot will be examined microbiologically. $c = 0$ means that none of the samples may test positive for the pathogen (JAY, 1986; WARD and HACKNEY, 1991).

Whilst both sampling plans are suitable for a purchaser or verification of compliance to a standard by import authorities on finished batches or consignments, the 2-class plan does not allow for naturally occurring inconsistencies, for instance in the processing of fresh deepsea whitefish (SHEWAN, 1961; VENNEMANN, 1991; WARD and HACKNEY, 1991). Regarding psychrotrophic, marine bacterial counts, essential in estimating shelf life and quality of the product, the 2-class plan is not suitable. The 3-class plan, with values set for safety or shelf life only, is also not suitable for a fish processing factory. A sample consisting of five individual units, taken together at any time off the processing line cannot be relevant, since the process may be continuing for a day or more and this sample represents only a moment in time of production, with many variables over time and individual fish (GEORGALA, 1958; SHEWAN, 1961; VENNEMANN, 1991). In order to be truly random in this situation, samples must be

taken at regular time intervals of the same continuous production on a random basis (selected at random off the processing line). The ranges of bacteriological counts, having been obtained over a period of time, under conditions of GMP and then incorporated into the 3-class plan, offer a more realistic approach:

2.4.2   The process of setting a bacteriological standard

Traditionally a standard implies a specific numerical value, but should be replaced by the concept of reference values or ranges (MOSSEL and VAN NETTEN, 1991). Such ranges should be developed by ecologically justified surveys of food specimens capable of supporting microbial survival and growth. The ranges should be incorporated into 3-class sampling plans (m,M,c) specifically designed for each individual commodity (MOSSEL and VAN NETTEN, 1991). A manner in which to arrive at relevant reference values is demonstrated via 5 essential sequential steps:
* selecting target organisms;
* choosing and standardizing a method to be
used for their enumeration, alternatively use an already approved official standard method;
* carrying out surveys of samples taken from the production environment adhering to GMP;
* deriving acceptable numerical values from the survey data guided by an HACCP analysis, meaning the evaluation of the microbiological analysis data in terms of identified potential hazards and the setting of safe tolerance levels;
* establishing a policy for dealing with consignments which fail the target values (acceptable counts) (MOSSEL and VAN NETTEN, 1991).

The above concept may, in terms of food microbiology, also be utilized to derive *end-product specifications* which are usually part of the purchase agreement and may be set by the retailer,

consumer, or the legal authority (Port health authorities). Similarly, this concept can be used to establish *guidelines*, usually set internally by the producer, and microbiological *standards* which are legally enforceable specifications usually adopted by individual countries (MOSSEL and VAN NETTEN, 1991).

Another factor for setting bacteriological standards for foods is the recently recognized "Minimal Infective Dose" (MID) concept. The idea behind this concept is that foodborne disease is dose related. For example: the human response depends on the numbers of pathogenic microorganisms ingested. It is recognized that these numbers may vary according to species and pathogenicity of the bacteria, the type of food consumed and the susceptibility of the consumer. The goal of this concept is to establish the levels of specific microorganisms that can be tolerated when consumed by the various categories of consumers (VAN SCHOTHORST, 1994). Thus the emphasis, as indicated earlier for sampling plans, is on developing standards matched to the specific type of food, its associated microorganisms and the requirements of customers, rather than being a legal specification constrained by limited perspective. A specification of such "unsuitable" nature is the current EU/FDA standard of establishing total viable bacterial counts at mesophilic incubation temperatures(30 or 35°C) on PCA, when applied on uncooked, frozen, deepsea whitefish such as Cape hake, where bacterial community structures are dominated by psychrotrophic, marine bacteria.

2.4.3 The role of index/indicator bacteria in setting standards

However, regarding the microbiological safety of seafoods, industry and authority based monitoring is an important activity. In such cases it is desirable to test seafoods, directly, for the presence of pathogenic organisms. This is possible mainly where the food has caused an actual outbreak of foodborne illness with

known symptoms, through stool samples (VAN SCHOTHORST, 1994).
More often, the requirement is for tests that will indicate the
absence, or presence in small numbers, of pathogenic organisms in
wholesome food. In such cases use is frequently made of tests for
index/indicator organisms. Index organisms have traditionally
been bacteria which are assumed to be associated with the
presence of pathogenic bacteria (JAY, 1986). For example
Escherichia coli has been used as an index of faecal
contamination, and therefore the possible presence of intestinal
pathogens such as *Salmonella* spp. The index organisms should be
present in much higher numbers than the pathogen and are
therefore easier to detect (JAY, 1986; HOBBS, 1987; GARRETT and
HUDACK-ROOS, 1990, 1991).

Indicator organisms are those, whose presence in numbers above
certain limits indicate inadequate processing or the absence of
GMP in respect of, for example, microbiological safety
(Enterobacteriaceae) and "lack of freshness" (aerobic,
psychrotrophic bacteria incubated at 20°C on SWA) of the fish
during processing (MOSSEL and VAN NETTEN, 1991; VENNEMANN, 1991).
The presence of an index organism such as *E. coli* in a precooked,
ready to eat seafood, may serve to indicate both the possible
presence of pathogens as well as an unhygienic manufacturing
process. The limitations of index organisms for regulatory
standards in view of improved methods of identification of
specific foodborne pathogens may seriously impede their use in
future regulatory standards (BROWN, 1991). However, their use as
indicator organisms may continue to be applicable to the
assessment or validation of thermal and other types of processing
designed to appreciably or totally reduce the microbiological
populations of foods (BROWN, 1991). Since indicator organisms are
generally composed of a broad spectrum of microbes (total counts,
enterobacteriaceae, etc.) and are easier to isolate, measure and
their destruction being analogous to the destruction of
pathogens, their use in regulatory standards can still be

supported (BROWN, 1991). It is common for food industries to use bacteriological "index/indicator" organisms in order to assess, control and ensure effective plant sanitation practices and, therefore, ensure a food product that is microbiologically safe and of an acceptable quality to the consumer (MOSSEL and VAN NETTEN, 1991; VAN SCHOTHORST, 1994). In order to be meaningful, however, it is important that the sampling procedure, the method of recovery of organisms from the samples and the interpretation of the analyses results be standardised along the five point concept indicated above (MOSSEL and VAN NETTEN, 1991). In addition, the number of microbiological criteria to be used for the estimation of safety and quality of foods must be limited for at least two reasons: firstly, reducing the number of tests to be carried out, enables more samples to be examined. This markedly increases the accuracy of the results, particularly since contaminating microbial populations might be unevenly distributed. Secondly, the use of criteria which stem from an imaginary microbiological problem does little to improve the credibility of the food microbiologist (MOSSEL and VAN NETTEN, 1991).

2.4.4 Application problems with some bacteriological food standards

Current world standards for seafoods include the testing for total viable bacteria (TVB) per gram on PCA incubated at 30 - 35°C, faecal coliforms/*E. coli* in most probable numbers (MPN) per 100 grams, co-agulase positive *Staphylococcus aureus* per gram, *Salmonella*, *Shigella* and *Clostridium*. More recently, *Listeria monocytogenes* was added to the list by several countries. However, since there is limited knowledge about *Listeria monocytogenes* only a few countries have laid down specific enforceable standards for this bacterium (JAY, 1986; WARD and HACKNEY, 1991; BONNELL, 1994).

The maintenance of microbiological standards for seafoods has often been a dilemma for authorities. For instance, the legal limit for total viable bacteria on raw, frozen fish products is 10^6 cfu/g tested at 30- or 35°C on PCA (m), with a tolerance of 2 (c) out of 5 (n) samples showing counts of $\geq 10^6$ cfu/g (M). On cooked, ready-to-eat seafood products the standard is stricter at $m = 10^5$ cfu/g, $M =$ either 2 X 10^5 or 5 X 10^5 cfu/g in 2 or only 1 (c) out of 5 (n) samples respectively (SABS 1973, 1987; EEC, 1991, 1993; CODEX ALIMENTARIUS, 1993; FDA, 1994). However, since raw foods of marine origin usually support a diversity of natural marine bacteria, PCA is not a suitable recovery medium, but could be replaced with Salt Water Agar (SWA) (SHEWAN and HOBBS, 1967; HODGKISS, 1980; CHANDRASEKARAN et al., 1985; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991). Since much of the world's seafood is caught in cold or temperate waters, an incubation temperature of 30- or 35°C is unsuitable to recover marine psychrotrophic bacteria as indicators for GMP, temperature control and estimation of shelf life (HOBBS, 1983, 1987; VENNEMANN, 1991). Furthermore, in todays competitive markets, new products made from combinations of micro-ecologically diverse raw materials, such as combinations of cheese and fish are increasingly marketed. Mature cheddar cheese may show a total aerobic bacterial count of $10^8 - 10^{10}$ cfu/g. Frozen, raw fish products are not permitted to show more than 10^6 cfu/g (SABS, 1987). The question arises, whether the standard for fish is still meaningful and applies to the combined uncooked product. It is therefore clear, that any attempt to generalize bacterio-logical product specifications with a blanket statement, such as a standard, is not possible, nor scientifically justifiable under all conditions (MOIR, 1991). Even at public authority level a standard and the breaking of it, should therefore be treated with circumspection, which warrants further investigation rather than a product recall.

The total coliforms, previously used as indicators for poor

processing and process sanitation are no longer a standard requirement (BRODSKY, 1991). This group has taxonomically grown so diverse that effectively no difference exists to testing for total viable bacteria at 35 $\pm$ 2°C (BRODSKY, 1991; MOSSEL and VAN NETTEN, 1991). Faecal coliforms and *Eschericia coli*, however, are a predominant part of the warm blooded animal and human intestinal bacterial populations and thus, at low numbers, serve as indicators for either direct or indirect faecal contamination of the product and at higher numbers, serve as indices for the likely presence of intestinal pathogens like *Salmonella* and *Shigella* (MOIR, 1991; MOSSEL and VAN NETTEN, 1991).

2.4.5 Current standards for bacterial pathogens in seafoods

The current standard for faecal coliforms (*E. coli*) is absent in raw, frozen seafoods (*m*) with no more than 300 cfu/100g (*M*) in 2 (*c*) out of 5 (*n*) samples. In cooked, ready-to-eat seafoods *m* = absent, *M* = <100 cfu/100g in 1/5 samples. The standard for pathogens like *Salmonella*, *Shigella*, *Vibrio cholerae* and *Campylobacter jejuni* is "absent" in 30 grams for both raw, frozen and cooked, ready-to-eat seafood products (SABS, 1973, 1987; EEC, 1991, 1993; CODEX ALIMENTARIUS, 1993; FDA, 1994).

Staphylococus aureus may produce a heat stable enterotoxin when permitted to grow to a level >10^4 cfu/g (RHODEHAMEL, 1992). The foodborne intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (>60°C) or cold enough (<10°C) (RHODEHAMEL, 1992). The organism is commonly isolated from hands (infected cuts, pimples and sores) and nasal/throat passages of humans. Thus foods requiring reprocessing or extensive handling are at risk. This organism can grow at low water activity (a_w of 0.86) and at high salt concentrations (RHODEHAMEL, 1992). The current standard for *Staphylococcus*

aureus on seafood products is <30 cfu/g, although there is a tendency to move towards a three point sampling plan (n,M,c) and acceptance limits: $n = 5$, $c = 1$, $m = 10^3$ cfu/g, $M = 10^4$ cfu/g (BONNELL, 1994). However, the Canadian Fisheries Inspectorate report that buyer specifications tend to be much stricter at $n = 5$, $c = 2$, $m = 10$ cfu/g, $M = 100$ cfu/g on raw, frozen seafood products (BONNELL, 1994; FDA 1994).

The standard regarding *Listeria monocytogenes* is, due to the ubiquitous nature of this organism and its ability to grow under stressed conditions ($3^{o}C - 45^{o}C$, pH of 5.0 - 9.6, a_w of 0.94 and less, salt $\geq$ 10%) more complex (BONNELL, 1994). Few countries have yet established a standard, however the Canadian government through its Department of Fisheries and Oceans have taken the lead in order to define safety related guidelines, enforceable in the first multifaceted standard of its kind. The standard is expressed as a "compliance policy" which has evolved due to the controversy surrounding this organism's epidemiological history (BONNELL, 1994). With more sophisticated science and greater knowledge about *Listeria*, several realizations became apparent. Firstly, there are only few cases of listeriosis per year in the USA with a population that exceeds 250 million, and the majority of these cases involve individuals who are immunocompromised, due to disease, medical treatment or pregnancy. Secondly, the organism is extremely widespread in nature, having been isolated from human and animal faeces, vegetables, soil, poultry, dairy and marine foods (BONNELL, 1994). Thirdly, most sporadic cases of listeriosis are epidemiologically linked to few foods, notably soft cheeses, undercooked chicken and poorly re-heated hot dogs. Fourthly, some ready-to-eat foods (green salads, cold smoked salmon) are prepared without listericidal processing steps, yet apparently were not sources of infection in a case control study performed by the Centre for Disease Control (CDC) in the USA. Due to these facts, the "FDA's" and other countries "zero tolerance" standards are being vigorously challenged (MADDEN, 1994). Even

aureus on seafood products is <30 cfu/g, although there is a tendency to move towards a three point sampling plan (n,M,c) and acceptance limits: $n = 5$, $c = 1$, $m = 10^3$ cfu/g, $M = 10^4$ cfu/g (BONNELL, 1994). However, the Canadian Fisheries Inspectorate report that buyer specifications tend to be much stricter at $n = 5$, $c = 2$, $m = 10$ cfu/g, $M = 100$ cfu/g on raw, frozen seafood products (BONNELL, 1994; FDA 1994).

The standard regarding *Listeria monocytogenes* is, due to the ubiquitous nature of this organism and its ability to grow under stressed conditions ($3^{o}C - 45^{o}C$, pH of 5.0 - 9.6, a_w of 0.94 and less, salt $\geq$ 10%) more complex (BONNELL, 1994). Few countries have yet established a standard, however the Canadian government through its Department of Fisheries and Oceans have taken the lead in order to define safety related guidelines, enforceable in the first multifaceted standard of its kind. The standard is expressed as a "compliance policy" which has evolved due to the controversy surrounding this organism's epidemiological history (BONNELL, 1994). With more sophisticated science and greater knowledge about *Listeria*, several realizations became apparent. Firstly, there are only few cases of listeriosis per year in the USA with a population that exceeds 250 million, and the majority of these cases involve individuals who are immunocompromised, due to disease, medical treatment or pregnancy. Secondly, the organism is extremely widespread in nature, having been isolated from human and animal faeces, vegetables, soil, poultry, dairy and marine foods (BONNELL, 1994). Thirdly, most sporadic cases of listeriosis are epidemiologically linked to few foods, notably soft cheeses, undercooked chicken and poorly re-heated hot dogs. Fourthly, some ready-to-eat foods (green salads, cold smoked salmon) are prepared without listericidal processing steps, yet apparently were not sources of infection in a case control study performed by the Centre for Disease Control (CDC) in the USA. Due to these facts, the "FDA's" and other countries "zero tolerance" standards are being vigorously challenged (MADDEN, 1994). Even

though *Listeria* have been isolated from various seafood products such as shrimps, oysters, black mussels, clams, lobster tails and fish roe, no seafood has yet been responsible for a listeriosis outbreak (BONNELL, 1994; MADDEN, 1994). The current most popular standard for this organism in uncooked seafood products is $\leq$ 100 cfu/g (MADDEN, 1994) and "not present" on all ready-to-eat foods which will support the growth of this bacterium (pasteurized milk, soft cheeses).

2.5 APPLICATION OF HURDLE TECHNOLOGY TO SEAFOOD PROCESSING

Most food processing operations will be at risk from one or more biological hazards, either from the raw materials or during the process, and the HACCP plan will be designed to control these (MORTIMORE and WALLACE, 1994). The most common and important biological hazard is microbiological through its potential to generate foodborne disease (MORTIMORE and WALLACE, 1994; VAN SCHOTHORST, 1994). Therefore, in food processing operations the prevention of "hazardous" microbes from contaminating the product is a primary concern. Some stresses, or hurdles, such as canning, ultra high temperature (UHT), radappertization (radiation sterilization) are extreme, removing all microbes from foods (JAY, 1986). Other hurdles are less extreme, such as pasteurization, radurization, vacuum packing, drying, salting and smoking. Whilst some industries may not have many alternatives to preserve products, such as the dairy and canning industries, the whitefish industry has another alternative, ie.: that of prevention of bacterial growth rather than cure. This means, that at all times during all stages of processing, the deterioration of the fish must be controlled to be as slow as possible through Good Manufacturing Practice (GMP) (LORD, 1991; VENNEMANN 1991; ANON., 1992; CODEX ALIMENTARIUS, 1993; FDA, 1986, 1994).

2.5.1 Precautionary measures or controls in fish processing to prevent bacterial growth and contamination

In a whitefish processing factory, processing from fresh to the finished frozen product, involves such precautionary activities, like strict handwashing routines, good line, surface and tool sanitation, as well as temperature and time controls ($<10^{o}C$). These should ensure the prevention of contamination of product with pathogenic, human associated bacteria and the rapid build up of marine psychrotrophic bacteria, which could cause premature spoilage (GEORGALA, 1957; SHEWAN, 1961, 1977; HODGKISS, 1980; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; SHIPMAN and WYLER, 1989). However, the effectiveness of these precautions during processing ashore is limited by the quality and state of the fish as landed by the trawlers. In fact, the most effective precautions are those, which control the actual trawling techniques, such as drag length and volume and the speed, handling and temperature with which the catch is worked away. The manner of storage of the headed and gutted Cape hake onboard the trawler is important for texural quality as well as limiting bacterial growth. This requires the correct ratio of fish-to-ice, to prevent crushing and to facilitate rapid cooling of the fish by melting ice. The time of the catch spent onboard the trawler is also an important determining factor for the quality of the landed catch (GEORGALA, 1957; SHEWAN 1961, 1977, SIMMONDS and LAMPRECHT, 1985; LORD, 1990; WARD and HACKNEY, 1991; VENNEMANN, 1991; CODEX ALIMENTARIUS, 1993; BONNELL, 1994).

When using GMP in an industry such as the above, it is desirable to install controls for quality as close as possible to the living resource, or the start of the process flow. Some of these might be enacted *before* the fish is caught by preventing abusive practices by the trawler's crew and skipper, such as excessive trawling times and volumes, which cause textural damage and increase the total microbiological population on the skin of the

fish in the cod end (closed end of trawling net) (WARD and HACKNEY, 1991, BONNELL, 1994).

Knowledge about the practices of retail outlets as well as the final consumer will also assist with placing hurdles to make the product last as long as possible and be free of microbiological hazards (WARD and HACKNEY, 1991; VENNEMANN 1991; ANON., 1992; BONNELL, 1994).

2.5.2 Hurdles used in fish processing to extend shelf life and reduce the risk of pathogenic bacterial proliferation

Hurdles employed by the whitefish industry are temperature control (chilling and freezing), radurization, vacuum packing, salting and smoking, to name a few typical methods specifically designed to slow the spoilage process and eliminate proliferation of pathogens (SHEWAN, 1961, WARD and HACKNEY, 1991).

2.5.2.1 Smoking

In the traditional and still widely used practice of smoking for instance, there are at least 3 important steps in a process, which may have in excess of thirteen steps that are critical in controlling microbial and pathogen growth/toxin elaboration (HODGKISS, 1980; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; HOBBS, 1987; WARD and HACKNEY, 1991), ie.:

* Brining to ensure salt penetration of the product, effectively lowering the a_w.
* Smoking to ensure thermal penetration as well as penetration of antimicrobial substances such as phenols.
* Storing to ensure refrigeration temperatures to prevent microbiological growth and toxin production by pathogens.

2.5.2.2 <u>Gamma-irradiation</u>

Radurization is still controversial and not generally accepted by the consumers (HOBBS, 1987; WARD and HACKNEY, 1991). Raduri-zation is best applied to packaged or sealed products in order to prevent regrowth or re-contamination of the products after the treatment. Radurization is effective in reducing the total number of bacteria as well as species diversity. Under certain conditions, such as resistance to radurization, the eliminiation of interspecific competition of natural or endemic bacteria might lead to accelerated growth of the survivor species (*Moraxella* spp and *Micrococcus* spp) on fresh, unfrozen whitefish products. Thus extension of shelf life may not necessarily be achieved (MIYAUCHI, 1972; HOBBS and HODGKISS, 1982; TIWARI and MAXCY, 1982; JAY, 1986; HOBBS, 1987). Product safety might also be compromised, especially where consumers are not well educated: if endogenous non-pathogenic bacteria are reduced or damaged by a process such as radurization, the perishable product, contaminated through handling by a food service establishment or home consumer, might have toxins present before the product is noticeably spoiled.

There are three degrees or levels of control relating to seafood irradiation (JAY, 1986; WARD and HACKNEY, 1991; MILLER JONES, 1992), ie.:

*   *Radappertization* is a high intensity irradiation which eliminates or inactivates all micro-organisms and effectively sterilizes the product. The process is named after "Appert", who developed the traditional thermal canning process. The doses required to achieve sterility may be as high as 50 kGy.
*   *Radurization* is a treatment with an effect similar to that of heat pasteurization by inactivating 90-95% of spoilage micro-organisms. This reduction may extend the shelf life of refrigerated, fresh seafoods. The dose required is considered

to be <10 kGy in the range 1-5 kGy, since dose levels in excess of 5 kGy have been observed to dtrimentally affect the sensory qualities of seafoods.

* *Radicidation* is used for the specific inactivation of non-spore-forming pathogenic bacteria sometimes present in seafoods, such as *Salmonella, Shigella,* Vibrionaceae and *Staphylococcus*. Members of the Vibrionaceae are amongst the most sensitive and may be inactivated with doses as low as 0.5 kGy. However, higher doses may be necessary to inactivate *Salmonella* and especially *Staphylococcus* where the most effective level is 5-8 kGy (JAY, 1986; WARD and HACKNEY, 1991; MILLER JONES, 1992).

It is important to note, that standard pathogenic bacterial sampling and analysis procedures may not be applicable to radurized seafoods: *E. coli* is often used as an indicator/index organism for feacal pathogens. This common bacterium of the human intestine is as sensitive to irradiation as are the Vibrionaceae and is therefore inactivated before pathogens such as *Salmonella* and enteric virusses, effectively eliminating its usefulness as an indicator organism in radurized seafoods (WARD and HACKNEY, 1991).

2.5.2.3 <u>Modified atmoshere packaging</u>

Since most spoilage and pathogenic bacteria require oxygen to grow, the reduction of oxygen's partial pressure from the environment is a stress or hurdle to aerophilic microorganisms and can result in prolonging shelf life of fresh chilled seafood products (WARD and HACKNEY, 1991). This is especially the case if vacuum packaging is applied in conjunction with refrigerated storage. However, because of the anaerobic conditions in vacuum packaging, the possibility of encouraging the growth of *Clostridium botulinum* must be considered (CANN *et al.*, 1965;

HOBBS and HODKISS, 1982; MILLER JONES, 1992). Another variant of the above is the use of different gases inside the packaging in modified atmosphere packaging (MAP). Different mixtures of gasses are chosen here to delay or inhibit microbial and oxidative spoilage. Storage in carbon dioxide atmospheres is effective for increasing shelf life in a variety of fish products, since pseudomonads are one of the most sensitive bacteria to elevated (80% CO_2/20% air) concentrations of this gas (JAY, 1986; WARD and HACKNEY, 1991). Since the Gram negative part of the microbial community on fish products is more sensitive towards CO_2 and the Gram positive (micrococci, lactobacilli, clostridia) more resistant, the effect of MAP using this gas results in a change regarding the predominance of these two groups (WARD and HACKNEY, 1991). In fact, CO_2 can act as a stimulant for clostridial spore germination, especially at a lower pH due to hydration of the gas to carbonic acid (JAY, 1986; WARD and HACKNEY, 1991). Apart from the food safety issue, which may thus be negated, the lowering of the pH of the fish in the package may cause textural damage by breaking down sensitive connective tissue binding muscle segments and cause extensive "gaping" in soft textured whitefish species such as hake (SHEWAN and HOBBS, 1963; JAY, 1986; WARD and HACKNEY, 1991). Thus this hurdle, MAP, may not be an effective safety measure nor may it preserve the eating quality (texture) of the whitefish product.

Controlled storage temperature ($<1^{O}C$) is the single most critical consideration regarding the success of MAP or vacuum packaging in prolonging shelflife and safety of fish products (WARD and HACKNEY, 1991). In Cape hake fillets, vacuum packaging and MAP at 40/30/30, $CO_2/N_2/O_2$ and 60/40, CO_2/O_2, was not observed to change the predominance of the microbial population from Gram negative to Gram positive and spoilage was still caused by a predominance of *Pseudomonas* spp, although this could have been due to high initial bacterial counts on the fillets. Furthermore, storage of prepacked MAP Cape hake fillets at $1^{O}C$ yielded prolonged shelf

life when compared to storage at 5°C (LAMPRECHT *et al.*, 1984).
Therefore, to effectively prolong shelf life, CO_2 concentrations
in excess of 40% should be considered only in conjunction with
refrigerated storage.

2.5.2.4 "Sous Vide" or minimal processing

Minimally processed seafoods or "sous vide" products are a recent
development. This process uses cooking under vacuum (temperatures
under 100°C) for short durations, to prepare pasteurized and
vacuum packed foods which are kept refrigerated (WARD and
HACKNEY, 1991). However, the safety of these products has been
questioned, because they are only minimally processed and do not
contain preservatives to control microbial growth. Furthermore,
the cooking does not eliminate *C. botulinum* spores and there is
some question as to whether it may allow other organisms such as
Listeria to survive. The safety and shelf life of these products
is solely dependent on refrigeration, therefore it is critical
that psychrotrophic pathogens, like *Listeria* and *Yersinia* do not
survive and grow (WARD and HACKNEY, 1991).

2.5.2.5 Salting and drying processes

Other processes such as salting, drying and fermentation by
lactic acid bacteria are also still widely used to prolong shelf
life. Although these are restricted to areas of the world where
people traditionally prefer strong flavour development. These
processes are effective in reducing the water activity (a_w)
and/or pH to levels unfavourable for microbial growth. However,
halophilic bacteria might still cause premature spoilage of
salted fish products, whereas yeasts and moulds as well as
halophilic or halotolerant *Micrococcus* spp are usually
responsible for spoiling dried fish products, especially under

humid conditions (HOBBS and HODGKISS, 1982; HOBBS, 1987; JAY, 1986, MILLER JONES, 1992). The drying of white, low fat fish such as cod (the original "stockfish") or hake is preceded by a salt cure, where fillets are layered, skin side up, interspersed with coarse salt. When sufficient brine has formed (after ca. 24 h) the fish are submerged with a heavy weight or stone. They are kept like this for a period of another 48-72 h and are then removed, stacked and either artificially dried in a kiln or in the sun. Such processes usually result in a cured fish product having about 12% salt and 35% water content after drying. But drying after an even heavier salt cure, lasting for months may result in a salt content of about 25% and a water content of 35% in the final hard-dried stage (SHEWAN and HOBBS, 1967). The effect of this hurdle on the microbial ecology of the fresh fish is mainly that the salt cure seems to change the microbial ecology from psychrotrophic Gram negative, to mesophilic Gram positive bacteria, mostly *Micrococcus* and also some salt tolerant, pigmented halobacteria. The latter bacteria are associated with the well known "pink" condition of salt cured and dried fish causing characteristic sour or cheesy odours and eventual disintegration of the tissue. All these bacteria are non-pathogenic and disease after consumption of cured/dried fish is usually only caused by halotolerant *Staphylococcus aureus* strains (SHEWAN and HOBBS, 1967; HOBBS and HODGKISS, 1982; HOBBS, 1987).

2.5.2.6 Chemical preservatives

The addition of, chemical preservatives such as sorbate, benzoate, to a certain extent polyphosphate and others, is a controversial subject. They are restricted internationally with legal limits in quantities contained in products. No claims may be attached to any such food to be free of pathogen risks (JAY, 1986; MILLER JONES, 1992). They are, however, effective (MILLER

JONES, 1992) in prolonging shelf life of fresh, quality raw
material, but usually only in conjunction with refrigerated
storage.

2.5.2.7 Freezing and frozen storage

Freezing is still the most favoured method of seafood
preservation and one of the most effective hurdles to microbial
growth. Freezing is considered the best medium term (6 - 18
months) food storage method available, because in most cases both
the eating quality (taste and texture) remain unaltered and the
nutritional (minimal protein denaturation and loss of nutrients)
quality is maintained (MILLER JONES, 1992). There are two ways of
freezing foods: quick and slow freezing. For a product to be
called "quick frozen" the minimum requirement is a freezing rate
of 25 mm product thickness per h, to below $-5^{o}C$ in a period not
exceeding 4 h and a core temperature of $-20^{o}C$ at the completion
of the process (SABS, 1977; EEC, 1991). This may be achieved by
direct immersion into refrigerants such as liquid nitrogen or the
use of blast or plate freezers. Slow freezing refers to the
process whereby the desired temperature is achieved at a rate of
6mm or less product thickness per h, with the process requiring
in excess of 24 hours to achieve $-20^{o}C$ (SABS, 1977, 1987). This
type of freezing is considered to be poor manufacturing practice
in the industry (SABS, 1977; FDA, 1986; CODEX ALIMENTARIUS,
1993), whereas it is the usual practice in the home freezer (JAY,
1986). Quick freezing prevents the formation of large intra-
cellular ice crystals which disrupt the cellular membranes,
leading to excessive drip- and nutrient loss upon defrosting or
cooking. Instead, many small crystals are formed during this
process which cause less damage to cells during the freezing
process (JAY, 1986).

A number of microorganisms have been reported to grow at and

below 0°C (JAY, 1986). In order to prevent this growth the temperature of frozen storage has to be low enough to restrict the free water available to microbes. Enzymic reactions are also slowed or stopped, delaying oxidative changes in the stored seafoods. The international standards community has set the specification for the storage and distribution of frozen whitefish products at a maximum of -18°C (EEC, 1991; CODEX ALIMENTARIUS, 1993) which stops most microbial activity and slows enzymic reactions in whitefish. However, most larger seafood packers and distributors using GMP (FDA, 1986) store at or below -24 to -30°C, with a few exceptions such as tuna for the Japanese market, being stored at -60°C (SIMMONDS and LAMPRECHT, 1985; JAY, 1986; LORD, 1990).

Although freezing is known to reduce the viability of many food spoilage bacteria as well as pathogens, quick freezing improves the viability of bacteria. Even though some bacteria are killed by freezing, this process should never be used as a safeguard against bacterial pathogens, nor can it be seen as an effective tool to reduce spoilage and potentially harmful bacteria (SIMMONDS and LAMPRECHT, 1985; JAY, 1986; WARD and HACKNEY, 1991).

Today in the seafood processing industry there are different activities, which are designed to enhance product shelf life and safety to the consumer. Some of these processes have been indicated above, however, none of the hurdles from irradiation to freezing can be successfull without appropriate preventative steps. These are control points placed at critical stages in the process flow, being monitored from the trawler to the processing plant, into the package and supermarkets. The hurdle concept can therefore never rely on a single hurdle placed at the end of the process, but must be supported by a systematic approach or a series of controlled steps (HACCP) within the process flow. Each of the control points is designed to prevent and control:

physical damage, contamination with hazardous chemicals and foreign objects, microbial growth and contamination with potentially harmful bacteria. Hurdles refer to specific steps taken to reduce and/or prevent bacterial growth within a food processing system.

Standardization of quality and safety monitoring is an important issue and an integral part of monitoring the effectiveness of the critical control points in a seafood processing system. Current bacteriological standards occasionally are meaningless such as an aerobic plate count as a part of food safety legislation. In addition the "zero tolerance" on *Listeria monocytogenes*, which is unrealistic (unattainable for many raw foods) amd the total coliforms as indicators of process sanitation and total viable bacterial counts at 30 - 35°C on PCA, for deepsea whitefish products are equally unfounded. Particularly questionable is the "blanket" standard of total aerobic bacterial counts at 30- or 35°C on PCA, which the EU and the FDA apply to all foods, regardless of type or origin. For standards to be meaningful they must in themselves consist of careful evaluations, as highlighted by the five essential steps (MOSSEL and VAN NETTEN, 1991) in initiating bacteria standards.

The objective of the subsequent research was to motivate introducing a more suitable standard in respect of a marine, psychrotrophic environment by applying the above five step concept to a whitefish processing system. The current directives of the EU and the FDA include total aerobic bacterial counts in their standard requirement of HACCP for countries importig seafood products. The results of this study were used to establish a system of processing point controls (Critical Control Points, CCP) and monitors, as well as demonstrating the suitability of aerobic, marine, psychrotrophic bacterial counts at 20°C on SWA for initiating an new international standard. In addition, the hurdle concept was used in various applications in

an attempt to find a way of extending the shelf life of Cape hake and other deepsea whitefish products to confirm the suitability of the proposed bacterial standard as a tool to estimate the bacterial quality (freshness).

CHAPTER 3

Bacteriological standards of
Merluccius capensis / paradoxus (Cape hake)
and other deepsea whitefish products

3.1 INTRODUCTION

In a whitefish factory processing between 120 and 150 tons of
Cape hake per day, many different products are routinely screened
on a daily basis for compliance with certain minimum
bacteriological standards or legal specifications. Six product
groups were selected for studying, based on different levels of
processing intensity (Figs. 1 - 6). Since most of the processing
is manual (GEORGALA, 1957; VENNEMANN, 1991), it follows that with
each processing step, time, temperature and therefore the
microbiology of the product may be affected. Absence of
microbiological and other hazards to consumer health at the
trawling and on board processing stage was ascribed to the fact
that deepsea whitefish, such as Cape hake, is intrinsically free
from microbiological and other hazards when caught, headed and
gutted and laid in ice from unpolluted, cold ocean current
ecosystems. Microbiological hazards of public health significance
are usually associated with the terrestial or human environment
of processing and handling (SHEWAN, 1961, 1971; CONNELL and
SHEWAN, 1980; HOBBS and HODGKISS, 1982; NICKELSEN and FINNE,
1984, ICMSF, 1986, VENNEMANN, 1991; WARD and HACKNEY, 1991).

Currently the international standard for total aerobic bacterial
counts ($m = 10^5$ cfu/g; $M = 10^6$ cfu/g; $c = 3$ and n = 5; on PCA
incubated at 30° or 35°C for 48h) on uncooked, fresh and frozen
deepsea whitefish, does not allow bacterial counts to exceed 1
million cfu/g. It was derived from the meat standards in Europe
and the USA which address aerobic bacterial counts of products
derived from a terrestial or warm-blooded source (EEC, 1991,
1993; CODEX ALIMENTARIUS, 1993; FDA, 1994). However, the
psychrotrophic nature of the inherent, natural bacterial
community structure places a question mark over the suitability
of the current international standard methodology.

When a bacteriological standard is first determined, several key

aspects have to be considered (MOSSEL and VAN NETTEN, 1991). These aspects are the careful selection of the target organisms and methods for their isolation taking their environment into account. These in turn rely on the ecology of the source or product which is then to be tested under GMP and non adherence to GMP. Had the system of MOSSEL and VAN NETTEN been applied to deepsea, whitefish the current international standard would not have been in existence.

Under these conditions, a standard relying on a test method for psychrotrophic, marine bacteria on uncooked, fresh and frozen deepsea whitefish could be more suitable than a standard relying on a test method for enumeration of mesophilic bacteria. The objective of this study was therefore, that by applying the system recommended by MOSSEL and VAN NETTEN a more relevant standard could be determined for testing deepsea whitefish products from cold and temperate marine environments. Under conditions of varying processing intensity and GMP total aerobic bacterial counts on SWA incubated at $20^{O}C$ were compared to total aerobic bacterial counts on PCA incubated at $35^{O}C$ on the same products in order to confirm the hypothesis of a more relevant bacteriological standard for deepsea whitefish products.

3.2 MATERIALS AND METHODS

3.2.1 Sample preparation and bacteriological analysis

Fish muscle ($\pm$20g) was cut with a sterilized knife from products randomly sampled twice daily, either before or after quick freezing. These were suspended in 180 ml quarter strength Ringer's solution at $20^{O}C$ and $35^{O}C$. Frozen product was allowed to defrost for 30 min at room temperature, in order to facilitate cutting. The soft texture of Cape hake facilitated the break up

of the samples in the diluent by vigorous shaking in a 250 ml "Schott" sterilizable bottle (continuous for 60 s). Stomaching was therefore not necessary. For sea frozen product, samples were taken randomly upon discharge of the vessel. Normally this was after six weeks of production at sea.

The sampled products were split into 6 different groups according to the intensity of processing as illustrated in Fig. 1-6. Fig. 6 illustrates the "Frozen-At-Sea (FAS) fillets" process flow (filleting, trimming, deboning, skinning and defatting). This product was different from the other products (Fig. 1-5) in that fish was processed and frozen within two hours of catching at sea. The other products were processed on land from wet landed, headed and gutted Cape hake averaging 2 to 6 d old on ice (VENNEMANN, 1991). Each of these products were sampled twice daily (when manufactured), 5 d per week, over a 19 month period from January 1993 to July 1994, except the seafrozen fillets which were sampled ca. every 6 weeks, upon discharging of the factory trawler. The samples consisted of frozen, packed fillet blocks (5 Kg, individually wrapped fillets frozen in plate freezers, in a frame of rectangular shape) taken randomly from the discharging conveyor at regular intervals throughout the two or three day offloading process. The blocks were "shattered" (fillets loosened) by bending the interleaved fillet blocks over the diagonal on a table corner whilst in their packaging and the loose fillets randomly selected for bacteriological analysis.

Standard bacteriological analysis methods were used for the enumeration of aerobic psychrotrophic bacteria incubated at 20°C and 35 ± 2°C, for 48h (AOAC, 1990). Plate Count Agar (PCA) used for standard 35°C incubation and enumerations, was modified to Sea Water Agar (SWA) for the enumerations of aerobic, marine, psychrotrophic bacteria (ZOBELL, 1941, 1946; SHEWAN and HOBBS, 1967; SIMIDU and HASUO, 1968a, b; LEE and PFEIFFER, 1974; MORITA, 1975; HORSLEY, 1977; SIMMONDS and LAMPRECHT, 1985). Both methods

were used to analyse samples since the legal standard at the time prescribed enumerations of mesophilic total aerobic bacterial counts on PCA incubated at 35°C (SABS, 1977). The pour-plate method was used for aerobic SWA, as well as the 35°C PCA bacterial enumerations (AOAC, 1990; VENNEMANN, 1991). A sample dilution of 10^{-3} was done in quarter strength Ringer's solution, before SWA and PCA pour-plates were prepared according to the method described above. Routine analyses included faecal coliforms, *Escherichia coli,* and *Staphylococcus aureus.* Random analysis of several products, for *Listeria monocytogenes* were done monthly for export products in accordance with export requirements (EEC, 1991). Methods used for these analyses were standard AOAC methods and consisted of the 5 tube - MPN test for faecal coliforms and *E. coli* as well as the positive-negative (indole and gas positive) indicator method for *E. coli.* *Staphylococcus aureus* was isolated and enumerated on Baird Parker agar plates, this included testing for DNAse and coagulase according to standard AOAC methodology (AOAC, 1990; VANDERZANT and SPLITTSTOESSER, 1992).

Listeria monocytogenes were isolated using pre-enrichment (UVM1 AND UVM2/Frazer's broth) as well as Palcam agar (Merck) plates. Positive colonies were identified using the API Biochemical *Listeria* kit (AOAC, 1990; VANDERZANT and SPLITTSTOESSER, 1992).

3.2.2   Construction of product group flow charts

The processing flow charts of all the different product groups were constructed using a detailed manufacturing specification, defect description and HACCP analyses regarding both, critical control points (HCP = CCP) as well as quality (subcritical) control points (QCP) (Figs. 1 - 6). By the authors definition, Hazard Control Points are those critical to consumer safety. Quality Control Points are those critical to consumer satisfaction.

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time and Volume	1.5 h; 5t max
Heading and Gutting	QCP2	Time and Temperature	1.5 h; 20°C max.
Washing	QCP 3	Prevention of autocatalysis	Belly cavity washing
Icing and Stowage	QCP 4	Ratio fish to ice and total quantity per crate	2 : 1 Fish to ice, max 29 kg Fish per crate
Discharge into Factory Chiller	QCP 5	Fish age in d	Less than 8d
IN FACTORY ASHORE			
De-icing / Descaling / Washing	-	-	-
Size Grading	QCP 6	Sizing	8 sizes by mass / length 80g - 2.4Kg
Intermediate Chilled Storage	QCP 7	Time and temperature stock rotation	5h max ; 5°C max
Filleting (Baader 188 / 210L)	-	-	-
Bellywashing and Trimming	HCP 1	Removal of fin bones	Remove all fin bones
Textural and Colour Grading	QCP 8	1st and 2nd grade only	Slight gaping only (Separation of muscle segments)
Deboning / Blemish Removal	HCP 2 / QCP 9	No bones and removal of small bloodspots / bruises	No bones / blemishes
Pouching and Packing	HCP 3 / QCP 10	Coding for recall / massing	Day code inner, mass within legal tolerance
Sampling for Bacteriological Analysis	HCP 4	To comply with minimum standard	SABS, 1977
Platefreezing (Quickfreezing)	QCP 11	Temperature	-22°C max core temp; day code outer
Coldstorage / Distribution	QCP 12	Time and temperature	-18°C max 6 months max.

Fig. 1 Flow chart for the production of prime fillets of hake
(basic processing)

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time & Volume	1.5 h; 5t max
Heading and Gutting	QCP2	Time and Temperature	1.5 h; 20°C max.
Washing	QCP 3	Prevention of autocatalysis	Belly cavity washing
Icing and Stowage	QCP 4	Ratio fish to ice and total quantity per crate	2 : 1 Fish to Ice, max 29 kg Fish per crate
Discharge into Factory Chiller	QCP 5	Fish age in d	Less than 8d
IN FACTORY ASHORE			
De-icing / Descaling / Washing	-	-	-
Size Grading	QCP 6	Sizing	8 sizes by mass / length 80g - 2.4Kg
Intermediate Chilled Storage	QCP 7	Time and temperature stock rotation	5h max ; 5°C max
Filleting (Baader 188 / 210L)	-	-	-
Bellywashing and Trimming	HCP 1	Removal of fin bones	Remove all fin bones
Textural and Colour Grading	QCP 8	1st and 2nd grade only	Slight gaping only (Separation of muscle segments)
Deboning / Blemish Removal	HCP 2 / QCP 9	No bones and removal of small bloodspots / bruises	No bones / blemishes
Manual Portioning and Resorting			Shape, size, quality
Pouching and Packing	HCP 3 / QCP 10	coding for recall procedures / pack and portion massing	Day code inner, mass within legal tolerance
Sampling for Bacteriological Analysis	HCP 4	To comply with minimum standard	SABS, 1977
Platefreezing (Quickfreezing)	QCP 11	Temperature	-22°C max core temp; day code outer
Coldstorage / Distribution	QCP 12	Time and temperature	-18°C max. case temp.; 6 months max.

Fig. 2 Flow chart for the production of steaks and loins (medium processing)

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time & Volume	1.5 h; 5t max
Heading and Gutting	QCP2	Time and Temperature	1.5 h; 20°C max.
Washing	QCP 3	Prevention of autocatalysis	Belly cavity washing
Icing and Stowage	QCP 4	Ratio fish to ice and total quantity per crate	2 : 1 Fish to Ice, max 29 kg Fish per crate
Discharge into Factory Chiller	QCP 5	Fish age in d	Less than 8d
IN FACTORY ASHORE			
De-icing / Descaling / Washing	-	-	-
Size Grading	QCP 6	Sizing	8 sizes by mass / length 80g - 2.4Kg
Intermediate Chilled Storage	QCP 7	Time and temperature stock rotation	5h max ; 5°C max
Filleting (Baader 188)	-	-	-
Bellywashing and Trimming	HCP 1	Removal of fin bones	Remove all fin bones
Textural and Colour Grading	QCP 8	1st and 2nd grade only	Slight gaping only (Separation of muscle segments)
Deboning / Blemish Removal	HCP 2 / QCP 9	No bones and removal of small bloodspots / blood-vessels / bruises	No bones / blemishes
Deepskinning / Final Trimming / Blemish Removal	HCP 3	Parasite inspection and removal	Only slight fat residue no parasites
Fingerlaying of Block / Blockmassing	HCP 4 / QCP 10	Fillet lay / general defect inspection / microbiological sampling	SABS, 1977
Platefreezing (Quickfreezing)	HCP 5 / QCP 11	Coding for recall procedure / temperature / finished product inspection and sampling	Day code inner & outer; -22°C max, core temp. mass within legal tolerance
Coldstorage / Distribution	QCP 12	Time and Temperature	-18°C max core temp; 3 months max.

Fig. 3 Flow chart for the production of cape hake blocks
(medium high processing)

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time & Volume	1.5 h; 5t max
Heading and Gutting	QCP2	Time and Temperature	1.5 h; 20°C max.
Washing	QCP 3	Prevention of autocatalysis	Belly cavity washing
Icing and Stowage	QCP 4	Ratio fish to ice and total quantity per crate	2 : 1 Fish to Ice, max 29 kg Fish per crate
Discharge into Factory Chiller	QCP 5	Fish age in d	Less than 8d
IN FACTORY ASHORE			
De-icing / Descaling / Washing	-	-	-
Size Grading	QCP 6	Sizing	8 sizes by mass / length 80g - 2.4Kg
Intermediate Chilled Storage	QCP 7	Time and temperature stock rotation	5h max ; 5°C max
Filleting (Baader 188 / 210)	-	-	-
Bellywashing and Trimming	HCP 1	Removal of fin bones	Remove all fin bones
Textural and Colour Grading	QCP 8	1st and 2nd grade only	Slight gaping only (Separation of muscle segments)
Deboning / Blemish Removal	HCP 2 / QCP 9	No bones and removal of small bloodspots / bruises	No bones / blemishes
Deepskinning / Final Trimming / Blemish Removal	HCP 3	Parasite inspection and removal	Only slight fat residue no parasites
Cutting / Portioning / Folding and Pouching	HCP 4 / QCP 10	Microbiological sampling / texural and visual quality	SABS, 1977
Packing / Sealing	HCP 5	Coding for recall procedure	Day code inner, mass within legal tolerance
Platefreezing (Quickfreezing)	QCP 11	Temperature	Day code outer; -22°C max, core temp.
Coldstorage / Distribution	QCP 12	Time and Temperature	-18°C max core temp; 3 months max.

Fig. 4 Flow chart for the production of cape whiting steaks (highly processed)

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time & Volume	1.5 h; 5t max
Heading and Gutting	QCP2	Time and Temperature	1.5 h; 20°C max.
Washing	QCP 3	Prevention of autocatalysis	Belly cavity washing
Icing and Stowage	QCP 4	Ratio fish to ice and total quantity per crate	2 : 1 Fish to Ice, max 29 kg Fish per crate
Discharge into Factory Chiller	QCP 5	Fish age in d	Less than 8d
IN FACTORY ASHORE			
De-icing / Descaling / Washing	-	-	-
Size Grading	QCP 6	Sizing	8 sizes by mass / length 80g - 2.4Kg
Intermediate Chilled Storage	QCP 7	Time and temperature stock rotation	5h max ; 5°C max
Filleting (Baader 188 / 210)	-	-	-
Bellywashing and Trimming	HCP 1	Removal of fin bones	Remove all fin bones
Textural and Colour Grading	QCP 8	1st and 2nd grade only	Slight gaping only (Separation of muscle segments)
Deboning / Blemish Removal	HCP 2 / QCP 9	No bones and removal of small bloodspots / bruises	No bones / blemishes
Cutting / Portioning			
Deepskinning / Final Trimming / Blemish Removal	HCP 3	Parasite inspection and removal	Only slight fat residue no parasites
Handfilling 8 Portions per Tray	QCP 10	Portion minimum mass and piece number control	8 portions 20g minimum mass
Packing / Sealing	HCP 4 / 5	Coding for recall procedure Microbiological sampling	Day code inner; mass within legal tolerance SABS, 1977
Platefreezing (Quickfreezing)	QCP 11	Temperature / finished product sampling and inspection	Day code outer; -22°C max, core temp.
Coldstorage / Distribution	QCP 12	Time and Temperature	-18°C max core temp; 3 months max.

Fig. 5 Flow chart for the production of fiori
(highly processed)

PROCESS STEPS	QCP / HCP	CONTROL	SPECIFICATION
AT SEA			
Trawling / Catching	QCP 1	Time; volume	1,5 h; 5t max
Size Grading	QCP 2	Sizing	8 sizes
Descaling / Washing			Remove slime & scales
Filleting / Gutting / Deboning (Baader 182)	HCP 1	Removal of pin bones	No bones in fillets
Deepskinning / Washing	QCP 3	Skin and fat removal	Slight fat residue No skin
Trimming and Blemish removal	HCP 2	Parasite inspection and removal.	No parasites
Washing	QCP 4	Prevention of autocatalysis of finished product.	Belly lining removal & washing
Drying Conveyor	QCP 5	Remove excessive water	Dry appearance
Prepacking Conveyor / Size grading of Fillets	QCP 6	Size grading	Visual fillet size grading 6 categories
Massing / Weighing / Hopper filling	QCP 7	Block mass control	To be within legal limit.
Packing (Shatterpacking) / Final blemish removal	QCP 8	Colour texture and defect control.	No blemishes 1st & 2nd grade textural quality
Platefreezing (Quickfreezing)	HCP3 / QCP 9	coding for recall procedure / temperature	-22°C max core temp. day code inner
Coldstorage onboard Factory Trawler			-18°C core max. day code outer; 6 weeks max.
Discharging of Trawler once / six weeks	HCP 4 / QCP 10	Finished product inspection and microbiological sampling.	Finished product audit.
Coldstorage and Distribution	QCP 11	time and temperature / stock rotation.	-18°C max core temp. 6 months max.

Fig. 6 Flow chart for the production of frozen at sea hake fillets
(highly processed)

3.2.3 Statistical evaluations

All statistical analyses (frequency distributions, means,
variance and standard deviations) were done using the Software
package of "Quattro Pro" Version 5.0, 1993.

In total 6 689 x 2 (20°C, SWA and 35°C, PCA) aerobic bacterial
counts, covering a 19 month period, from January 1993 to July
1994, were compiled according to processing intensity in their
respective product categories (Fig. 1 - 6). Each one of the
product group counts were classified in frequency (y-axis)
columns along class intervals (x-axis)(Figs. 7 - 12). The number
of samples for each product was determined by production criteria
such as market demand, fish availability and textural quality.
The most basic product used for this analysis was "Prime Hake
Fillets" (Fig. 1, 7) and a total of 476 x 2 (20°C, SWA and 35°C,
PCA) enumerations were done over the entire time span. Whereas
2971 x 2, 976 x 2 and 1178 x 2 enumerations were done,
respectively, for "Steaks and Loins"(Fig. 2, 8), "Cape Whiting
Steaks"(Fig. 4, 10) and "Fiori" (Fig. 5, 11). "Steaks and Loins"
actually consist of two distinct products which are, however, cut
simultaneously from the same fillet. On the "Cape Hake Blocks"
(Fig. 3, 9) 605 x 2 enumerations were done and 483 x 2
enumerations of the "Frozen-At-Sea (FAS) Fillets" (Fig. 6, 12)
were carried out.

The manufacture of each product is dependent on the size of the
market and on various quality attributes such as texture, colour,
bruising and fish size availability, which may change during the
year. Hence, there may be a month to month variation in the
manufacture of the different products resulting in variations in
sampling and analysis frequencies (Fig. 7 - 22).

3.3 RESULTS AND DISCUSSION

3.3.1 Introduction

According to MOSSEL AND VAN NETTEN (1991) when setting
microbiological standards a 5 point plan should be adhered to:
(*) selecting target organisms;
(*) choosing and carefully standardizing a method to be
used for their enumeration, alternatively an already approved
official standard method could be used;
(*) carrying out surveys of samples taken from the production
environment adhering to GMP;
(*) deriving acceptable numerical values from the survey data
guided by an HACCP analysis;
(*) establishing a policy for dealing with consignments which
fail the target values.

Mossel and van Nettens five point plan was followed during this
study and the results will be discussed accordingly.

3.3.1.1 Selecting target organisms and choosing and standardi-
zing a method for their enumeration

A total aerobic bacterial count is useful only for the estimation
of freshness or shelf life of the product sampled. Since spoilage
in deepsea whitefish is brought about by aerobic, marine
psychrotrophic bacteria (GEORGALA, 1957; SHEWAN, 1961; LISTON,
1980; SIMMONDS and LAMPRECHT, 1985; MOSSEL and VAN NETTEN, 1991;
VENNEMANN, 1991), the choice of the target group of
microorganisms was aerobic, marine, psychrotrophic bacteria. The
choice of medium was SWA (SIMONDS and LAMPRECHT, 1985) and the
incubation temperature 20°C since the optimum growth temperature
for marine psychrotrophic bacteria is at 20°C (SHEWAN, 1961;
LISTON, 1980; HOBBS, 1987). This would ensure that the first two
steps of Mossel and van Nettens five point plan were covered.

Psychrotrophic bacterial counts of the different product groups were compared by frequency interval. The frequency intervals of psychrotrophic, marine bacterial counts on whitefish products from January '93 to July '94 (Figs. 7 - 12) indicate similar trends for all product groups. The highest count frequency had bacterial counts between < 10 - 50 000 cfu/g (Figs. 8, 10, 11 and 12) (<10 - 60 000 cfu/g for Figs. 7 and 9). For most product groups this was more than double that of the next interval, which was between 50 000 - 100 000 cfu/g (60 000 to 120 000 cfu/g).

The more complex landfrozen products "Cape Whiting Steaks" (Fig. 10), "Fiori" (Fig. 11) and "Cape Hake Blocks" (Fig. 9) indicated that just more than 50% of the bacterial counts were below 150 000 cfu/g. For "Fiori", the most complex product, only 43% fell within this interval (Fig. 11). For "Prime Hake Fillets" and "Hake Steaks and Loins" ca. 65% and 75% of their bacterial counts were below 150 000 cfu/g, respectively. The "FAS Fillets" (Fig. 12) had the highest percentage (88%) of bacterial counts below 150 000 cfu/g. This was ascribed to FAS fillets being processed and frozen within a few hours of being caught. Processing then took place under more favourable conditions to that of landfrozen product. Processing begins with the fish still virtually alive, its immune system still intact and the flesh sterile. The total time of processing for FAS fillets varied between 2 and 4 h, from the time of catching to freezing. The critical factor, that determined these results, was time, since in the other aspects, little difference to the shorebased products existed (Figs. 1 - 6). Landfrozen product, on the other hand, is made from fish headed and gutted at sea and then kept in ice onboard the trawler for 6 to 7 d. On the fresh fish trawlers, the natural marine psychrotrophic bacteria infiltrate and increase their numbers in the flesh of the headed and gutted Cape hake through cut surfaces (SHEWAN, 1961; LISTON, 1980; VENNEMANN, 1991).

Fig. 7 Frequency distribution of aerobic bacterial counts of prime fillets of hake
for the period January 1993 to July 1994 (476 X 2 samples)
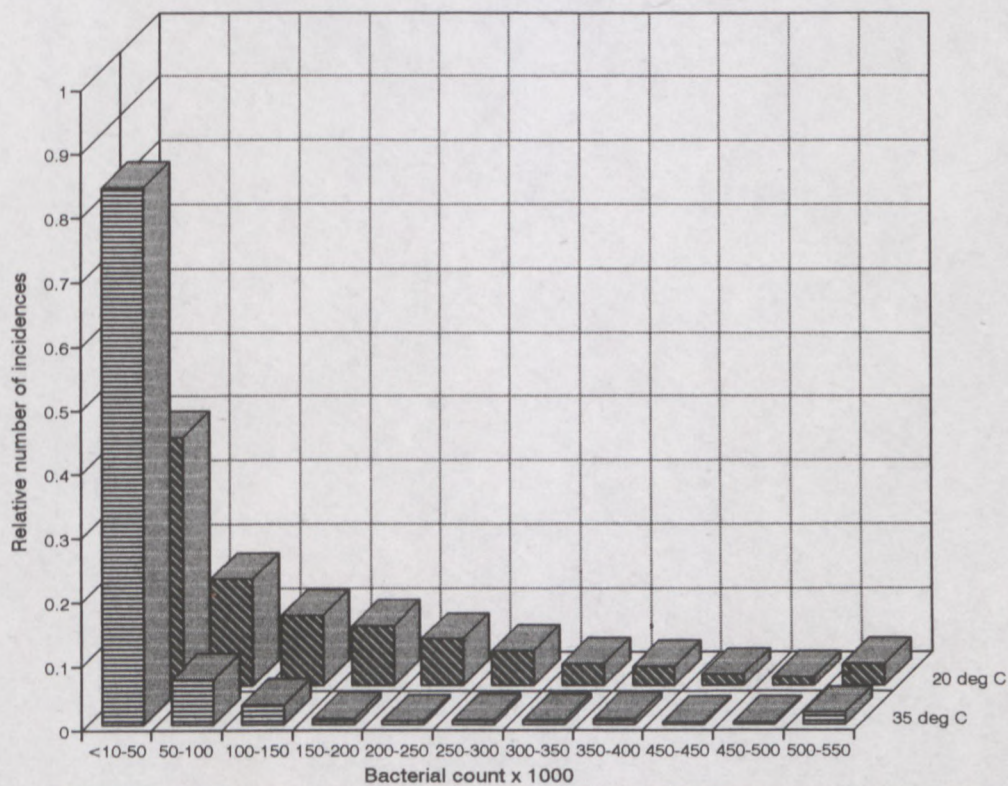
Fig. 8 Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period January 1993 to July 1994 (2971 X 2 samples)

Fig. 9   Frequency distribution of aerobic bacterial counts of cape hake blocks
for the period January 1993 to July 1994 (605 x 2 samples)

Fig. 10   Frequency distribution of aerobic bacterial counts of cape whiting steaks
for the period January 1993 to July 1994 (976 x 2 samples)
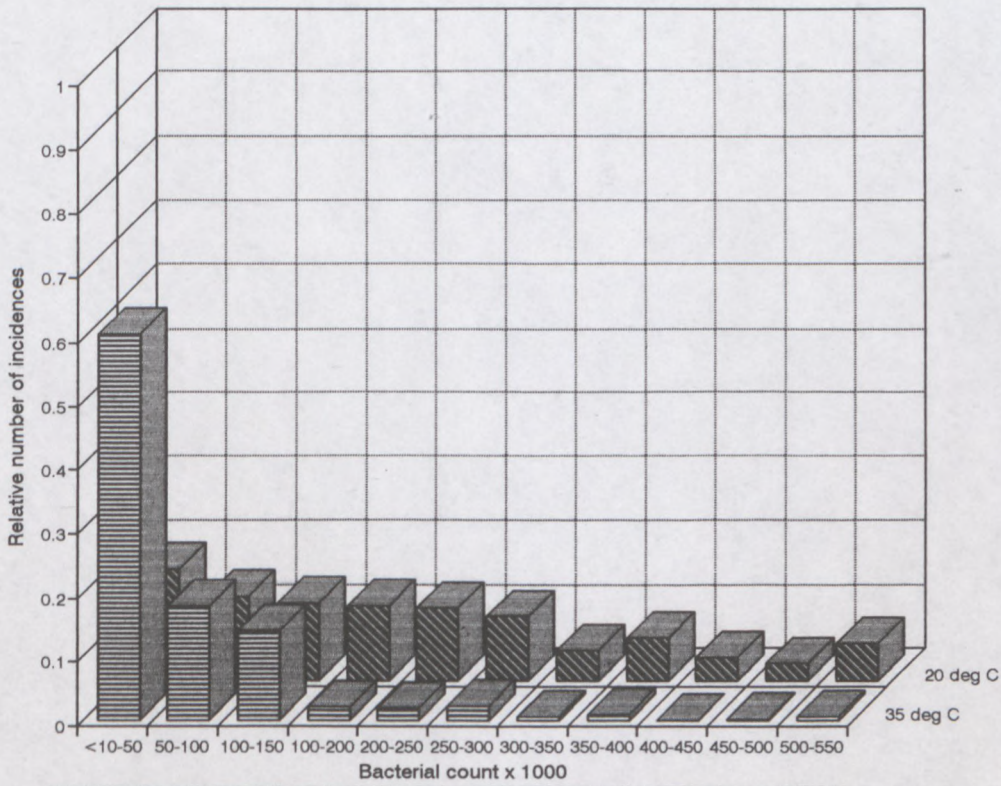
Fig. 11   Frequency distribution of aerobic bacterial counts of fiori
for the period January 1993 to July 1994 (1178 x 2 samples)
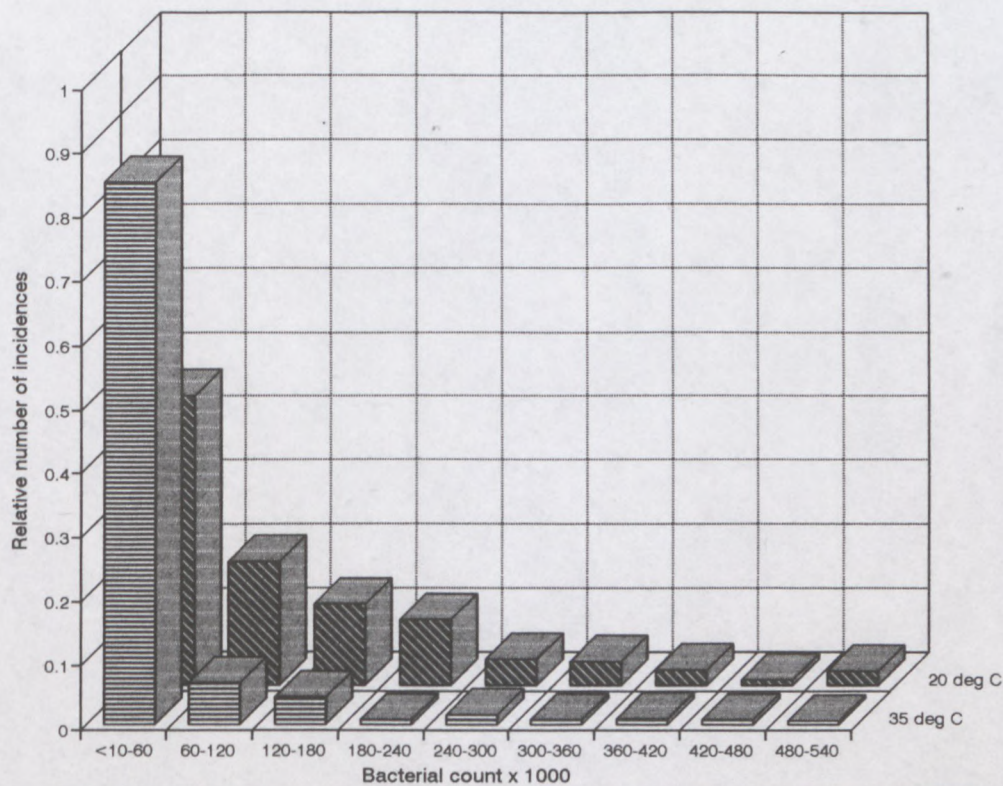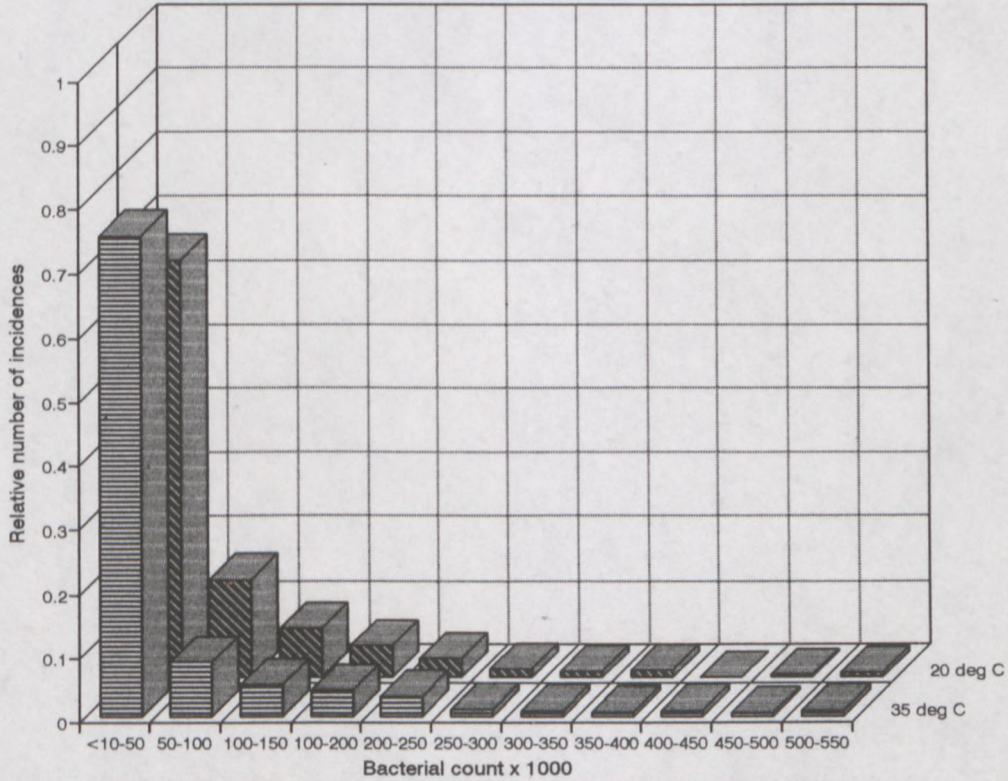

Fig. 12   Frequency distribution of aerobic bacterial counts of FAS fillets
for the period January 1993 to July 1994 (483 x 2 samples)

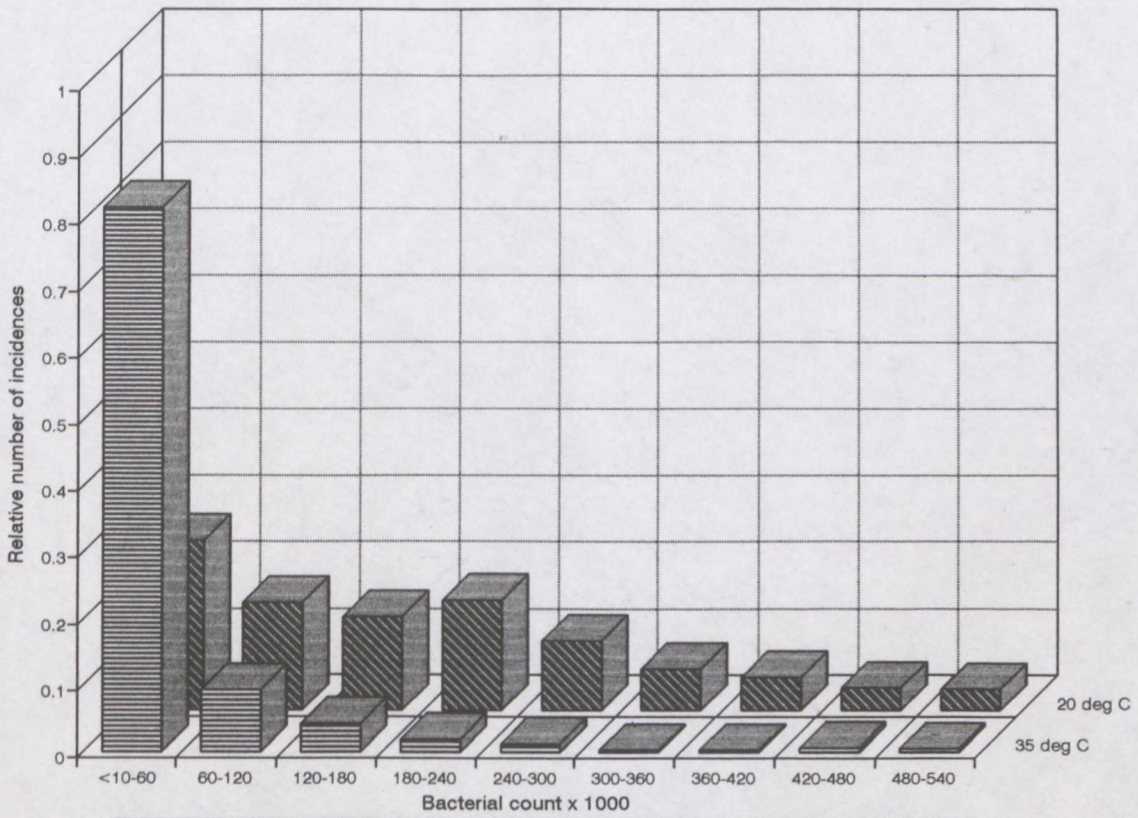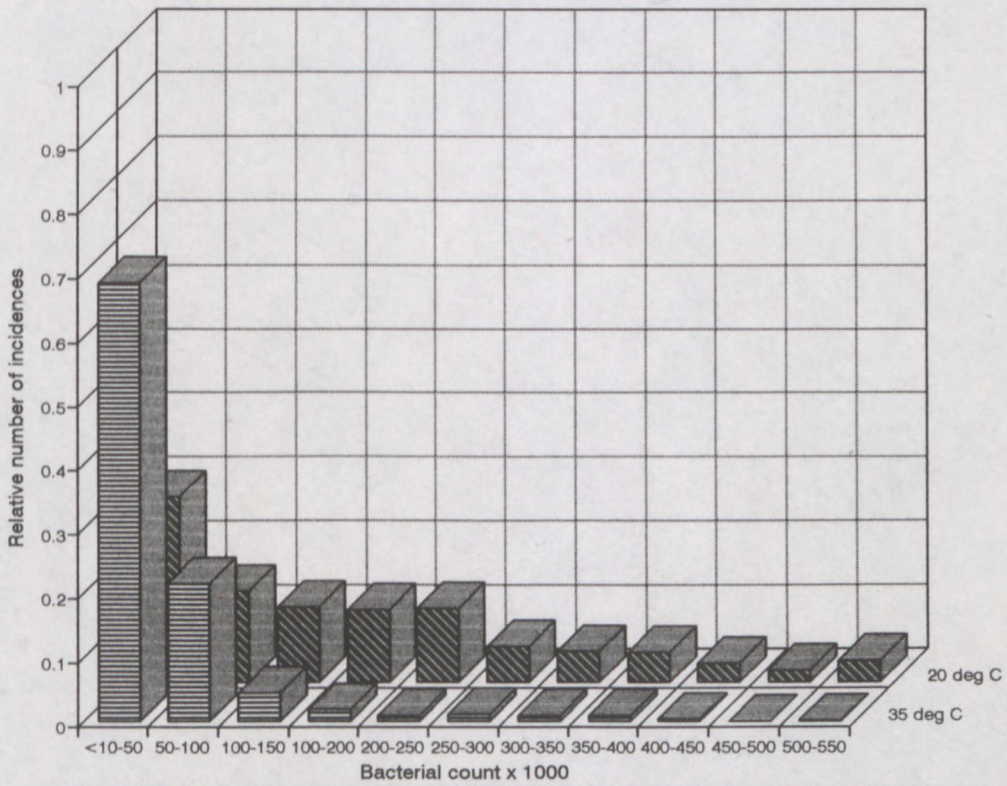The mesophilic bacterial counts on PCA incubated at 35°C, (part of the current international standard) did not indicate an increase in the higher intervals with the complexity of processing (Figs. 9, 10 and 11), but remained constant predominating only in the lowest interval (<10 - 50 000 cfu/g for Figs. 8, 10 11 and 12 and <10 - 60 000 cfu/g for Figs. 7 and 9). This indicated that the mesophilic bacterial counts were not as sensitive to the production complexity as the psychrotrophic marine bacterial counts and were therefore an inferior measurement of the bacterial condition of the product. The similar pattern for the products of lower complexity and the FAS fillets (Figs. 7, 8 and 12) were ascribed to the low bacterial counts, absence of additional processing steps and time on these products.

The most highly processed product group, "FIORI", indicated the highest psychrotrophic, marine bacterial count frequency above 150 000 cfu/g (67%) (Fig. 11). This was ascribed to its processing which was the most complicated (Fig. 5). Most of the processing in the factory was done manually with build ups and standing times between processing steps causing cross contamination and temperature increases. The total processing time for this product, including freezing, after 5 to 7 d at sea averaged ca. 7 - 9 h. These made time and temperature difficult to control during processing and the result was an increase in psychrotrophic bacterial counts (Fig. 11).

The findings discussed above confirmed the choice of the target organism. Since the results have been recorded over a period of time, aerobic marine psychrotrophic bacterial counts could be used to define bacterial quality of the products as well as achievable and meaningful bacterial standards for each product category which vary according to processing complexity. Mesophilic bacterial counts were unsuitable for these interpretations in whitefish products.

3.3.1.2 The use of bacteriological analysis at 20°C and 35°C
         for monitoring GMP

Between October 1993 and June 1994, the fresh fish distribution
and raw material receiving division, as well as the largest part
of the actual processing division of the whitefish factory was
translocated to newly built premises, located *ca.* 500m away from
the old processing division. This resulted in logistical
problems, causing in poor stock rotation. Product had to be
handled more frequently. Problems with sanitation were also
regularly encountered during this time. This created an ideal
opportunity to compare the psychrotrophic (20°C, SWA) method with
the mesophilic (35°C, PCA) method for monitoring GMP's.

The monthly frequency distributions of landfrozen product
categories are indicated in Figs. 13 - 22. Deterioration of the
bacterial quality of the product was indicated only using the
psychrotrophic bacterial counts and not the mesophilic bacterial
counts (Figs. 13 - 19). This progressive deterioration peaked in
March- to April '94 (Figs. 18, 19), where the frequencies of the
lowest interval (< 50 000 cfu/g) approached those of the highest
interval (> 500 000 cfu/g). The mesophilic bacterial counts
indicated only a marginal, if any, deterioration with slight
increases in the higher intervals. Specific problems contributing
to the worsening situation as indicated by the psychrotrophic
bacterial counts, included poor cleaning and rotation of factory
fish crates (automatic sanitizing equipment was not operative).
Furthermore problems were experienced with fresh fish chilling
and floor stock rotation. This was caused by faulty and wrongly
specified, newly installed equipment. Sanitation problems were
experienced to the absence of a qualified and dedicated hygiene
team. These factors contributed to the deterioration of the
bacterial problems. High (>150 000 cfu/g) psychrotrophic
bacterial counts on equipment and product handling surfaces were
indicated by hygiene audits conducted on a regular basis.

Fig. 13   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period October 1993 (166 x 2 samples)

Fig. 14   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period November 1993 (180 x 2 samples)

Fig. 15   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period December 1993 (129 x 2 samples)

Fig. 16   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period January 1994 (97 x 2 samples)
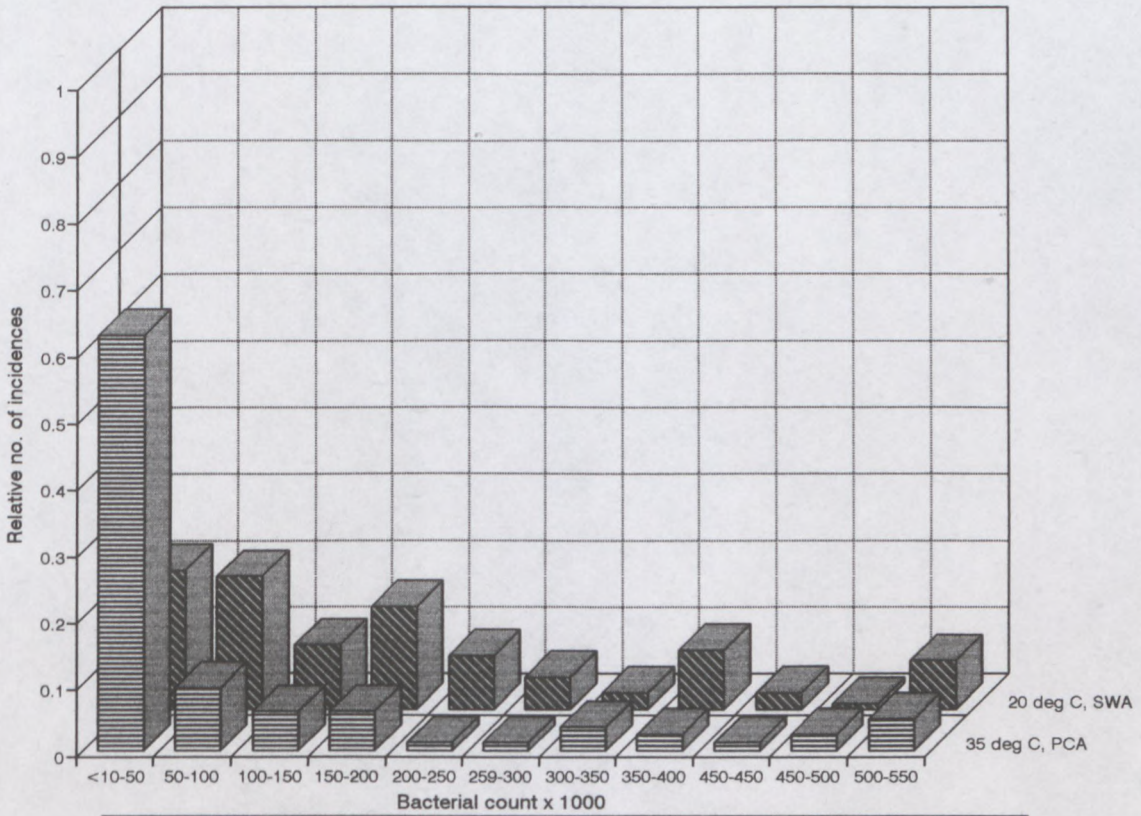
Fig. 17   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period February 1994 (125 X 2 samples)
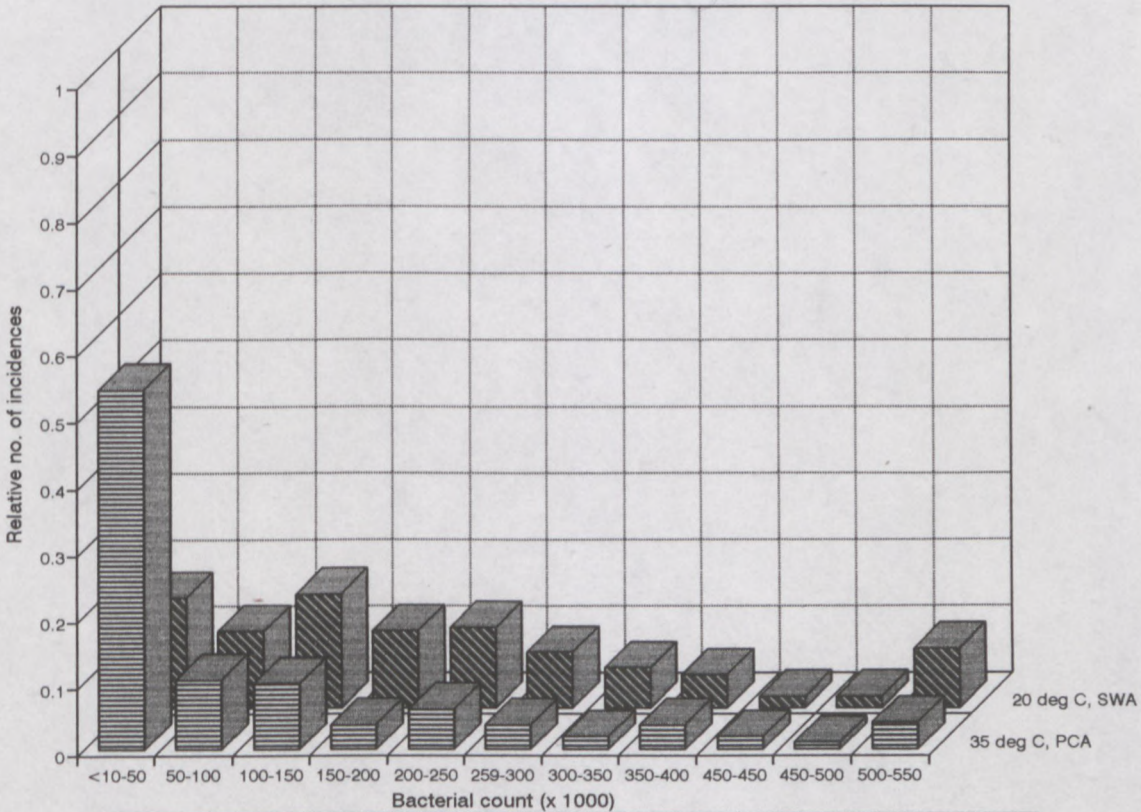
Fig. 18   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period March 1994 (182 X 2)
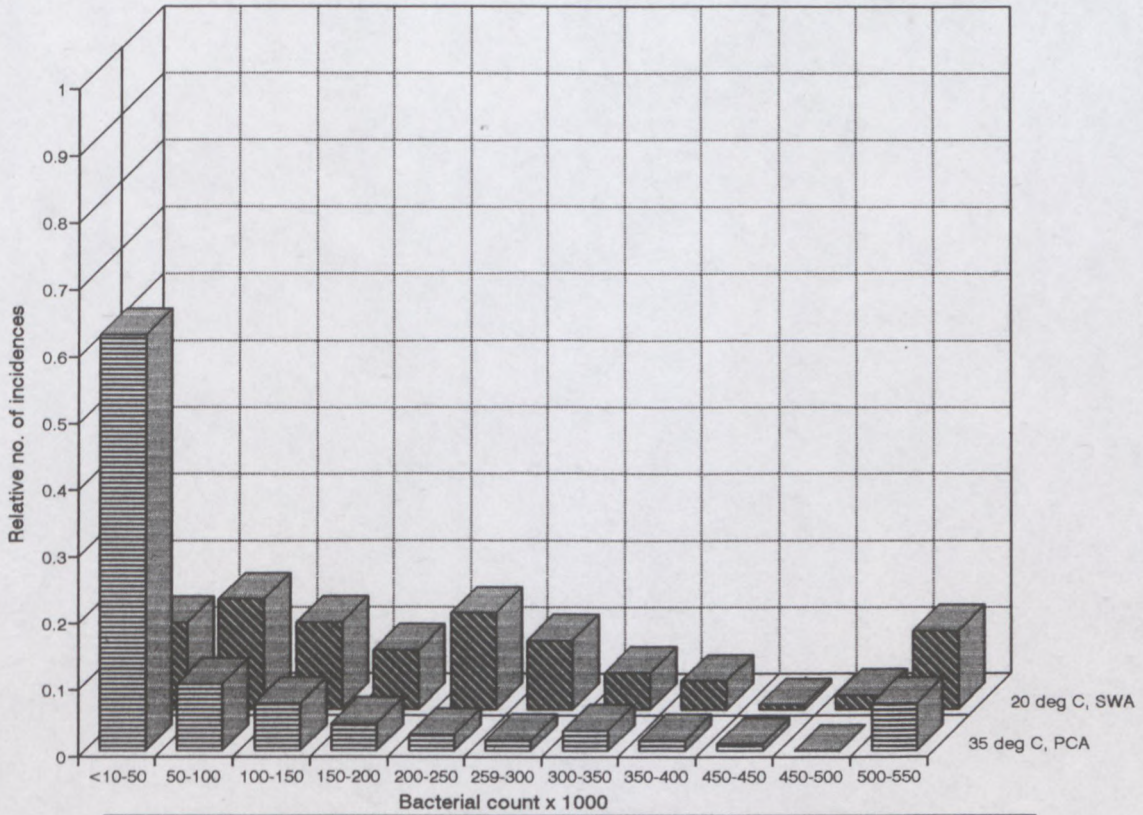
Fig. 19   Frequency distribution of aerobic bacterial counts of hake steaks and loins
for the period April 1994 (146 x 2 samples)
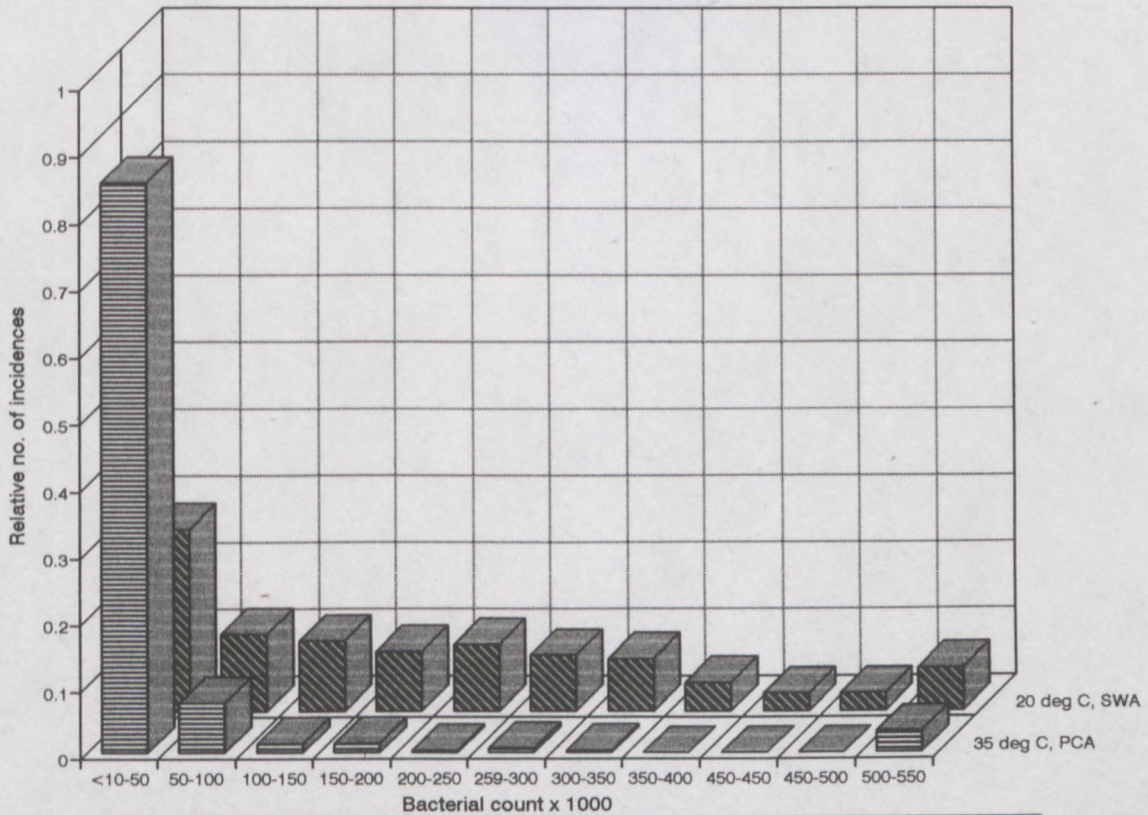
Fig. 20   Frequency distribution of aerobic bacterial counts of hake steaks and loins
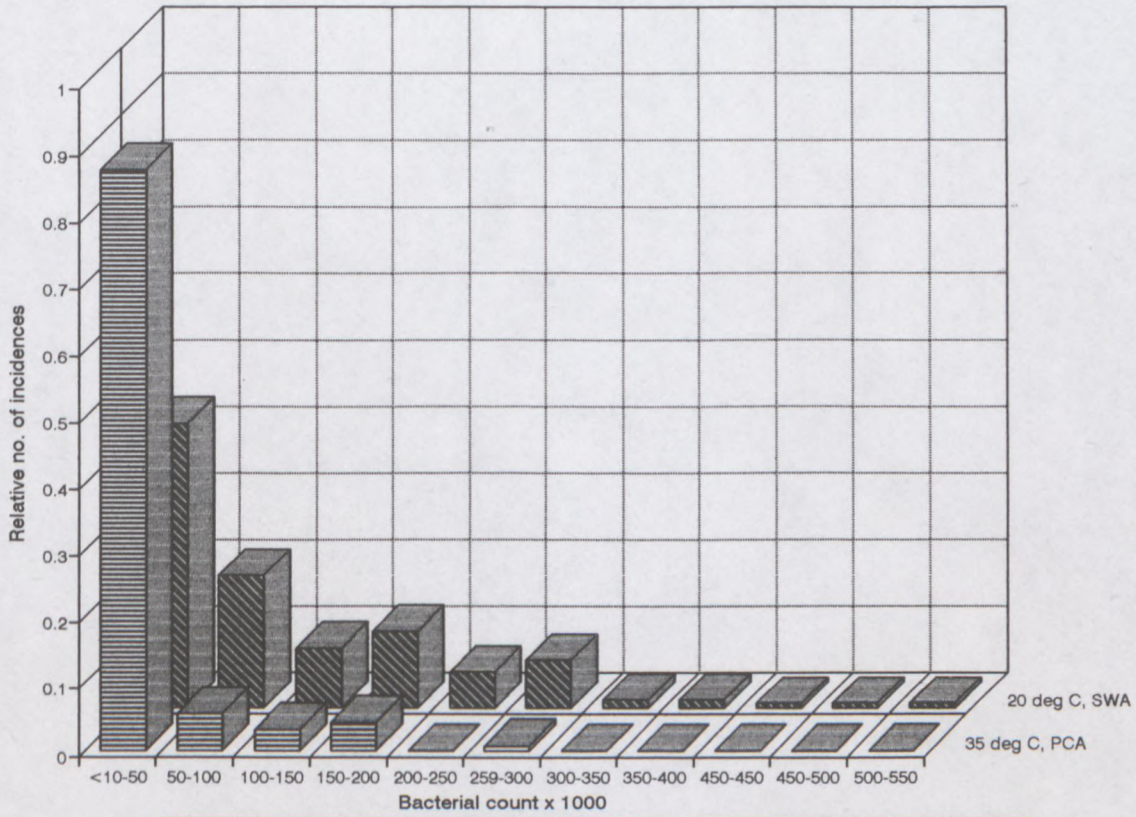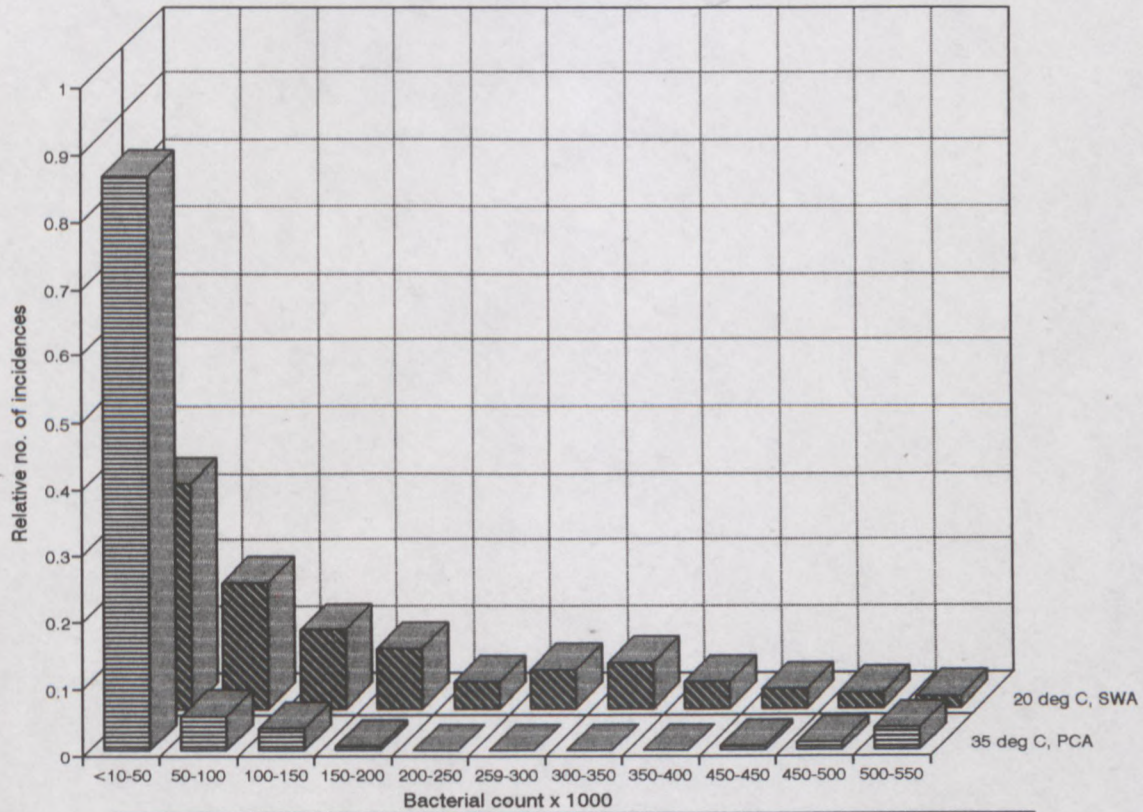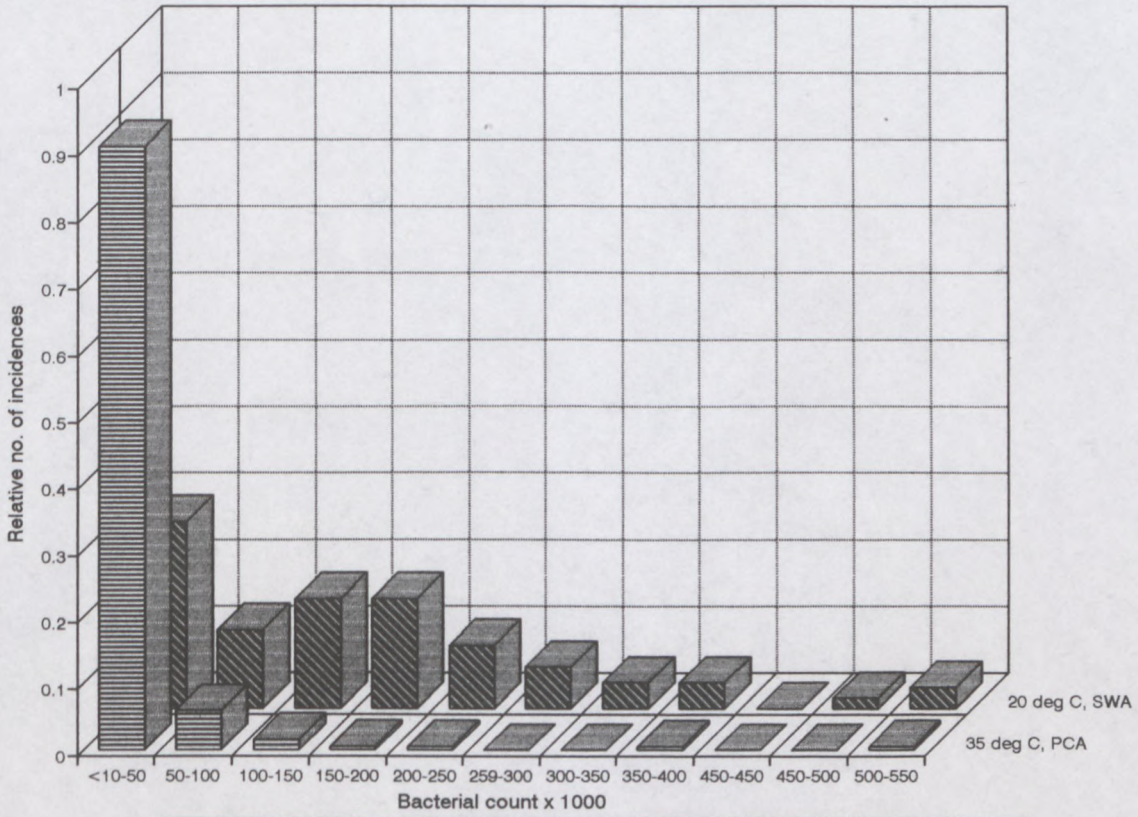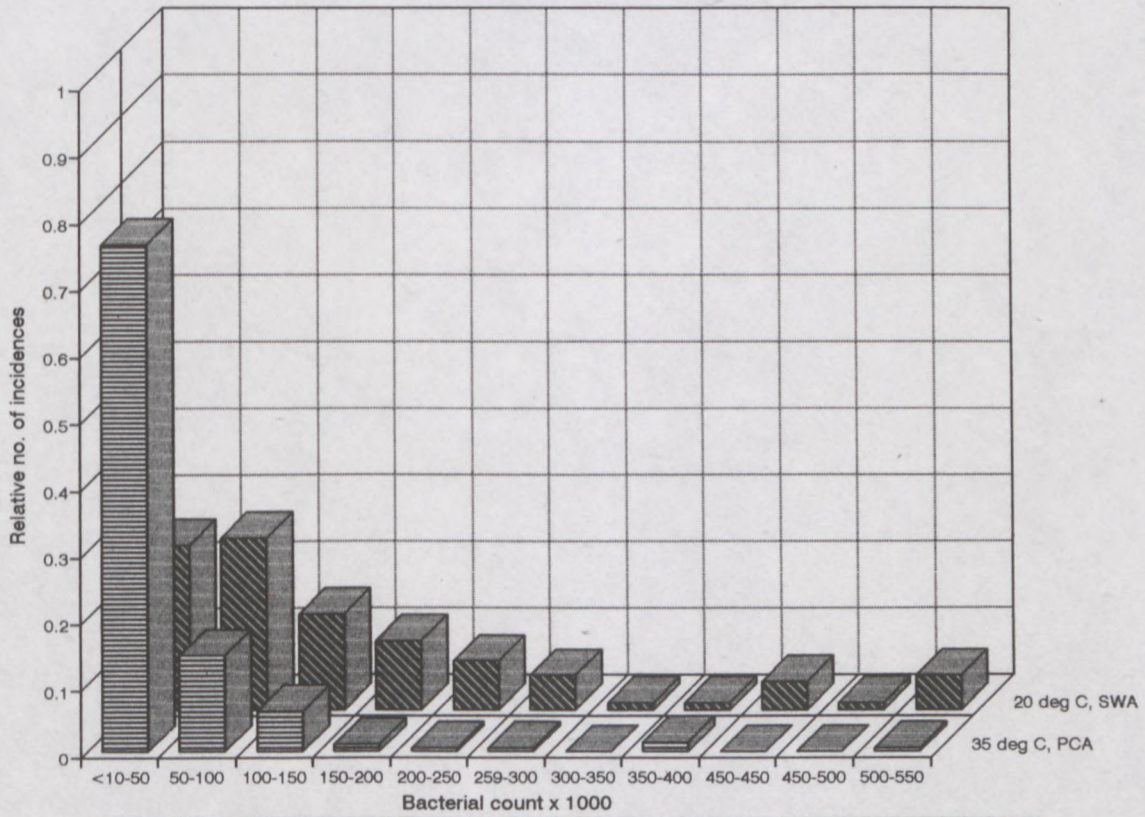for the period May 1994 (191 x 2 samples)
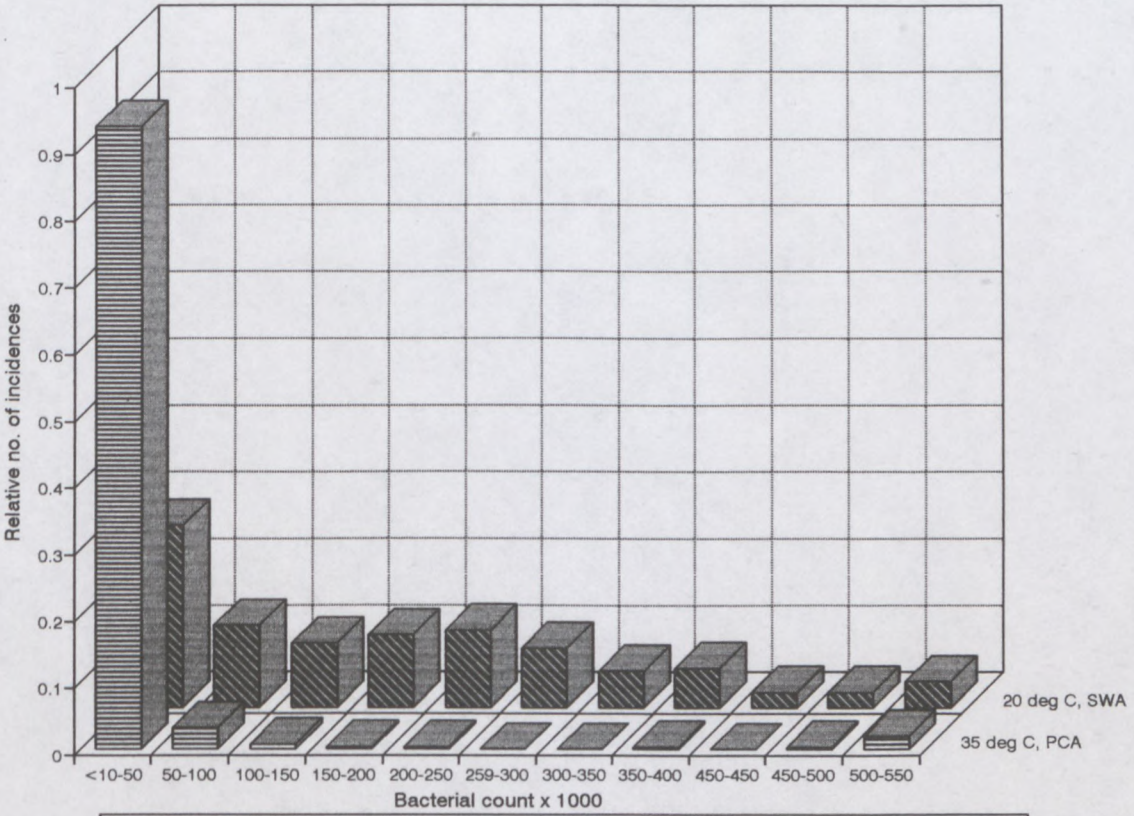
Fig. 21 Frequency distribution of aerobic bacterial counts of hake steaks and loins for the period June 1994 (260 x 2 samples)
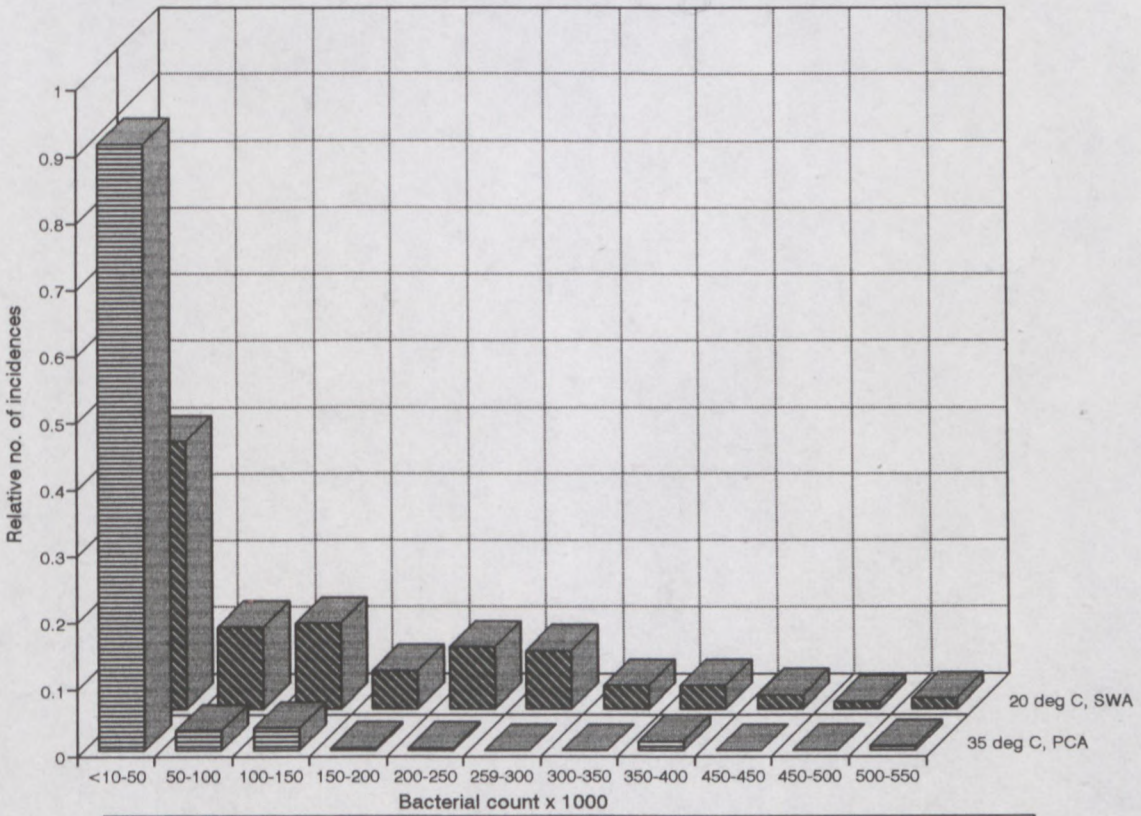
Fig. 22 Frequency distribution of aerobic bacterial counts of hake steaks and loins for the period July 1994 (197 x 2 samples)

An unexpected increase in fish catches during this period, also forced large scale employment of unskilled casual labourers. This was detrimental in a perishable food processing factory, demanding trained and experienced workers.

These problems were indicated by increases in psychrotrophic bacterial counts on products sampled from the production lines. Bacteriological analysis conducted at 35°C on PCA did not indicate this lapse in the bacterial quality of the fish products (Figs. 13 - 19). After normal processing conditions were re-established, the effect was again reflected by the psychrotrophic bacterial counts, returning to the pattern of October 1993 (Figs. 13, 20 - 22). The bacterial counts of the FAS fillets remained unchanged during the entire study period (Fig. 12), due to unchanged control parameters in seafrozen processing procedures.

These results indicated that psychrotrophic bacterial counts icubated at 20°C on SWA, were a more suitable means of assessing bacterial quality (shelf life) and were more sensitive indicators of GMP than mesophilic bacterial counts. Since the psychrotrophic bacteria also are endogenous on Cape hake and other deepsea whitefish, it is suggested that national and international bacteriological standards for uncooked, fresh or frozen deepsea whitefish products include psychrotrophic bacteria incubated at 20°C on SWA (GEORGALA, 1957; SHEWAN, 1961, 1977; LISTON, 1980; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991). Current international standards for uncooked, fresh or frozen, deepsea whitefish, target mesophilic bacteria, by determining aerobic bacterial counts incubated at 30 - 35°C on PCA (SABS, 1977; FDA 1986, 1994; EEC, 1991, 1993; CODEX ALIMENTARIUS, 1993). These bacteria are not endogenous to deepsea whitefish and do not play a role in spoilage under refrigerated storage conditions (GEORGALA, 1957; SHEWAN 1961, 1977; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT 1985, VENNEMANN 1991).

Standards using the latter assessment method are therefore inferior in terms of gauging bacterial quality, shelf life and GMP for uncooked, fresh or frozen deepsea whitefish products.

3.3.1.3 Deriving a bacterial count standard from survey data

The third point in MOSSEL and VAN NETTEN's five point plan, was satisfied in the above study, using the pre-October '93 results for the six product groups (Figs. 7 - 12), reflecting good GMP. The comparison of the bacterial product analyses data (survey data) of a product manufactured under both, "poor" (Figs 14 - 19) and "good" GMP (Figs, 13, 22) determined the limits for bacterial count standards. One the one hand, what could be expected under conditions of "poor" GMP, or where, in bacterial counts, does "poor" GMP become noticeable and on the other hand, how low could a bacterial count standard be set and still be achievable by a GMP processor. Furthermore, if such count standards were exceeded on the product sampled, the corrective action to the cause of bacterial count increases would be facilitated through the experience of the previous survey under "poor" GMP.

Point 4 of the 5 point plan, the translation of survey data into control standards, could therefore be addressed. An internal psychrotrophic aerobic bacterial count standard could be determined using a 3 point sampling plan: m = 100 000 cfu/g; M = 250 000 cfu/g, with c = 3 and n = 5 for basic and medium processed product categories (Figs 7 - 8) (Where m = *desirable limit, M = absolute limit, c = number of samples permissible between m and M and n = total number of samples*), since 70% of the bacterial counts on these groups were less than 150 000 cfu/g. However, m = 250 000 cfu/g; M = 500 000 cfu/g,; with c = 2 and n = 5 for highly processed landfrozen products (Figs. 9 - 11), since 57% of the bacterial counts under "good" GMP exceeded 150 000 cfu/g. The FAS fillets (Fig. 12) standard could be set to

m = 100 000 cfu/g; M = 250 000 cfu/g, with c = 2 and n = 5, due to its high frequency (88%) of bacterial counts under 150 000 cfu/g.

The current legal standard for total aerobic bacterial counts (incubated at 30 - 35°C, on plate count agar, PCA) in South Africa may not exceed 10^6 cfu/g (SABS, 1977, 1987). However, an aerobic bacterial count incubated at 30 - 35°C cannot be interpreted as an indicator for pathogens (MOSSEL and VAN NETTEN, 1991) and therefore is not safety related. Furthermore deepsea whitefish is caught in temperatures varying from 4 - 12°C, processed in temperatures of up to 15°C, and stored either frozen or refrigerated at 0 - 10°C (SHEWAN, 1961; VENNEMANN, 1991). The relevance of the current standard is therefore questioned.

Since the endogenous, bacterial community of deepsea whitefish has the capacity to produce strong spoilage odours (GEORGALA, 1957; SHEWAN, 1961; SHEWAN and HOBBS, 1963, 1967; MARTIN et al., 1978; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; SHIPMAN and WYLER, 1989; LORD, 1990; ANON., 1992; BONNELL, 1994) it can be argued that for fish processed under GMP, spoilage will be indicated by increasing bacterial counts and developing off odours in situations where the cold chain has been broken. Furthermore, since these bacteria grow optimally at 20°C (HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985), they are able to grow rapidly at chill temperatures (4 - 8°C) and spoil the fish product before pathogens (MARTIN et al., 1978; WARD, 1990; BONNELL, 1994; MORTIMORE and WALLACE, 1994) like Listeria monoytogenes and Yersinia enterolytica (slow growers at refrigeration temperatures) can become a hazard to consumers (WARD, 1990; BONNELL, 1994; MORTIMORE and WALLACE, 1994). Whitefish products with their natural bacterial population intact may therefore be considered low risk to the consumer. High total aerobic bacterial counts on SWA incubated at 20°C, could thus indicate possible temperature abuse and undesirability in terms

of shelf life or bacterial quality, but not a safety hazard. This further supports the hypothesis above, that standards on aerobic bacterial counts should be relevant to the environment, process and product. For the Cape hake industry this means bacteria deriving from the marine environment, being psychrotrophic in nature and therefore able to spoil uncooked product in refrigerated storage.

3.3.1.4 Establishing a policy dealing with products failing the target values

Point 5 of Mossel and Van Netten's five point plan deals with the formulation of a procedure for action to be taken for products not complying with the standard. Such a procedure for action may depend on the type of non-compliance. For example, exceeding the aerobic bacterial count of 500 000 cfu/g would result in an uncooked, whitefish product to be declared unfit for use in refrigerated distribution and storage. However, if the product is not spoiled (absence of off odours) and the enumeration does not exceed 10^6 cfu/g the product may still be used to produce quick-frozen products. Aerobic bacterial counts exceeding the above or product being spoiled due to odour development are unfit for any use, other than reduction to fishmeal. Should, however, a standard for pathogenic bacteria be exceeded (eg. Salmonella "+" where the standard is "-" in 25g of sample), the procedure will only permit the reduction to fishmeal. The procedure includes a microbiological audit into the source of the noncompliance to prevent a re-occurrence. If, for example a pathogen standard was exceeded, the likely source of the identified organism is audited along the route of the product's manufacturing flow (ie.: via handswabs taken from personnel handling the product with unwashed hands). If, on the other hand the total viable bacterial standard (10^6 cfu/g, 20°C, SWA) is exceeded, the processing line itself is audited for stock rotation or processing equipment cleanliness.

3.3.1.5 Incidence of bacterial pathogens on products tested
        from January 1993 to July 1994

Bacterial pathogens that were routinely checked for on the 6
product categories were *E. coli*, *Staphylococcus aureus* and
Listeria monocytogenes.

Table 1.   Bacterial pathogen incidence for each product
                category (Fig. 1 - 6) over three periods, January '93
                to January '94 (GMP); February to April '94
                (poor GMP) and May to July '94 (GMP).

Product	T_n	T_n pos	pos J93-J94	pos F94-A94	pos My94-Jy94
1	476	6	1	5	0
2	2971	32	5	26	1
3	605	7	3	4	0
4	976	14	2	12	0
5	1178	11	2	9	0
6	483	5	5	0	0

pos = positive for bacterial pathogens
T_n = Total number of samples

Over the 19 month period the incidence per product for any of the
above organisms was lower than 1.5% of samples (total n of
samples = 6 689) being positive. Only one incidence *Listeria*
monocytogenes was found on "Fiori" and only 5 samples out of 605
of FAS fillets indicated *Staphylococcus aureus* in numbers
exceeding the standard of 10 cfu/g (FAS did not undergo any
change in operations). All other incidences were *E. coli* positve
samples, recorded mainly in the three months of weakened
production controls from February to April 1994 (Table 1). On a
monthly basis the incidence of *E. coli* went up from less than
0.5% to 4.3% positive in March and April 1994. This coincided
with the period where the aerobic bacterial counts on SWA
incubated at 20°C indicated the worst conditions (Figs. 18, 19).
These results were confirmed by overseas customers through their
quality control acceptance analyses and in South Africa through

the South African Bureau of Standards (SABS), who sample products randomly on a weekly basis. This indicated that the standard for pathogens, including methodology, was not questionable and suited the framework of MOSSEL and VAN NETTEN (1991), as it confirmed the findings of the aerobic bacterial counts on SWA incubated at 20°C. Pathogens, although less sensitive through low incidence, were therefore also indicators poor GMP.

3.3.2 Special conditions for standardization of combinations of raw, fresh or frozen sea food products

Microbiological standards for cooked, ready to eat, whitefish products have a legal limit (M) for aerobic bacterial counts 1 log cycle less (10^5 cfu/g) than the standard for uncooked, raw whitefish products (WARD and HACKNEY, 1991; BONNELL, 1994). It should be noted, that raw products, whether fresh or frozen, containing a mixture of ingredients from diverse bacterial environments such as a fish and cheese combination, must be evaluated in an entirely different manner. Care must be taken to include aerobic bacterial counts relevant to the type of cheese used ($\geq 10^9$ cfu/g lactobacilli in cheddar) (JAY, 1986) when attempting to set a standard. In such cases, where there is a high probability of one or several genera of bacteria having been intentionally introduced to the food product (ie.: lactobacilli via cheese in a cheese - fish combination), a partial characterization of these organisms as the predominant ones in a high total count might be necessary.

Questions arising from these combined raw materials to produce a raw finished product relating to a practical international standard remain unsolved and no standard exists in this regard. However, under such conditions, any standard specification limiting aerobic bacterial counts to any one ingredient of the combination has no implication as an indicator for shelf life. It

was concluded that no practical purpose is served by testing demersal whitefish, such as Cape hake, on PCA incubated at 30 or 35°C. Conclusions drawn from such analyses results would be meaningless and could be challenged.

Bacterial standards should be determined for each of these new products using the framework of MOSSEL and VAN NETTEN (1991) as was highlighted in this study.

CHAPTER 4

Application of the hurdle concept
for shelf life extension of products from
Merluccius capensis / Merluccius paradoxus
and other deepsea whitefish species

4.1 INTRODUCTION

In the processing of marine whitefish there are several
techniques (hurdles), which aim to extend the commercial life of
the fresh, uncooked, chilled product. These attempt to minimally
affect the appearance, taste an texture of the product but reduce
bacterial populations or slow bacterial growth. Hurdles in
whitefish processing have been extensively researched and showed
some success under laboratory conditions (SHEWAN and HOBBS, 1967;
HOBBS and HODGKISS, 1982). However, most of this research was
carried out with northern hemisphere whitefish. Few reports exist
regarding the use and effectivity of hurdles on Cape hake
(*Merluccius capensis / paradoxus*) under commercial distribution
and storage conditions in South Africa. Cape hake has several
unique features, not generally present in typical northern
hemisphere species (Cod / *Gadus morhua*), which are conducive to
rapid spoilage such as high water activity (0.995), almost
neutral pH (6.6 - 7.0), and soft texture (SIMMONDS and LAMPRECHT,
1985).

The current international bacterial standard (10^6 cfu/g incubated
at 30 - 35°C, on PCA for 48h) was, in the previous chapter, shown
to be ineffective in determining shelf life or GMP. In this light
the primary objective of this study was to show whether the shelf
life of Cape hake and other whitefish products in terms of the
internal standard (maximum 10^6 cfu/g, incubated at 20°C on salt
water agar, SWA, for 48h) could be extended under commercial
storage conditions. Several preservation techniques (hurdles)
such as gamma irradiation, temperature, chemical preservation and
vacuum packaging were applied before distribution. The secondary
objective was to show that aerobic bacterial counts incubated at
20°C on SWA provide a sensitive measurement for testing the
effect of the preservation technique before the hurdle and
afterwards during the storage of the product under commercial
conditions.

4.2 MATERIALS AND METHODS

4.2.1 The effect of gamma-irradiation on the shelf life of fresh Cape hake fillets

4.2.1.1 Sample preparation

Seventy three headed and gutted, unfrozen Cape hake (300 - 500 g) less than 3d old, were randomly selected from iced crates. The fish were filleted and the fillets packed onto polyvinylchloride trays (2 fillets per tray) and shrinkwrapped (film drawn under vacuum) with an oxygen permeable film (oxygen transmission rate, (OTR), of 2 300 ml O_2/m^2/24h at 23°C and 720 mm Hg).

The filleted samples were stored in sealed polystyrene containers and kept below 5°C (with ice) until radurization 2-3 h later. The source of irradiation was a Co^{60} isotope with an activity of about 21 KCi. 42 fillet packs each were radurized at 0.5, 0.75 and 22 fillet packs at 1.0 KGy. Two controls (A, B) each with 20 fillet packs, were not irradiated. Control A remained in the factory chiller, whilst control B was taken with the fillets to be radurized but remained untreated.

The time taken for transport and radurization was about 4 h. The temperature increase during the radurization process varied between 0.5^{o}C and 2.5^{o}C (fillet core temperature) for the samples radurized at 0.5, 0.75 and 1.0 KGy. After radurization, the samples were repacked in layers of ice in the polystyrene containers, returned to the factory and immediately transferred into the same chiller as above. Air temperatures varied between 0° and 5°C (calibrated thermograph) with an average product core temperature of 2 - 3°C in the first experiment. A re-run of the first experiment using the same protocol except no 1 KGy radurization, was conducted and allowing storage temperatures to

vary between 1.7° and 8.9°C with a product core temperature of 5.0 - 8°C. This was done to simulate commercial storage and display conditions.

4.2.1.2   Sample evaluation after gamma-irradiation

Organoleptic assessment

Samples from all treatments were taken daily and used for cooking and organoleptic evaluation by a trained taste panel consisting of 7 persons. Shelf life termination was taken as the presence of off odours emanating from the fish either before or during its preparation. All fillets were steamed and considered cooked as soon as the flesh flaked easily indicated by individual muscle segments of the fillet separating.

Bacteriological analysis

Bacteriological analyses were carried out in accordance with AOAC methods (AOAC, 1990; VENNEMANN, 1991). The Pour plate method was used for the enumeration of the aerobic psychrotrophic, marine bacteria (VENNEMANN, 1991). Counts exceeding 6 log cfu/g indicated spoilage.

Characterization of bacterial populations after the radurization treatment were carried out as described by VENNEMANN, (1991). Two-hundred and seventy isolates (10 randomly selected colonies from 27 plates, resulting from 5 treatment variations (0.5, 0.75, 1 KGy and Controls A and B), two experiments and a maximum of 7 d shelf life) were characterized from SWA plates using a 10^{-3} dilution, incubated at 20°C for 48h and counting all colonies. The twenty seven SWA plates were prepared from 27 fillets of the different treatments (0.5, 0.75 and 1.0 KGy) taken every day of the storage including one sample from the untreated controls until shelflife expired.

4.2.2 The effect of storage temperature on the shelf life of brined, smoked and untreated whitefish products

4.2.2.1 Sample preparation

Headed and gutted Hake (84) and Kingklip (*Genypterus capensis*) (42) of medium size (620 - 800g) and less than 3d from catch were randomly selected from iced crates. After descaling and washing in a drum descaler the Hake and Kingklip were filleted. Centre cuts (steaks) were prepared from the fresh fillets. Half of the of Hake steaks (84) were further processed by brining in a 30° NaCl solution (20 min.) and stained by submerging in a freshly prepared 1% annato dye solution for 5 min. Hereafter, the steaks were cold smoked for 2.5h at 25 ± 2^OC in an Afos kiln using hardwood saw dust. This brining and smoking process is traditional from the United Kingdom and the end product in South Africa called "haddock". The steaks prepared in that way are referred to as "haddock steaks".

In total 252 steaks (samples) consisting of 84 Hake-, 84 Haddock- and 84 Kingklip steaks were prepared. Two steaks to a tray, shrinkwrapped under vacuum with an oxygen permeable film (OTR = 2 300ml O_2/m^2/24h at 23^OC and 720mm Hg) on a polyvinylchloride tray. 168 (even parts of the three types) of these samples were kept at the factory, half of which were stored under refrigerated conditions controlled to $0 - 2^O$C. The other half was kept under commercial chill conditions, controlled to $5 - 7^O$C, averaging at 5.5^OC product core temperature, to study the effect this difference in storage temperature would have on the product's shelf life. As a control, the remaining 84 samples were sent to a major retail chain, where the same storage procedure was followed.

The reason for using two different species (Cape hake and Kingklip) and one treatment (Haddock) was that all three are

products processed and prepacked every day in this form. The study was also attempting to establish wether storage temperature had the same effect on bacterial counts in the different species and whether brining and smoking in hake had a shelf life extending effect (SHEWAN and HOBBS, 1967; HOBBS and HODGKISS, 1982; HOBBS, 1987).

4.2.2.2 Sample evaluation

This was done exactly as previously decribed (para. 4.2.1.2), excepting that no bacterial community structure analyses were conducted.

4.2.3 The effec of chemical preservatives on the shelf life of Cape hake fillets

4.2.3.1 Sample preparation

Seventy headed and gutted, fresh Cape hake (300 - 500 g), were randomly selected less than 3d from cath. The fish was filleted and then submerged in a saturated carbon dioxide solution (40 fillets) prepared by bubbling carbon dioxide through iced fresh water. The other fillets were subemerged in a composite of 0.75% potassium sorbate and 6% sodium pyrophosphate solution (40 fillets) and a saline (5% NaCl) solution (40 fillets) for 1 min. each and allowed to drain on racks for 5 min. 20 fillets remained untreated as a control. All solutions were prepared with iced, fresh water ($0^{o}C$). The temperature of both the solutions and the fillets were taken before and after the dip. The fillets were then packed onto polyvinylchloride trays (2 fillets per tray) and shrinkwrapped with an oxygen permeable film (OTR = 2 300 ml O_2/m^2/24h at $23^{o}C$ and 720 mm Hg). The oxygen transmission rate of this film was enough to inhibit the germination and growth of *Clostridium* spores (JAY, 1986; WARD and HACKNEY, 1991). Product packed into this film is thus not under complete vacuum.

All samples (140 fillets) were stored at 5 - 8°C product core temperature in an industrial chiller in order to assess the role of storage temperature after preservative treatments under commercial conditions.

4.2.3.2 Sample evaluation

This was carried out as described previously (para. 4.2.1.2) excepting that no bacterial community structure analyses were conducted.

4.2.4   The effect of vacuum packaging on the shelf
         life of deepsea whitefish products

4.2.4.1   Sample preparation

Fourty headed and gutted (H+G) Kingklip (800 -1400g), 20 from stock frozen at sea and defrosted in air for 14 hours and 20 fresh from wetfish trawlers, as well as 40 headed and gutted Cape hake (300 - 500g), were randomly selected and filleted. Fourty fillets (20 from defrosted and 20 from fresh Kingklip) were then packed onto a tray (2 fillets per tray) which was vacuum shrunk with oxygen permeable film (OTR = 2300 ml $O_2/m^2/24h$ at 23°C and 720 mm Hg). The other 40 fillets were packed into a non-permeable, full barrier vacuum pouch (2 fillets per pouch). Samples were stored between 4° and 6°C. The 80 Hake fillets were split in two and and packed the same as the Kingklip fillets with permeable and nonpermeable films.

Since the marine resource for Kingklip is restricted in South African fishing waters, occasional use is made of frozen at sea production which is defrosted in the factory for chilled distribution and resale. The objective of this additional variable was to determine wether any difference existed in the

shelf life of fresh (never frozen) and defrosted Kingklip under the same storage conditions.

4.2.4.2 Sample evaluation

The organoleptic assessment was carried out as described in para. 4.2.1.2. The microbiological enumerations were carried out as described for the other tests as well as the characterizations except that 420 isolates (10 randomly selected colonies from 42 plates were characterized from each storage day (VENNEMANN, 1991).

4.3 RESULTS AND DISCUSSION

4.3.1 The effect of gamma irradiation on the shelf life of fresh Cape hake fillets

4.3.1.1 Organoleptic assessment

After 1 d of refrigerated storage following irradiation, strong "earthy" or burnt flavours were detected on Cape hake fillets which were radurized at 1.0 KGy (Table 2). This "tainting" following gamma irradiation has also been reported elsewhere (WARD, 1991; MILLER JONES, 1992). These odours and flavours were found to increase in intensity with each day of storage. However, they were considered "unacceptable" after the 1st day of chilled storage following the radurization (Table 2). Slight tainting was noted from the product radurized at 0.75 KGy after the 2nd day of 5 - 8°C product storage and became unacceptable after the 3rd day (Tsable 2). Product stored at 2 - 3°C did not exhibit any tainting until the 3rd day, but became unacceptable after the 6th day (Table 2).

Slight tainting after the 3rd day was noted from the product radurized at 0.5 KGy and stored at 5 - 8°C. Tainting became unacceptable after the 4th day at the above storage temperature. Slight tainting was noted from product stored at 2 - 3°C after the 4th day. The fillets were spoiled after the 7th day due to off odours, which overshadowed tainting due to radurization. The untreated controls indicated slightly "fishy" odours/flavours after the 3rd day of storage at both storage temperatures (5 - 8°C and 2 - 3°C) (Table 2). The off odours became unacceptable after the 4th day of storage at 5 - 8°C and after the 5th day of storage at 2 - 3°C.

Table 2.   Organoleptic characteristics of fresh Cape hake
fillets radurized at 0.5, 0.75 and 1.0 KGy as well as
untreated controls (A; B) during refrigerated
storage

Storage d	A a	b	B a	b	0.5 a	b	0.75 a	b	1.0 a	b
1	*	*	*	*	*	*	*	*	X	X
2	*	*	*	*	*	*	*	(*)	X	X
3	(*)	(*)	(*)	(*)	*	(*)	(*)	X	X	X
4	(*)	X	(*)	X	(*)	X	(*)	X	X	X
5	X	X	X	X	(*)	X	(*)	X	-	-
6	X	X	X	X	(*)	X	X	X	-	-
7	X	X	X	X	X	X	X	X	-	-

a. 2 - 3°C product core temperature
b. 5 - 8°C product core temperature
 * = good, fresh, untainted
(*) = slightly tainted/fishy
 X = badly tainted/off

It was concluded that radurization at 1.0 KGy for fresh Cape hake
fillets was unsuitable due to excessive tainting. Radurization at
0.75 and 0.5 KGy indicated that temperature played a role in the
extension of shelflife in that storage at refrigerated (2 - 3°C)
temperature had an additional day of shelf life with only slight
tainting as opposed to the samples stored at chilled temperature
(5 - 8°C) (Table 2). The shelf life extension amounted to 1 d for
the 0.75 radurized Cape hake fillets and 2 d for the fillets
radurized at 0.5 KGy when compared to the controls. In terms of
the organoleptic results it was concluded that the radurization
treatment did not provide shelf life extension desired by
retailers and consumers of fresh Cape hake fillets.

4.3.1.2 Microbiological counts

Psychrotrophic bacterial counts decreased by more than 1.5 log
cfu/g after radurization at 1 KGy (day 2, Fig. 23, Fig. 24). At
0.5 KGy radurization decreases in bacterial counts were less than
0.5 log cfu/g. After three days of storage at 2 - 3$^{\text{O}}$C, an
increase of bacterial counts was noted and after the 6th day
bacterial counts exceeded 10^6 cfu/g (Fig. 23), which was regarded
as the spoilage level (SABS, 1977). The non-radurized controls
reached bacterial counts of 5 x 10^5 cfu/g within 3 - 4d and
spoilage levels (10^6 cfu/g) after the 4th day of storage at 2
- 3$^{\text{O}}$C. The radurized samples (0.5; 0.75 and 1.0 kGy) exceeded the
spoilage level after 7 d of chilled storage.

At a storage temperature of 5 - 8$^{\text{O}}$C) no difference was noted
between the controls and radurized fillets in terms of bacterial
counts (Fig. 24). Bacterial counts in exceeded 5 x 10^5 cfu/g
after 3 - 4d and the spoilage level was reached after the 5th day
(Fig. 24). This was ascribed to the higher storage temperatures
(5 - 8$^{\text{O}}$C, product core temperature) compared to 2 - 3$^{\text{O}}$C product
core temperature storage. Storage at 5 - 8$^{\text{O}}$C more accurately
reflected commercial storage and display conditions in South
Africa (OLLEY and RATKOWSKY, 1973; SHIPMAN and WYLER, 1989; LORD,
1990; ANON., 1992). This represented a more practical assessment
for radurization as a means of shelf life extension of fresh Cape
hake. These findings were in agreement with the organoleptic
characteristics for the products radurized at 0.5 KGy. Odours and
flavours became unacceptable after the same number of days in
which bacterial counts exceeded the spoilage level for both
storage at 2 - 3$^{\text{O}}$C and 5 - 8$^{\text{O}}$C (Table 2). 0.75 and the 1.0 KGy
irradiation prevented bacteriological spoilage. Unacceptable
tainting was therefore due to the radurization process rather
than bacteriological spoilage.

Fig. 23 Bacterial counts incubated at 20 deg.C on SWA for 48h of Cape hake fillets stored at 2 - 3 deg.C product core temperature.

During storage at 2 - 3°C, the controls showed a decrease in bacterial counts (Fig. 23). This was probably due to the micro-aerophilic environment caused by the "shrinkwrapping" of the product. At 5 - 8°C storage, control A reached a temperature of 9°C for 4 - 5 h (Fig. 24). This explained why no decrease of bacterial counts was detected for control A on the first day of chilled storage. The variation in bacterial counts between the different samples before radurization (Figs. 23 and 24) is a natural occurrence and derives from variations in bacterial counts between individual fishes (VENNEMANN, 1991).

Studies by SIMMONDS and WESSELS, (1986) and AVERY et al., (1987) did not find tainting of Cape hake at 0.75 and 1.0 KGy gamma irradiation. Radurization resulted in a shelf life extension of 14 - 20d in northern hemisphere commercial fish species (SHEWAN and HOBBS, 1967; HOBBS and SHEWAN, 1969; HANNESSON and DAGBJARTSSON, 1972; MIYAUCHI, 1972; HOBBS and HODGKISS, 1982; HOBBS, 1987; VAN DER WATT and VAN DER MERWE, 1988; DYMSZA et al., 1990; ALUR et al., 1991). The results obtained in this study contradict these findings. The reason may be the naturally soft texture, high water activity (0.995) and high pH (6.6 - 7.0) of Cape hake, when compared to northern hemisphere whitefish species (HOBBS and SHEWAN, 1969; HANNESSON and DAGJATRSSON, 1972; HOBBS and HODGKISS, 1982; VENNEMANN 1991). Northern hemisphere whitefish species, such as Cod (Gadus morhua) and Haddock (Melanogrammus aeglefinus) have firmer texture and lower water activity than Cape hake (SIMMONDS and LAMPRECHT, 1985). The storage temperature after radurization was of importance in determining whether shelf life extension was achieved in this study. Radurized samples stored at 2 - 3°C had a day extra shelf life in comparison to radurized samples stored at 5 - 8°C (Table 2) and aerobic bacterial growth after radurization took up to two days longer to achieve spoilage levels (Fig. 23 and 24).
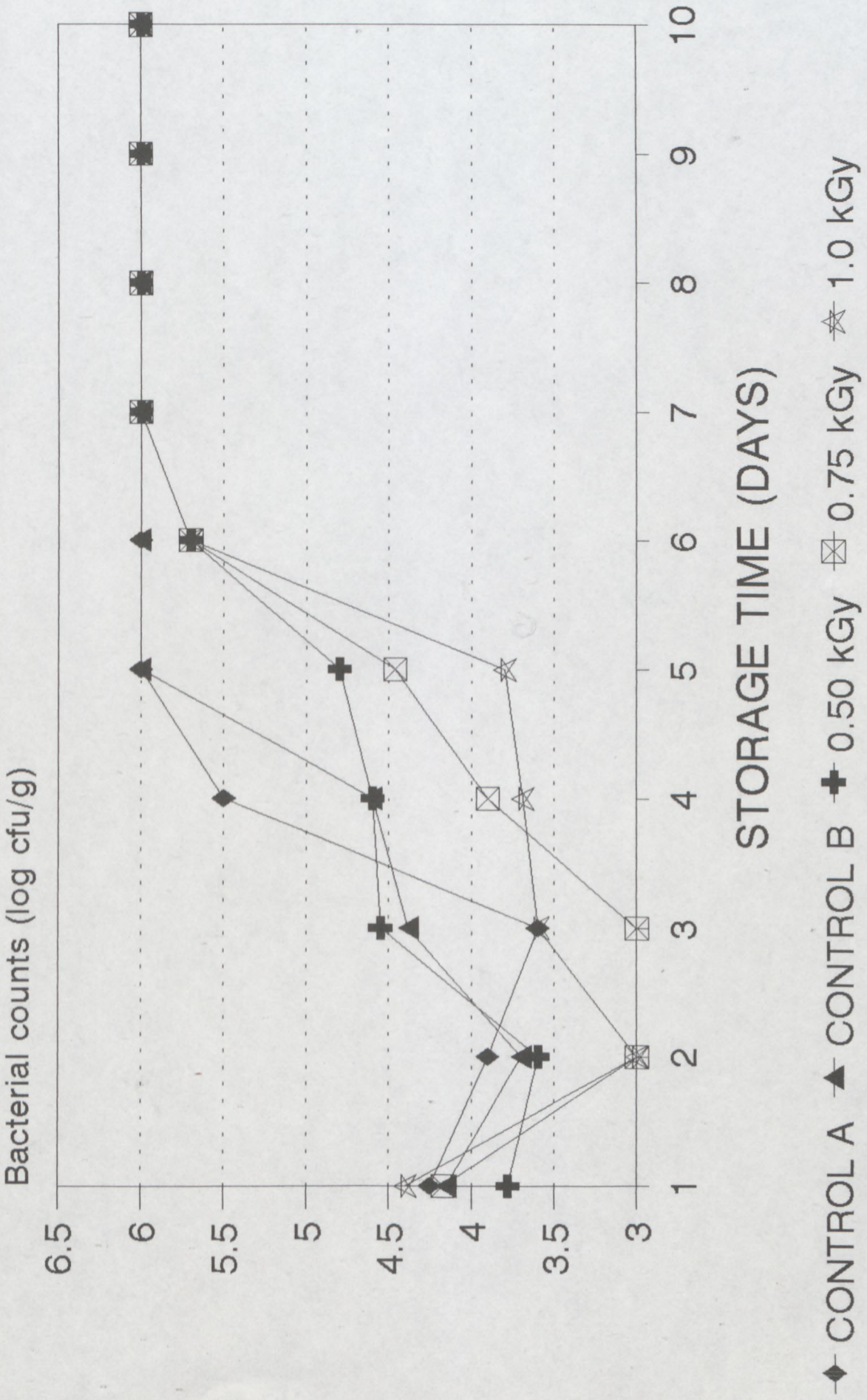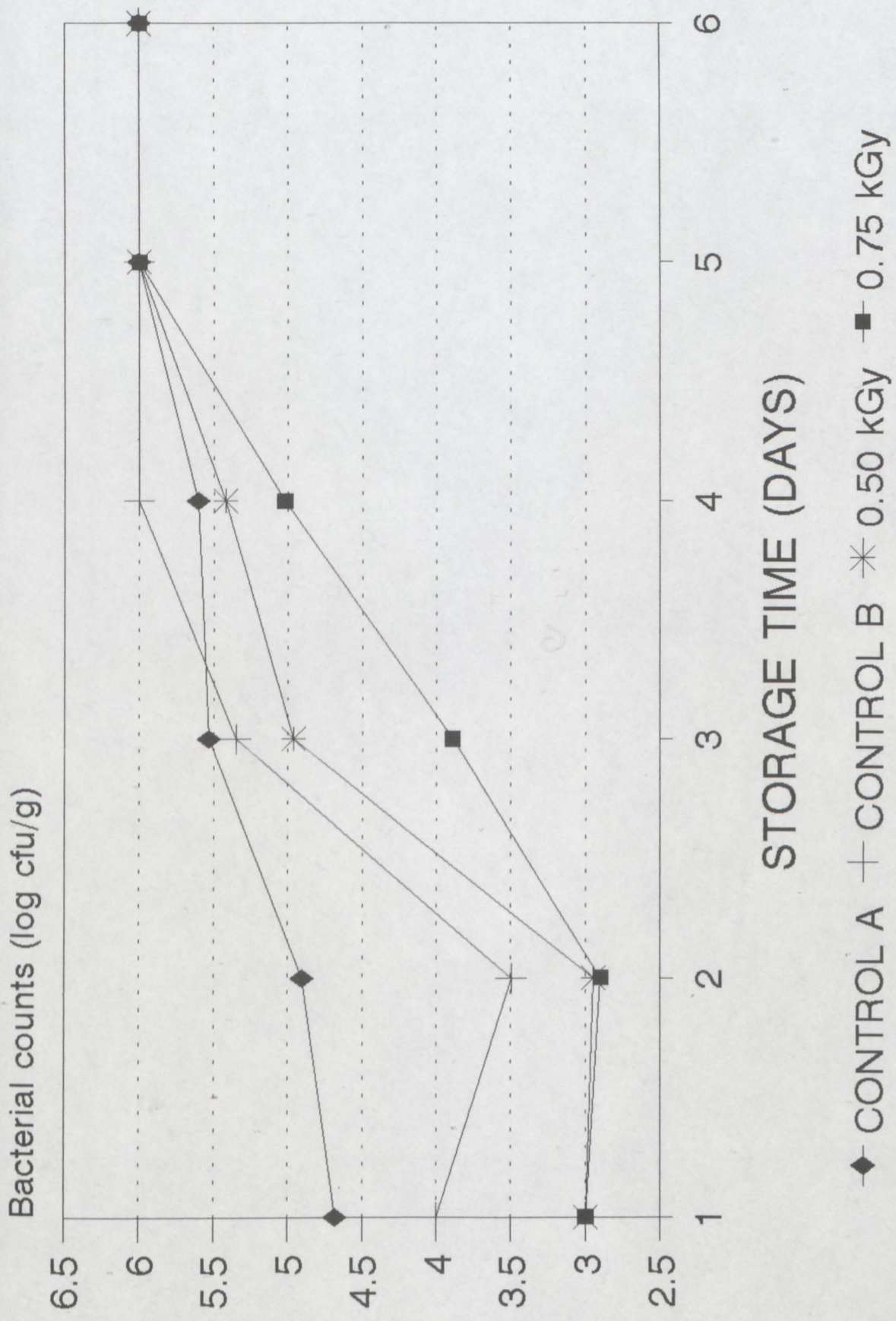
Fig. 24 Bacterial counts incubated at 20 deg. C on SWA for 48h
of Cape hake fillets stored at 5 - 8 deg. C
product core temperature.

The objective of this study was to test the value of radurization to extend the shelf life of fresh Cape hake products under conditions simulating the retail trade, which are more abusive in terms of temperature control, than laboratory conditions. Measured by aerobic, marine psychrotrophic bacterial counts ($20^{O}C$, SWA), radurization of Cape hake fillets was an effective hurdle in conjunction with storage below $5^{O}C$. Radurization at 0.5 KGy yielded an effective shelf life extension of Cape hake fillets under refrigerated ($<5^{O}C$) storage. Tainting rendered 0.75 KGy and 1 KGy radurization impractical.

4.3.1.3   Characterization of the bacterial communities

The predominant bacteria after radurization of Cape hake fillet samples at 0.75- and 1.0 KGy were *Moraxella spp* (83% of 0.75 and 1.0 KGy radurized sample isolates), followed by *Micrococcus spp* (17% of all isolates) (Table 3). Characterization results of isolates from the untreated control samples indicated that aerobic bacterial community structures were typical for fresh, untreated Cape hake fillets (Table 3) (VENNEMANN, 1991).

Table 3.   Characterization of bacterial spoilage populations after radurization of the Cape hake fillet samples.

Genus	(A: B)	0.5 KGy	(0.75 - 1.0 KGy)
Moraxella	38%	41%	83%
Micrococcus	2%	13%	17%
Pseudomonas	60%	46%	-

A = untreated control kept in chiller
B = untreated control travelling with samples

In samples radurized at 0.5 KGy *Pseudomonas* predominance was reduced from 60% to 46% (Table 3), which indicated sensitivity of this genus to irradiation. The predominance of *Moraxella*

increased to 41% and was followed by *Micrococcus* at 13% (Table 3). This, in turn, indicated the relative resistance of *Moraxella* spp to irradiation.

The increased predominance of *Moraxella* in radurized whitefish has been reported before (LEE *et al.*, 1967; KUMTA and MAVINKURVE, 1971; MIYAUCHI, 1972; HUSSEIN *et al.*, 1976; HOBBS and HODGKISS; 1982; JAY, 1986; AVERY *et al.*, 1987; HOBBS, 1987; FARKAS, 1991: WARD, 1991). However, in most cases the increase of *Moraxella* counts, after radurization was slow enough to yield prolonged storage times (14 - 20 d) at refrigerated (<5°C) storage. In this study, the bacterial counts predominated by *Moraxella spp* increased to > 10^6 cfu/g (the spoilage limit), 6 to 7 d after radurization and at the lower storage temperature (2 - 3°C, Fig. 23). However, bacterial counts dominated by *Moraxella* increased to > 10^6 cfu/g 3 - 4 d after radurization and higher temperature storage (5 - 8°C, Fig. 24), indicating the importance of low (<5°C) storage temperature after radurization to achieve an extension of the shelf life of the product.

4.3.1.4   Conclusions

Organoleptic characteristics and bacterial counts, indicated that radurization was only effective in extending shelf life at 0.5 KGy and under refrigerated conditions (<5°C). This indicated that multiple hurdles such as radurization and storage at low temperature are more effective in the extension of shelf life than a single hurdle (radurization followed by higher temperature storage). Table 3, indicates that *Pseudomonas* predominance was only slightly reduced after radurization at 0.5 KGy. The 0.75 and the 1.0 KGy radurization eliminated *Pseudomonas* and *Moraxella* predominated spoilage. Bacterial counts exceeded the spoilage limit without off odour production. Off odours were a result of "tainted" radurization odours and flavours (Table 2). Tainting of the fish at higher intensity radurization remains an obstacle in

using radurization as an hurdle for extending the shelf life of uncooked, fresh Cape hake products.

Bacterial counts indicated increases after radurization from samples incubated at 20°C on SWA. The characterization of the isolates from the SWA plates indicated species normally associated with marine whitefish. This highlighted the suitability of marine psychrotrophic bacterial counts incubated at 20°C on SWA for determining shelf life of marine whitefish products. This was further supported by the increase in aerobic, marine bacterial counts, which compared well with organoleptic findings.

Relating to consumer safety, another problem may arise. Under abusive storage conditions the natural fast growing bacterial spoilage community may be damaged by radurization, 0.5 - 1.0 KGy). The result could be accelerated growth by resistant, normally slow growing pathogens such as *Clostridium botulinum* Type E, *Listeria monocytogenes, Salmonella* spp and *Staphylococcus aureus*. These organisms require radurization doses exceeding 2 - 5 KGy, in order to be eliminated (HOBBS and SHEWAN, 1969; WARD and HACKNEY, 1991). It was therefore concluded that gamma irradiation was not an option for the extension of the shelf life of fresh Cape hake fillets.

4.3.2 The effect of storage temperature on the shelf life of brined, smoked and untreated whitefish products

The previous study indicated the importance of storage temperature in order to gain shelf life after radurization. The effect of temperature as a hurdle was therefore investigated. Fresh Cape hake products are marketed nationally and internationally from fish shops and fish counters in supermarkets. These are mostly refrigerated or chilled with ice.

This normally gives a shelf life of at least 3 - 4 days. The first objective of this study was to establish the degree to which temperature needs to be reduced to achieve shelf life extension, without freezing the products. The second objective of this study was to investigate whether brining and smoking contributed to shelf life extension in combination with temperature.

4.3.2.1 Organoleptic characteristics

Hake and Kingklip steaks were stored at 5 - 7°C and at 0 - 2°C core temperature in both, the factory (Table 4) and a retail store (Table 5). In addition to the two plain cut portions (Hake and Kingklip steaks), a processed cut, "Haddock" steaks, which were salted (brined) and cold-smoked Hake was tested under the same conditions. The reason for the additional hurdle was that brining and smoking have inhibitory effects on bacterial growth and potential shelf life extension needed to be assessed for the retail chain.

Table 4   Organoleptic characteristics of three fish products stored at different temperatures at the factory.

Day No.	Hake		Kingklip		Haddock	
	0 - 2°C	5 - 7°C	0 - 2°C	5 - 7°C	0 - 2°C	5 - 7°C
1	*	*	*	*	*	*
2	*	(*)	*	(*)	*	*
3	*	X	*	X	*	(*)
4	*	X	*	X	*	X
5	*	X	*	X	*	X
6	(*)	X	(*)	X	*	X
7	X	X	X	X	(*)	X
8	X	X	X	X	X	X

```
*    = fresh
(*)  = fishy
X    = off
```

The shelf life of all three product types was reduced by elevated temperature storage. Off odours were noted after 2 - 3d when

stored at 5 - 7°C (Table 4.), compared to 6d to 7d at a storage temperature of 0 - 2°C. The results were similar for Hake and Kingklip steaks. However, the brined, cold smoked haddock steaks had an additional shelf life of one day at both storage temperatures. This finding confirmed the inhibitory effects of brining and smoking to spoilage. This was not surprising since this was a traditional way of extending shelf life of whitefish products (SHEWAN, 1961; HOBBS and HODGKISS, 1982).

Table 5   Organoleptic characteristics of three fish products stored at different temperatures at a retail store.

Day No.	Hake		Kingklip		Haddock	
	0 - 2°C	5 - 7°C	0 - 2°C	5 - 7°C	0 - 2°C	5 - 7°C
1	*	*	*	*	*	*
2	*	*	*	*	*	*
3	*	(*)	*	(*)	*	*
4	*	X	*	X	*	(*)
5	*	X	*	X	*	X
6	(*)	X	(*)	X	*	X
7	X	X	X	X	(*)	X
8	X	X	X	X	X	X

```
*   = fresh
(*) = fishy
X   = off
```

The untreated steak products stored at the retail outlet at 0 - 2°C (Table 5) were spoiled after the 6th day as were the corresponding steak products stored at the factory. The steaks stored at 5 - 7°C were spoiled after 3 - 4 days the same as for the factory trial. The haddock steaks also indicated a similar result for the 0 - 2°C and 5 - 7°C storage. Thus temperature increases of 3 - 5°C in chilled storage, may result in reducing the shelf life by half, which was confirmed findings of other researchers (SHEWAN, 1961; SHEWAN and HOBBS, 1963; OLLEY and RATKOWSKY, 1972; LISTON, 1980, 1982; SIMMONDS and LAMPRECHT, 1980; LAMPRECHT et al., 1984; LORD, 1990).

Fig. 25 Marine, psychrotrophic bacterial counts (cfu/g) during storage of fresh Cape hake steaks at different temperatures.

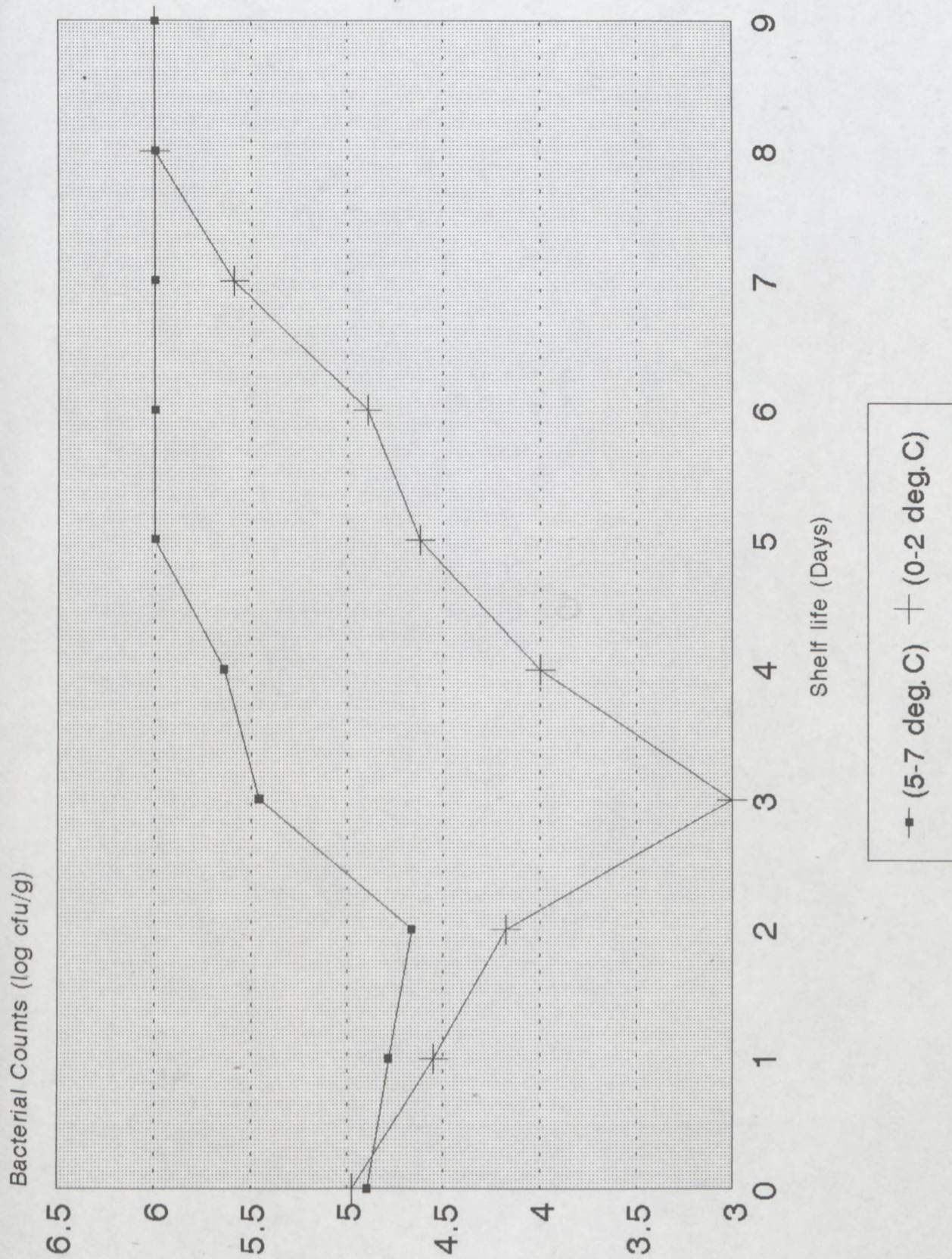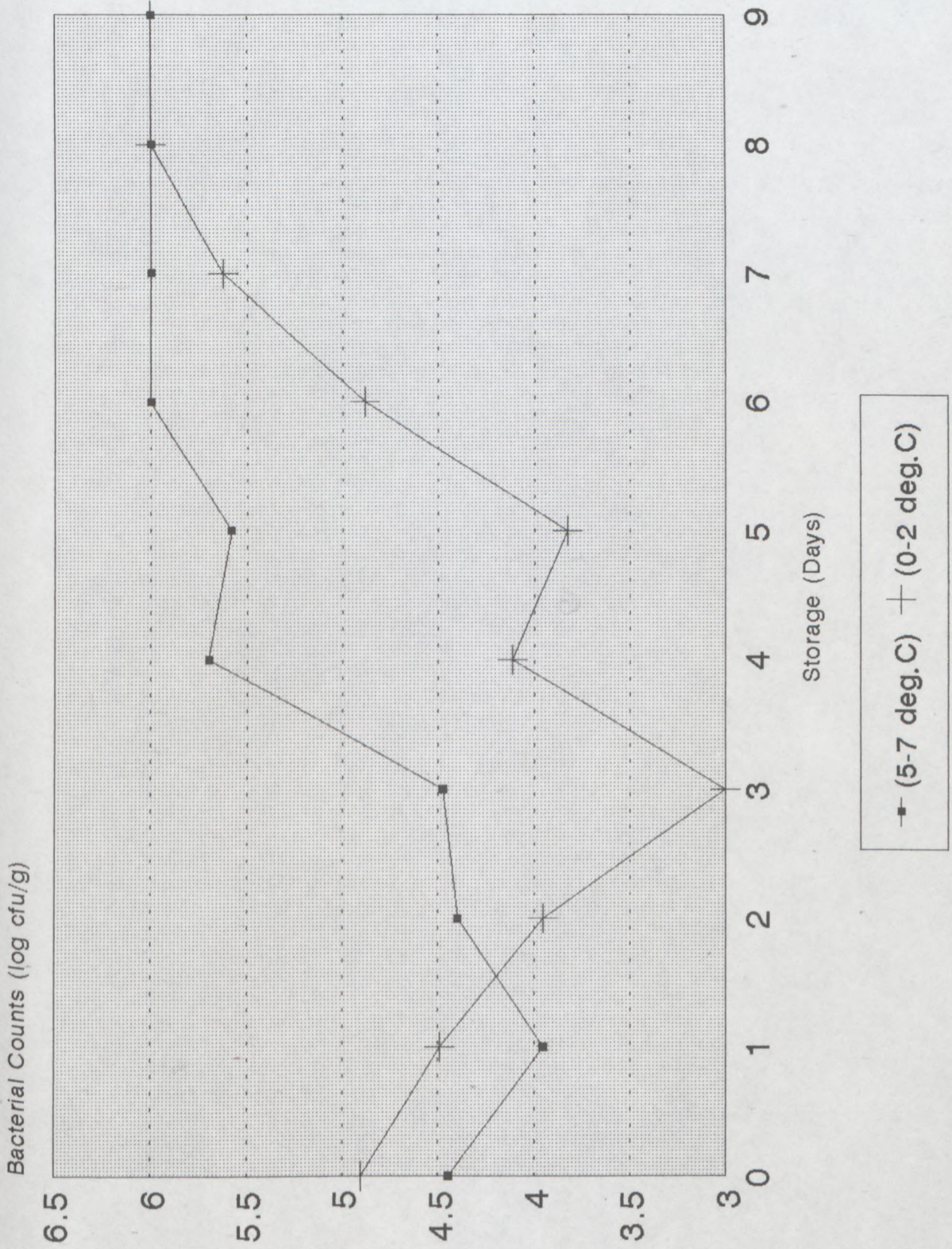Bacterial Counts (log cfu/g)

Shelf life (Days)

■ (5-7 deg.C) + (0-2 deg.C)

Fig. 26 Marine, psychrotrophic bacterial counts (cfu/g) during storage of fresh Kingklip steaks at different temperatures.

Bacterial Counts (log cfu/g)

Storage (Days)

■ (5-7 deg.C) + (0-2 deg.C)
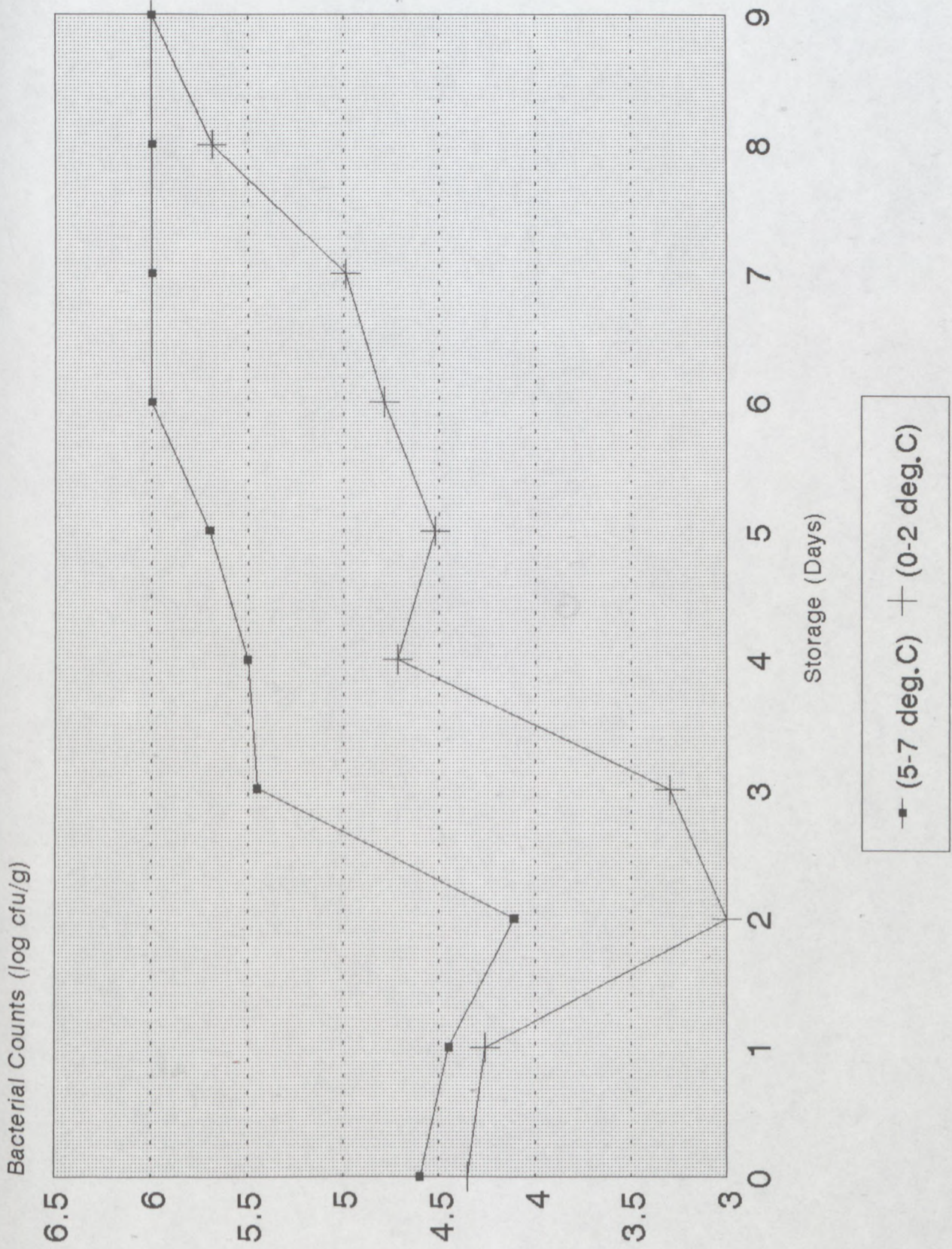
Fig. 27 Marine, psychrotrophic bacterial counts (cfu/g)
of Haddock Steaks during storage at different
temperatures.

4.3.2.2 Bacterial counts

Increases of bacterial counts (Figs 25, 26, 27), corresponded
with organoleptic deterioration (Tables 4, and 5) for all three
steak products. Off odours were noticed when bacterial counts
exceeded 10^6 cfu/g (Table 4 and 5). At 5 - 7°C, both trials at
the factory and at the retail store showed an increase in
bacterial counts on Hake (Fig. 25) and Kingklip (Fig. 26) steaks
after 2d, which exceeded the spoilage limit after 5d. However,
the bacterial counts of haddock steak only exceeded 10^6 cfu/g
after the 6th day of storage at 5 - 7°C (Fig. 27). In contrast,
the bacterial counts of the products stored at 0 - 2°C increased
gradually from day 4 on Hake and Kingklip steaks and only
exceeded 10^6 cfu/g after 7d. Bacterial counts on haddock steaks
exceeded the spoilage limit only after the 8th day of storage at
0 - 2°C. Therefore, at both storage temperatures at the factory
and the retail outlet, the bacterial counts on haddock steaks
consistently increased more slowly than those of the untreated
products. Explanations for this finding are the brining process
in a salt brine, as well as the bacteriostatic effect of phenolic
substances in the woodsmoke (SHEWAN, 1961; LISTON, 1980).

4.3.2.3 Conclusions

At storage temperatures of 5 - 7°C, Hake and Kinklip steaks had a
shelf life of 2 - 3d. However, at 0 - 2°C, the same products had
a shelf life of 6d. The Haddock steaks showed a similar pattern,
with a further day added to the organoleptic and
"microbiological" shelf life, confirming the inhibitory nature of
brining and smoking to bacterial growth. Storage temperature was
the most effective hurdle for controlling growth of spoilage
bacteria and one of the most important control points for the
preservation of quality of refrigerated whitefish products (LUPIN
et al., 1980; SHIPMAN and WYLER, 1989; LORD, 1990; ANON., 1992).

4.3.3 The effect of chemical preservatives on the shelf life of Cape hake fillets

There are many traditional ways of preserving fish products. Almost all of these involve processes of reducing the water activity (salting and drying) and also acidifying (pickling). Many preservatives are used in the food industry to delay spoilage and prolong the shelf life of perishable food products (JAY, 1986; MILLER JONES, 1992). The objective of this study was to determine whether it would be possible to prolong the shelf life of fresh (unfrozen), prepacked Cape hake fillets with a selection of chemicals such as CO_2, NaCl and a combination of Potassium Sorbate and Sodium Pyrophosphate. These substances were previously reported to be suitable for preserving fresh fish products (SHEWAN and HOBBS, 1967; WESSELS *et al.,* 1971; SIMMONDS *et al.,* 1981) under commercial (5 - 8°C product core temperature) conditions.

4.3.3.1 Organoleptic characteristics

From the 1st day of storage at 5 - 8°C, the sensory properties of the carbonic acid- and potassium sorbate treated samples were adversely affected (Table 6). The side effects (soft, dry skin) distinctly detracted from the unprocesed "image" fresh Cape hake fillets have. These side effects intensified from the 3rd day, so that treated fillets became more undesirable, although the controls had also lost theirs characteristic fresh odour and flavor. At this stage no characterristic spoilage (sulphury, sour) odours, normally caused by marine spoilage bacteria, could be detected from any of the fillets. The carbon dioxide treated fillets had lost their natural glassy white colour and turned milky white, as well as being soft and pulpy in texture after cooking. The potassium sorbate/sodium pyrophosphate treated samples had all developed an unattractive dry and hard skin and the salt treated fillets had lost flavour and showed a slimy

protein exudate on the surface which became white after cooking.

Table 6. Organoleptic assessment of chemically preserved fresh Cape hake fillets stored under chilled (5 - 8°C product core temperature)

Storage d	A	CO_2	PS	NaCl
1	*	*(soft)	*(dry skin)	*(salty)
2	*	*(soft)	*(dry skin)	*(salty)
3	(*)	(*)(soft)	(*)(dry skin)	(*)(slimy)
4	X	(*)(soft)	(*)(dry skin)	(*)(slimy)
5	X	(X)	(X)	(X)
6	X	X	X	X

(A = untreated conrol)
(CO_2 = saturated carbonic acid)
(PS = 0.75% potassium sorbate/6% sodium pyrophosphate)
(NaCl = 5% sodium chloride)
 * = fresh
(*) = fishy/undesirable due to treatment
(X) = unacceptable due to treatment
 X = off

The fillets treated with the salt solution developed a brown colour on the 4th day in addition to the slimy surface. All samples, irrespetive of treatment, were found to be unacceptable after the 5th day of storage. The controls spoiled after the 4th day of storage. After the 6th day (7 to 10d from catch), all fillets were spoiled due to off odours. The chemical preservative treatments thus did not extend the shelf life of Cape hake fillets at 5 - 8°C. In addition, the fillets were undesirable due to treatment side effects, from the 1st day of storage (Table 6).

4.3.3.2 Bacterial counts

Bacterial counts (Fig. 28) increased comparably between untreated controls and treated samples. This indicated the lack of bacteriostatic action of the preservative treatments during storage at 5 - 8°C. Even though the treated fillets did not develope the characteristic bacterial spoilage odours
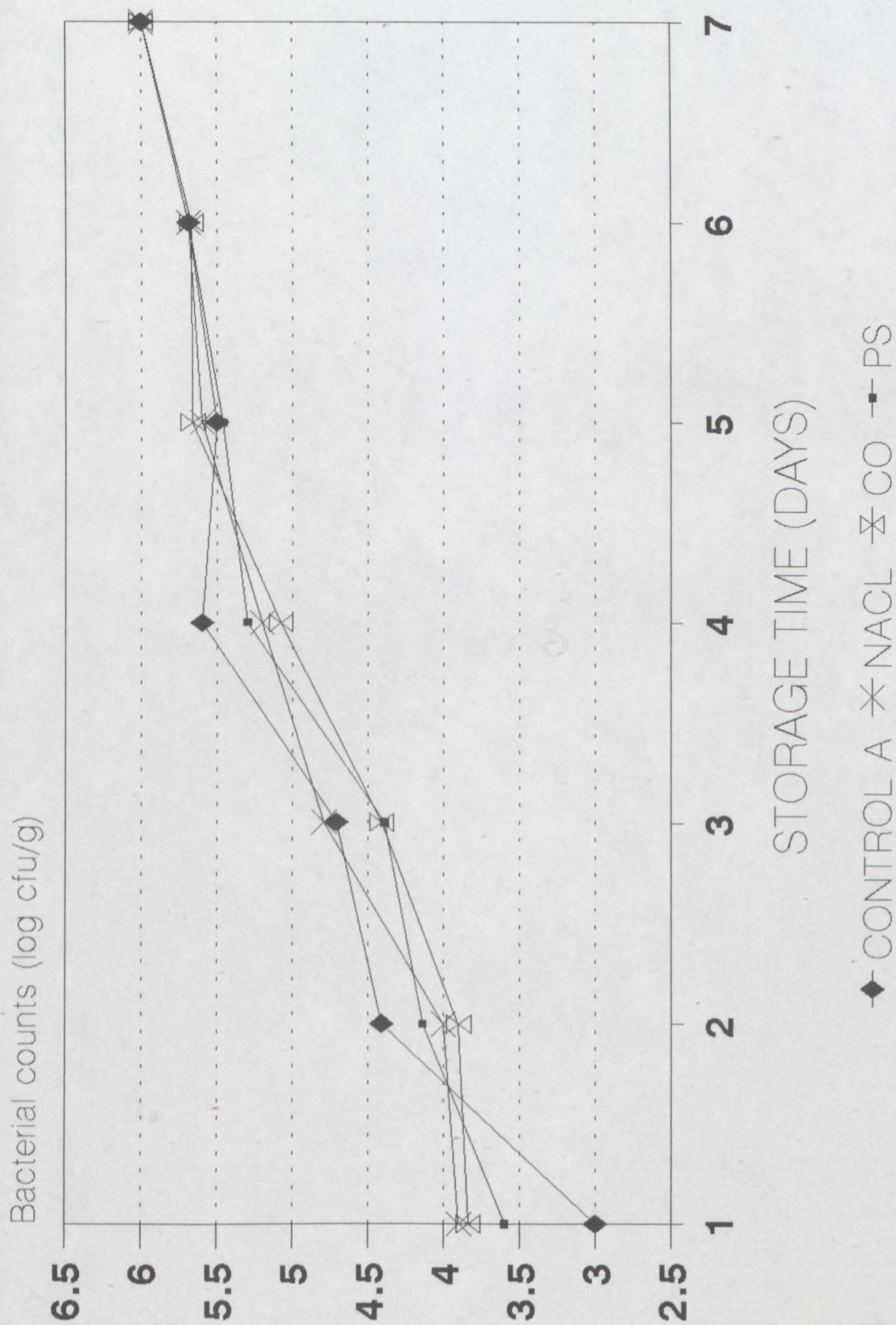
Fig. 28 Bacterial counts from SWA incubated at 20 deg. C of chemically treated Cape hake steaks during refrigerated storage at 5-8 deg. C

(sour, sulphury, ammoniacal) after the fourth day, the side effects of the treatments(Table 6) may have masked these. All samples reached the spoilage limit of 10^6 cfu/g after the 6th day of storage. This was also the time when bacterial spoilage became organoleptically evident and was no longer masked by the treatments (Table 6).

4.3.3.3 Conclusions

Treatments with especially saturated carbon dioxide solutions, have been successful in the past in extending the shelf life of Cape hake to 14d at refrigerated ($<5^{o}C$) temperatures (SIMMONDS et al., 1981). However, these tests used whole, headed and gutted Cape hake of the last days catch only, which were not filleted in a factory. These fish were continuously flushed with saturated carbon dioxide solution under iced conditions ($0^{o}C$) in a laboratory. Nevertheless, similar side effects (white, "dead" colour and soft, pulpy texture) to those observed in this study were reported.

The organoleptic characteristics (Table 6) did not indicate shelf life increases in the sampls treated with chemical preservatives. This was consistent with the microbiological counts (Fig. 28). Results also indicated that all fillets became unacceptable after the sixth storage day. This was also reflected by the psychrotrophic bacterial counts (Fig. 28). The relationship between organoleptic and bacterial results confirmed the suitability of aerobic, psychrotrophic marine bacteria counts incubated on SWA at $20^{o}C$ as a technique to determine the shelf life of Cape hake fillets exposed to different shelf life extending treatments.

Labelling regulations (SABS, 1977; FDA, 1986; EEC, 1991) today stipulate that all added preservatives must be declared on the product label. From the perspective of a consumer buying fresh

<u>fish</u>, it is difficult to associate chemically preserved fish with
a fresh or unprocessed image. As in most fresh foods today, the
marketing trend is away from chemically preserved foods. This is
especially valid for basic staple foods such as protein,
vegetables, fruit, bread and milk (JAY, 1986; MILLER JONES,
1992). It is important to recognize this trend, because it places
the emphasis on GMP and HACCP for the particular industry in
order to produce quality, shelf life and a safe food product.
This study, nevertheless, was conducted to determine whether
there was economic merit (ie.: a shelf life extension in excess
of 10 - 12d) with the use of well known preservatives.
Traditional means of preservation such as smoking, drying and
salting are perceived by today's consumer to be more "natural"
and therefore more acceptable, even though biochemically this
might not be true. This is partly the reason why such treatments
are still continuing today.

4.3.4   <u>The effect of vacuum packaging on the shelf life of</u>
        <u>deepsea whitefish products</u>

Packaging of foodstuffs, including fish provides an important
vehicle for supplying fresh, unfrozen fish to the consumer and
has the benefit of preventing further contamination, for as long
as the package stays intact (WARD and HACKNEY, 1991; MILLER
JONES, 1992). In addition, the consumer is able to select
according to size, visual appearance, mass and other criteria.
This offers superior adaptability towards a consumer's needs in
comparison with frozen, carton packed products (SHEWAN and HOBBS,
1963). As with other hurdle techniques, this practice relies on
GMP. Due to its nature, it is a visual pack and any abuse will
easily be recognizable by the consumer.

Since the predominant natural bacterial community on the white-
fish species from the Benguela ecosystem (Cape hake, *Merluccius*

capensis/paradoxus; Kingklip, *Genypterus capensis*; Monkfish, *Lophius upsicephalus*) is aerobic (SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991), it can be assumed that prepacking under vacuum would restrict growth of marine psychrotrophic spoilage bacteria and therefore extend shelf life. The purpose of this study was therefore, to determine whether additional shelf life may be gained by utilizing vacuum packaging as a hurdle.

4.3.4.1 Organoleptic characteristics

Table 7   Organoleptic assessment of deepsea whitefish products packed in oxygen permeable and full barrier film packs, stored at 4 - 6°C.

Day No.	Cape hake perm.	nonperm.	Fresh Kk perm.	nonperm.	Defrosted Kk perm.	nonperm.
1	*	*	*	*	*	*
2	*	*	*	*	*	*
3	*	*	*	*	*	*
4	*	*	*	*	*	*
5	(*)	(*)	*	*	*	*
6	X	X	(*)	(*)	(*)	(*)
7	X	X	X	X	X	X

perm. = permeable, 2 300ml O^2/m^2/24h
nonperm. = full barrier film
Kk = Kingklip (*Genypterus capensis*)
* = fresh
(*) = "fishy"
X = off

The organoleptic results (Table 6) showed no difference in shelf life between the samples packed in oxygen permeable and the full barrier film. Cape hake samples, packed into both, oxygen permeable and full barrier films, had lost their fresh natural odour and flavour after the fifth day and were off after the sixth day. The fresh (unfrozen) Kingklip fillets took one day longer to lose their natural fresh odour and flavour and were off after the sixth and seventh day of storage at 4 - 6°C. The fillets from the defrosted "frozen at sea" (FAS) Kingklip, indicated no difference in shelf life to the fresh Kingklip

fillets (Table 7). This finding was used by the factory to justify defrosting FAS Kingklip, for filleting and packing for sale under chilled conditions, as it did not decrease the shelf life. Kingklip naturally has a firmer texture and develops a lower pH (6.0 - 6.3) than the Cape hakes (6.4 - 6.8) during rigor mortis. The lower pH is reportedly inhibitory to bacterial growth and spoilage (SHEWAN, 1977; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991) and this could explain the additional day shelf life of Kingklip fillets compared to those of Cape hake in this study.

4.3.4.2 Bacterial counts

The results (Figs. 29 and 30) indicate that the bacterial counts on Hake fillets first decreased, but then increased from day three. Counts then levelled out after day 5 and decreased slightly after day 6 and 7 of chilled (4 - 6°C) storage. No explanation could be found for the difference in the rate of bacterial increase (Fig. 29).

The Hake fillets developed off odours and flavours after the 5th and 6th day of chilled storage. This corresponds with the logarithmic growth phase of Shewan's succession pattern of bacterial spoilage of fish (SHEWAN, 1977; VENNEMANN, 1991) where at the end of this phase bacterial counts level out to enter the stationary phase. Off odours may be noticed at the end of the log or rapid growth phase (SHEWAN, 1977).

A similar result was observed for the fresh and the defrosted Kingklip fillets packed into full barrier- and the same oxygen permeable films as the Hake fillets (Fig. 30). both types of Kingklip fillets showed a decline in psychrotrophic, marine bacterial counts after the 2nd day and again after the 5th day of chilled storage, with the exception of the defrosted Kingklip fillets packed into permeable film which indicated an increase in
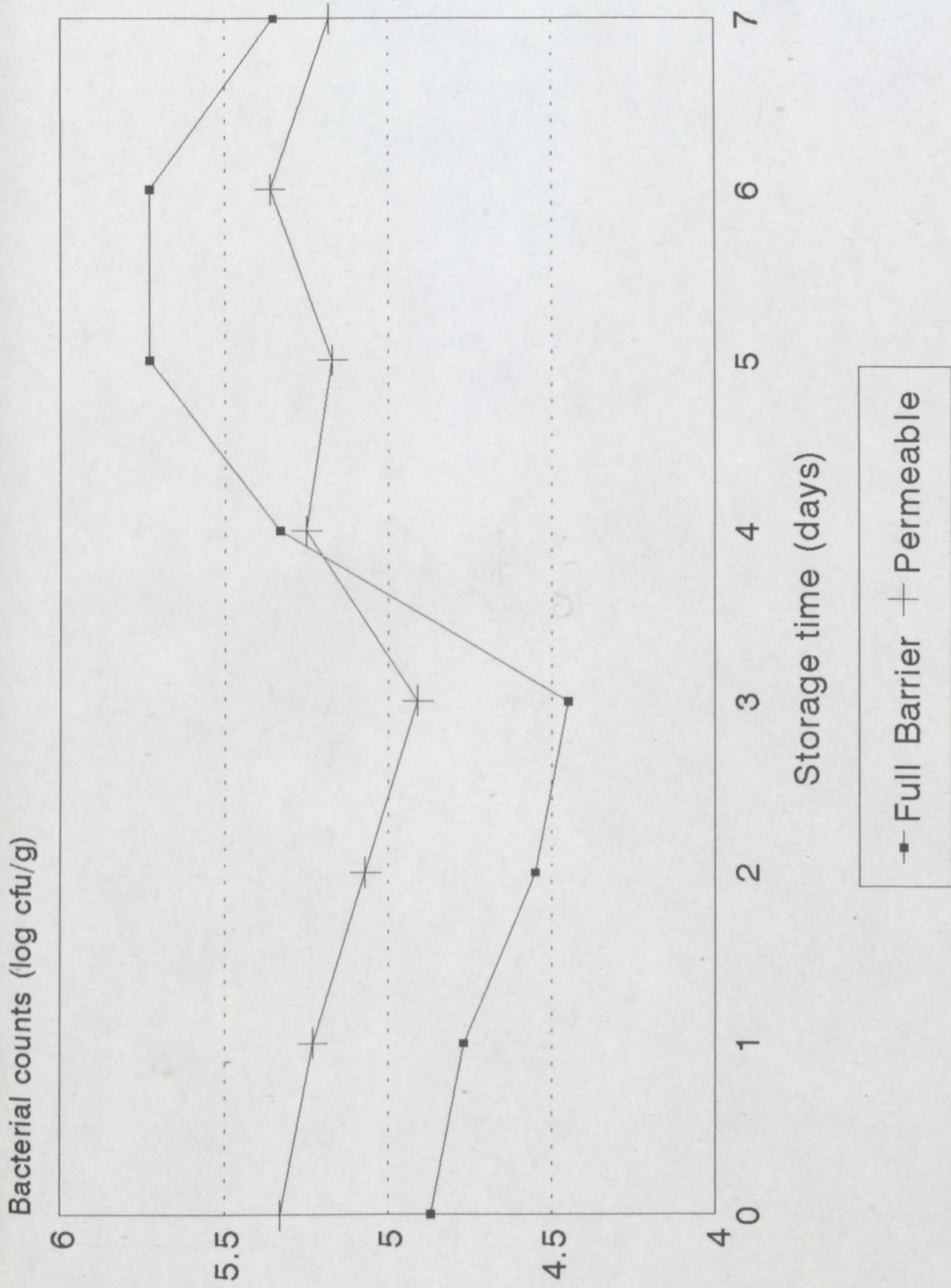
Fig. 29 Bacterial counts from SWA incubated at 20 deg. C
of Cape hake fillets packed in barrier and oxygen
permeable films, stored at 4-6 deg. C.

bacterial counts after the fifth day (Fig. 30). However, the
Kingklip fillets did not show a decline in bacterial counts
immediately after packing (Fig. 30), as did the bacterial counts
of the Hake fillets. A possible explanation for the different
trends in bacterial counts over the first 2 - 3 days after
packing between the Hake and the Kingklip fillets could be that
there may have been different bacterial populations for the two
different species of whitefish (Tables 8 and 9).

Bacterial counts below 10^6 cfu/g in spoiled fillets of both Hake
and Kingklip (Figs. 29, 30), were unexpected, since fish with off
odours and flavours usually has bacterial counts in excess of 10^6
cfu/g (SHEWAN, 1961, 1977; SHEWAN and HOBBS, 1963, 1967; LISTON,
1980; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985;
HOBBS, 1987; VENNEMANN, 1991). On the last day of storage, the
bacterial counts of Cape hake fillets varied from 5.2log to ca.
5.4log cfu/g for the permeable and the full barrier films,
respectively (Fig. 29). On the Kingklip fillets, bacterial counts
on the last day of storage varied from 3log to 4.29log cfu/g. The
lowest bacterial counts after the last day of storage, were
recorded from fresh fillets packed into permeable film and the
highest from the fillets cut from defrosted Kingklip packed into
full barrier film (Fig. 30). The difference in bacterial counts
between the two packing methods, could not be explained. No
statistically significant differences (p > 0.1) were observed
between the full barrier and permeable films, for the hake
fillets, as well as the fresh and defrosted Kingklip (Figs. 29
and 30). A possible explanation for this observation on the fresh
and defrosted Kingklip could be the fact that quick freezing on
board the factory trawler at sea preserved the viability of the
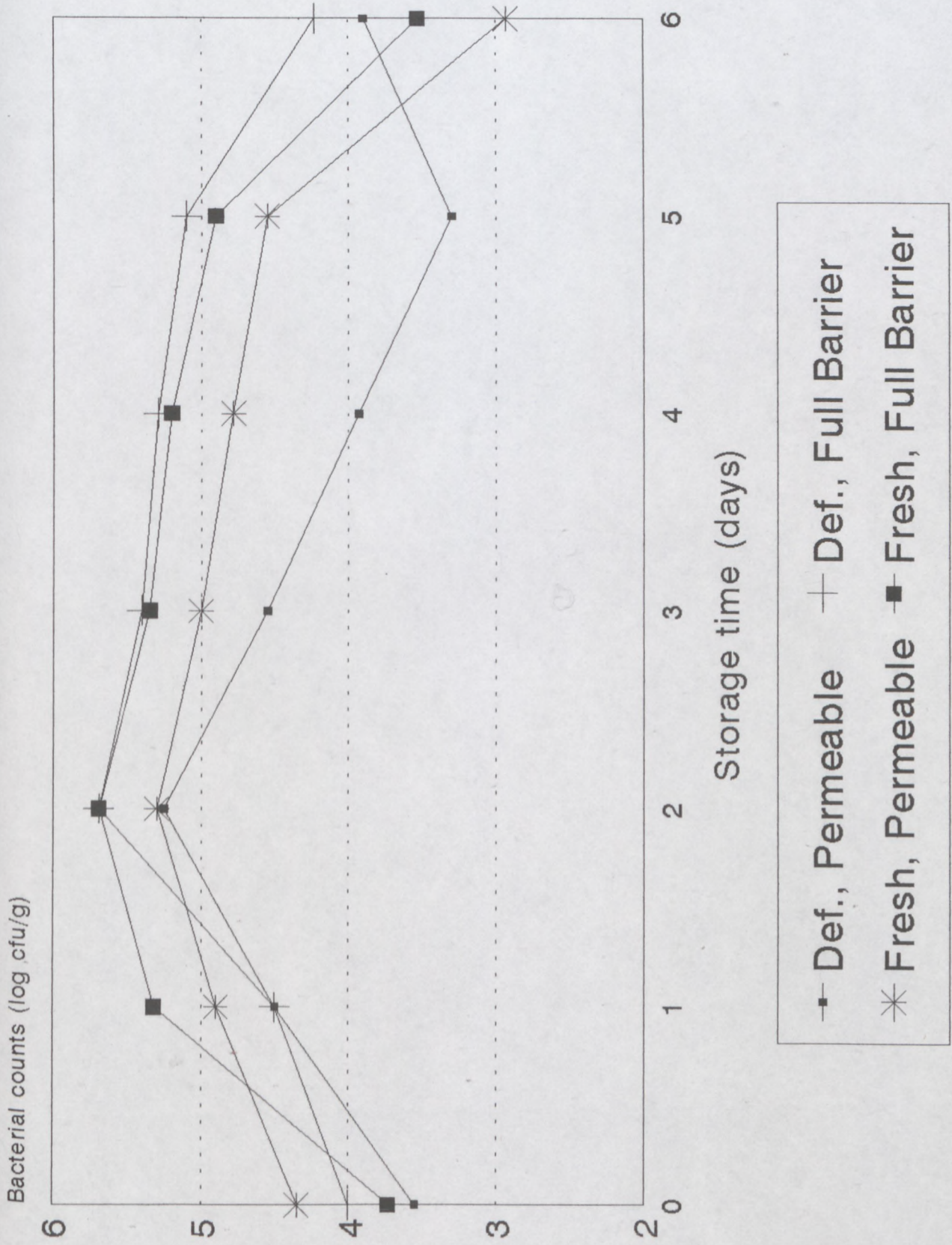bacteria on the fish skin.

Fig. 30 Bacterial counts from SWA incubated at 20 deg. C of fresh and defrosted Kingklip fillets packed in barrier and oxygen permeable films, stored at 4-6 deg. C.

4.3.4.3 Characterization of bacterial communities.

Characterization of predominant bacteria was carried out from the first to after the sixth day of chilled (>5°C) storage from Cape hake and Kingklip fillets (Tables 8 and 9).

Table 8   Characterization of predominant bacteria from packed Cape hake fillets from d 1 to d 6 of chilled (4 - 6°C) storage.

% Genera

Film	Day	Ps	Mo	Mi	Co	O
P	1	20	35	30	5	10
NP	1	50	40	10	0	0
P	2	75	25	0	0	0
NP	2	50	50	0	0	0
P	3	60	20	20	0	0
NP	3	80	20	0	0	0
P	4	50	50	0	0	0
NP	4	70	20	10	0	0
P	5	70	30	0	0	0
NP	5	90	0	10	0	0
P	6	80	20	0	0	0
NP	6	100	0	0	0	0

Ps = *Pseudomonas*, Mo = *Moraxella*, Mi = *Micrococcus*,
Co = *Corynebacterium*, O = unidentified
P = permeable
NP = nonpermeable

The Hake fillets during the 1st and 2nd day of chilled storage revealed a community structure typical for whitefish from cold and temperate marine environments for both types of packaging (Table 8)(GEORGALA, 1957; SHEWAN 1961; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991). Gram negative *Moraxella* and *Pseudomonas* predominated over the Gram positive *Micrococcus* and *Corynebacterium*. However, from the 3rd day of storage, *Pseudomonas* became increasingly predominant at the expense of *Moraxella*. When the Hake fillets were off (Table 7)

after the 5th and the 6th days of chilled storage, *Pseudomonas* predominated the bacterial populations at 70 - 100%. *Moraxella* represented only 0 - 30% of the populations on both types of packaging. No difference in bacterial population between the permeable and nonpermeable packaging could be found (Table 8).

The Kingklip fillets (Table 9) showed a similar pattern of predominance of Gram negative genera. The Gram positive genera did however occur in higher proportion on the Kingklip, compared to the Hake fillets until the 5th day of storage. After the 3rd day of storage, however, *Pseudomonas* became predominant over the other genera in similar proportion to that of the Hake fillets.

Table 9   Characterization of predominant bacteria from packed Kingklip fillets from d 1 to d 6 of chilled (4 - 6°C) storage.

% Genera

Film	Day	Ps	Mo	Mi	Co	O
P	1	0	85	15	0	0
NP	1	0	60	25	0	15
P	2	15	45	15	15	10
NP	2	10	45	20	20	5
P	3	20	55	0	15	10
NP	3	15	36	6	44	0
P	4	10	30	30	25	5
NP	4	65	20	10	5	0
P	5	0	50	40	10	0
NP	5	45	25	25	5	0
P	6	20	55	15	5	5
NP	6	90	10	0	0	0

Ps = Pseudomonas, Mo = Moraxella, Mi = Micrococcus,
Co = Corynebacterium, O = unidentified
P = permeable
NP = nonpermeable

Table 9 combines the results for fresh and defrosted Kingklip as
the structure of the bacterial populations were similar. However,
regarding the predominance of *Pseudomonas* between the different
packaging types this genus occurs in lower proportion in the
permeable than the nonpermeable films over most storage days,
including the last day. This was ascribed to the increased
proportion of Gram positive genera present in the same samples.
There was also no increase in *Pseudomonas* predominance over the
other genera in the permeable film packs. However, the
nonpermeable film packs indicated an increase of *Pseudomonas*
predominance in a similar fashion to the Cape hake fillets and
this genus predominated the bacterial populations on the last day
of storage (Table 9). This observation indicated a possible
higher sensitivity of *Moraxella* and the Gram positive genera to
vacuum packaging with full barrier films in the Kingklip fillets.

The spoilage pattern of chilled ($>4^{\circ}$C) whitefish normally follows
a succession of 4 distinct steps (SHEWAN, 1977; SIMMONDS and
LAMPRECHT, 1985; VENNEMANN, 1991). These correspond to the lag
phase, the rapid bacterial growth or logarithmic phase, where on
occasion musty and off odours might be detected with bacterial
counts between 10^5 and 10^6 cfu/g. The third stage is associated
with bacterial counts of 10^6 - 10^8 cfu/g and more evident off
odours. It is usually during this phase that *Pseudomonas* become
predominant in psychrotrophic, marine whitefish spoilage
populations and are responsible for the odour production. By the
fourth phase (10^8 - 10^9 cfu/g) *Pseudomonas* have become
predominant to the extent that only small fractions of the other
genera, chiefly *Moraxella* remain on the now putrid fish (SHEWAN
1961; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991).

The above results (Table 8 and 9) are consistent with the end of
the 2nd and the 3rd phase of the spoilage succession pattern
described above, including the bacterial counts at these stages
(Fig. 29 and 30) with the exception of the lack of predominance

of *Pseudomonas* over the other genera on the permeable film packed
Kingklip fillets. No reason for this exception, other than a
larger proportion of Gram positive genera in these fillets could
be found.

4.3.4.4   Conclusions

No shelf life extension was gained for either Cape hake or
Kingklip fillets when stored in a nonpermeable vacuum pack in
chilled storage (4 - 6°C) for 6 to 7 days. From the 3rd day of
chilled storage, the bacterial counts, as well as the bacterial
community structure on the samples, were consistent with Shewan's
(SHEWAN, 1977; VENNEMANN, 1991) spoilage succession pattern for
marine whitefish species. Spoilage odours could be detected in
the products prepacked after the 5th day for the Hake fillets and
after the 6th day for the Kingklip fillets (Table 7). This meant
that vacuum packing using a nonpermeable film to exclude oxygen
for Cape hake and Kingklip fillets, was not an effective hurdle
to psychrotrophic bacteria under chilled storage conditions.

The fact that the bacterial counts and characterization agreed
with Shewan's spoilage succession pattern (1977) confirmed that
the use of aerobic marine, psychrotrophic bacteria incubated on
SWA at 20°C on fresh and frozen, uncooked deepsea whitefish
products is a suitable technique to establish bacterial quality
(shelf life) for these products.

CHAPTER 5

CONCLUSION

CONCLUSION

1. Bacterial standards

According to MOSSEL AND VAN NETTEN (1991) when setting microbiological standards a 5 point plan should be adhered to:
(*) selecting target organisms;
(*) choosing and carefully standardizing a method to be used for their enumeration, alternatively an already approved official standard method could be used;
(*) carrying out surveys of samples taken from the production environment adhering to GMP;
(*) deriving acceptable numerical values from the survey data guided by an HACCP analysis;
(*) establishing a policy for dealing with consignments which fail the target values.

1.1 Target organism and choosing a technique

Spoilage in deepsea whitefish is brought about by aerobic, marine psychrotrophic bacteria (GEORGALA, 1957; SHEWAN, 1961; LISTON, 1980; SIMMONDS and LAMPRECHT, 1985; MOSSEL and VAN NETTEN, 1991; VENNEMANN, 1991). The choice of the target group of microorganisms was therefore aerobic, marine, psychrotrophic bacteria. The choice of medium was SWA (SIMONDS and LAMPRECHT, 1985) and the incubation temperature 20°C since the optimum growth temperature for marine psychrotrophic bacteria is at 20°C (SHEWAN, 1961; LISTON, 1980; HOBBS, 1987). This ensured that the first two steps of Mossel and van Nettens five point plan were covered.

Furthermore, the study indicated that with an increase in the number of processing steps, there was an increase in the marine, psychrotrophic bacterial counts. The mesophilic bacterial counts on PCA incubated at 35°C, (part of the current international

standard) did not indicate an increase in bacterial counts with the complexity of processing, but remained constant. This indicated that the mesophilic bacterial counts were not as sensitive to the production complexity as the psychrotrophic marine bacterial counts and were therefore an inferior measurement of the bacterial condition of the product. This result further supported the choice of marine psychrotrophic bacteria as target organisms and incubation on SWA at 20°C.

1.2 Carrying out surveys of GMP processes

The bacteriological monitoring of products under "good" and "poor" GMP over a period of 19 months indicated that bacterial counts of samples on SWA incubated at 20°C increased on products which had experienced "poor" GMP. However, mesophilic bacterial counts from the same samples incubated on PCA at 35°C increased only marginally. These results indicated that psychrotrophic bacterial counts incubated at 20°C on SWA, were a more suitable means of assessing bacterial quality (shelf life) and were more sensitive indicators of GMP than mesophilic bacterial counts.

1.3 Deriving bacterial count standards from survey data

The third point in MOSSEL and VAN NETTEN's five point plan, was satisfied. By using the bacterial counts from the six different product groups under "good" GMP. The comparison of the bacterial product analyses data (survey data) of a product manufactured under both, "poor" and "good" GMP determined the bacterial count standards. Internal standards for total marine psychrotrophic bacteria thus serve the purpose of monitoring processing controls in order to ensure fresh final product. An internal psychrotrophic aerobic bacterial count standard could be determined using a 3 point sampling plan: $m = 100\ 000$ cfu/g; $M = 250\ 000$ cfu/g, with $c = 3$ and $n = 5$ for basic and medium processed product categories (Where $m=$ *desirable limit, M=*

absolute limit, c= number of samples permissible between m and M and n=total number of samples). However, m = 250 000 cfu/g; M = 500 000 cfu/g,; with c = 2 and n = 5 for highly processed deepsea whitefish products. The sea frozen (FAS) standard was set at m = 100 000 cfu/g; M = 250 000 cfu/g, with c = 2 and n = 5.

1.4 Bacterial pathogen incidence and standards

Since most of the processing was manual, the addition of processing steps, increased the probability for product contamination with human environment pathogenic bacteria. During the 19 month GMP monitoring period, bacterial pathogen incidence was low. However, during the period were GMP was at its most compromised as indicated by the aerobic bacterial counts, the incidence of pathogens increased. This indicated that the standard for pathogens, including methodology, was not questionable and suited the framework of MOSSEL and VAN NETTEN (1991). Pathogens, although less sensitive through low incidence, were therefore also indicators of poor GMP.

1.5 Aerobic, marine, psychrotrophic bacteria

Whilst the presence of bacterial pathogens may pose a hazard for foodborne disease, marine, psychrotrophic bacteria are endogenous or natural on marine whitefish such as Cape hake and already present (SHEWAN, 1961, 1977; HOBBS and HODGKISS, 1982; SIMMONDS and LAMPRECHT, 1985; VENNEMANN, 1991). Ecologically they are therefore adapted to a cold environment and the product and are able to grow under psychrotrophic conditions. Pathogens deriving from the human environment, on the other hand are poor competitors under chilled conditions and grow slowly on refrigerated ($<4^{\circ}$C, but $>0^{\circ}$C) or chilled ($>4^{\circ}$C, but $<10^{\circ}$C) (JAY, 1986; WARD and HACKNEY, 1991; MILLER JONES, 1992) marine products. This was clearly indicated by the low occurrence of pathogenic bacteria on the fish products even during a period of

compromised GMP. The products will therefore spoil before
becoming hazardous under abusive conditions. It follows that
uncooked, fresh and frozen seafoods, utilizing south african
whitefish, such as Cape hake (*Merluccius capensis/paradoxus*) and
Kingklip (*Genypterus capensis*) as well as others, are low risk
foods.

It may therefore be argued, that minimum specifications for
aerobic bacteria regarding uncooked, fresh or frozen seafoods are
irrelevant in respect of the safety of consumers. Instead, their
presence in large numbers (> 5log cfu/g) may serve to protect the
consumer. Most of the legislation surrounding seafoods today,
refer to safety of the consumer (FDA, 1986, 1994; ICMSF, 1986;
BRODSKY, 1991; BROWN, 1991; MOIR, 1991; MOSSEL and VAN NETTEN,
1991; MILLER JONES, 1992; CODEX ALIMENTARIUS, 1993; BONNELL,
1994; MORTIMORE and WALLACE, 1994; TAYLOR, 1994; VAN SCHOTHORST,
1994) and standards regarding total viable numbers of bacteria on
these foods are therefore superfluous. Thus, these standards are
of importance to the industry and consumers only in terms of
shelf life or freshness, provided that the specific target
organisms and methods typical to the raw material process and
product are used for the enumerations.

Current international standards for uncooked, fresh or frozen,
deepsea whitefish, target mesophilic bacteria, by determining
aerobic bacterial counts incubated at 30 - 35°C on PCA (SABS,
1977; FDA 1986, 1994; EEC, 1991, 1993; CODEX ALIMENTARIUS, 1993).
These bacteria are not endogenous to deepsea whitefish and do not
play a role in spoilage under refrigerated storage conditions
(GEORGALA, 1957; SHEWAN 1961, 1977; HOBBS and HODGKISS, 1982;
SIMMONDS and LAMPRECHT 1985, VENNEMANN 1991). Standards using the
latter assessment method are therefore inferior in terms of
gauging bacterial quality, shelf life and GMP for uncooked, fresh
or frozen deepsea whitefish products. It is therefore motivated
that the current national and international standard method,

regarding total viable bacterial counts from uncooked, fresh or
frozen, marine, deepsea whitefish incubated on PCA at 30 - 35°C
for 48h testing for total aerobic, mesophilic bacteria, is
changed to a standard method testing for aerobic, marine,
psychrotrophic bacteria incubated on SWA at 20°C for 48h.

2. Application of the hurdle concept to extend shelf life

2.1 Radurization

Organoleptic characteristics and bacterial counts, indicated that
radurization was only effective in extending shelf life at 0.5
KGy and under refrigerated conditions (<5°C). This indicated that
multiple hurdles such as radurization and storage at low
temperature are more effective in the extension of shelf life
than a single hurdle (radurization followed by higher temperature
storage). *Pseudomonas* predominance was only slightly reduced
after radurization at 0.5 KGy. The 0.75 and the 1.0 KGy
radurization eliminated *Pseudomonas* and *Moraxella* predominated
spoilage. Bacterial counts exceeded the spoilage limit without
off odour production. Off odours were a result of "tainted"
radurization odours and flavours. Tainting of the fish at higher
intensity radurization remains an obstacle in using radurization
as an hurdle for extending the shelf life of uncooked, fresh Cape
hake products. It was therefore concluded that gamma irradiation
was not an option for the extension of the shelf life of fresh
Cape hake fillets.

Bacterial counts indicated increases after radurization from
samples incubated at 20°C on SWA. The characterization of the
isolates from the SWA plates indicated species normally
associated with marine whitefish. This highlighted the
suitability of marine psychrotrophic bacterial counts incubated
at 20°C on SWA for determining shelf life of marine whitefish
products. This was further supported by the increase in aerobic,

marine bacterial counts, which compared well with organoleptic findings.

2.2 Temperature

At storage temperatures of 5 - 7°C, Hake and Kinklip steaks had a shelf life of 2 - 3d. However, at 0 - 2^{O}C, the same products had a shelf life of 6d. The Haddock steaks showed a similar pattern, with a further day added to the organoleptic and "microbiological" shelf life, confirming the inhibitory nature of brining and smoking to bacterial growth. Storage temperature was the most effective hurdle for controlling growth of spoilage bacteria and one of the most important control points for the preservation of quality of refrigerated whitefish products (LUPIN *et al.*, 1980; SHIPMAN and WYLER, 1989; LORD, 1990; ANON., 1992).

2.3 Chemical preservatives

Organoleptic characteristics did not indicate shelf life extensions in the samples treated with chemical preservatives. This was consistent with bacteriological counts (Fig. 28). Results also indicated that all fillets became unacceptable at a time when bacterial counts reached and exceeded the spoilage limit. The relationship between organoleptic and bacterial results confirmed the suitability of aerobic, psychrotrophic marine bacteria counts incubated on SWA at 20°C as a technique to determine the shelf life of Cape hake fillets exposed to different shelf life extending treatments.

Labelling regulations (SABS, 1977; FDA, 1986; EEC, 1991) today stipulate that all added preservatives must be declared on the product label. From the perspective of a consumer buying <u>fresh fish</u>, it is difficult to associate chemically preserved fish with a fresh or unprocessed image. As in most fresh foods today, the marketing trend is away from chemically preserved foods. This is

especially valid for basic staple foods such as protein, vegetables, fruit, bread and milk (JAY, 1986; MILLER JONES, 1992). It is important to recognize this trend, because it places the emphasis on GMP and HACCP for the particular industry in order to produce quality, shelf life and a safe food product. This study, nevertheless, was conducted to determine whether there was economic merit (ie.: a shelf life extension in excess of 10 - 12d) with the use of well known preservatives. Traditional means of preservation such as smoking, drying and salting are perceived by today's consumer to be more "natural" and therefore more acceptable, even though biochemically this might not be true. This is partly the reason why such treatments are still continuing today.

2.4 Packaging

No shelf life extension was gained for either Cape hake or Kingklip fillets when stored in a nonpermeable vacuum pack in chilled storage (4 - 6°C) for 6 to 7 days. From the 3rd day of chilled storage, the bacterial counts, as well as the bacterial community structure on the samples, were consistent with Shewan's (SHEWAN, 1977; VENNEMANN, 1991) spoilage succession pattern for marine whitefish species. Spoilage odours could be detected in the products packed after the 5th day for the Hake fillets and after the 6th day for the Kingklip fillets (Table 7). This meant that vacuum packing using a nonpermeable film to exclude oxygen for Cape hake and Kingklip fillets, was not an effective hurdle to psychrotrophic bacteria under chilled storage conditions.

The fact that the bacterial counts and characterization agreed with Shewan's spoilage succession pattern (1977) confirmed that the use of aerobic marine, psychrotrophic bacteria incubated on SWA at 20°C on fresh and frozen, uncooked deepsea whitefish products is a suitable technique to establish bacterial quality (shelf life) for these products.

Further research is recommended, to investigate the modified
atmosphere packaging (MAP) technique. This is a vacuum drawn
combined with a make up of different gases (CO_2; N_2; O_2)
effective in retarding bacterial growth and spoilage (LAMPRECHT
et al., 1983, 1985).

3. Mossel and van Nettens concept and further work suggested

The application of Mossel and van Nettens concept has during this
study provided an exceptional opportunity to lay down the
foundations for the formulation of a new worldwide standard for
the fishing industry, indeed any bacterial standard for any food
industry may be derived at by utilizing this concept. Industry,
regulating bodies and customers alike are served with bacterial
standards which are relevant and meaningful.

Since this study mainly addressed the aerobic bacterial count of
whitefish products from a temperate, marine environment, further
work is necessary to address the HACCP concept in terms of
customer safety in the distribution and retail/catering
establishments. Since in this environment human contact is
intensified through increased handling and processing (cooking
and preparation), such a study would address the occurrence of
pathogenic bacteria on prepared seafoods.

A relatively new problem, which has arisen with increased human
effluent in the oceans is the impact of human origin pathogenic
virusses on the safety of seafoods particularly those which are
harvested or trawled in proximity of the shore (mussels, oysters,
shrimps, lobsters and crayfish as well as some linefish). This
issue will present further challenges to food microbiology
scientists for investigation.

REFERENCES

ANONYMOUS, 1992. Is our Fish Fit to Eat? From **Consumer Reports**, February 1992 ed.

ALUR, M.D., VENUGOPAL, V., NERKAR, D.P. and NAIR, P.M., 1991 Bacterial Spoilage Profiles to Identify Irradiated Fish. **J. Food Sci.** 56: 332 - 351.

AOAC, **Bacteriological Manual,** 1990. 15th Edition.

AVERY, K.W.J., LAMPRECHT, E.C. and FICK, M., 1987. The Shelf Life Extension of Fresh Hake through Gamma Irradiation. **South African J. Food Sci. & Nutrition** 1: 26 - 31.

BRODSKY, M.H., 1991. Regulatory Roundtable on Microbiological Standards for Foods - Realities and Fallacies. **J. Assoc. Off. Anal. Chem.** 74: 414 - 416

BONNELL, A.D., 1994. In: Quality Assurance in Seafood Processing: A Practical Guide. **Chapman & Hall.**

BROWN, B., 1991. Comments from Health and Welfare Canada. **J. Assoc. Off. Anal. Chem.** 74: 416, 417.

CANN, D.C., WILSON, B., HOBBS, G., SHEWAN, J.M., 1965. Growth and Toxin Production of *Clostridium botulinum* Type E in certain Vacuum Packed Fish. **J. App. Bact.** 28: 431 - 436

CHANDRASEKARAN, M., LAKSHMANAPERUMALSAMY, P. and CHANRAMOHAN, D. 1985. Fish Flesh Agar Medium - a Suitable Experimental Medium for the Detection of Spoilage Bacteria. **Antonie van Leeuwenhoek.** 51: 219 - 225.

CODEX ALIMENTARIUS, 1993. Guidelines for the Application of the Hazard Analysis Critical Control Point (HACCP) System **Codex Alimentarius, (CCFH) Alinorm 93/ 13A-Appendix II.**

CONNELL, J.J., SHEWAN, J.M., 1980. Past, Presence and Future of Fish Science. **Advances in Fish Science & Technology, Fishing News Books, Farnham, England:** 56 - 65.

DYMSZA, H.A., LEE, C.M., SAIBU, L.O., HAUN, J., SILVERMAN, G.J. and JOSEPHSON, E.S., 1990. Gamma Irradiation Effects on Shelf Life and Gel Forming Properties of Washed Red Hake (*Urophycis chuss*) Fish Mince. **J. Food Sci.** 55 : 1745 - 1748

EEC, 1991. European Council Directives **No. 492/493,** Standards
for Food Products Imported from third Countries.

EEC, 1993. European Council Directive for Hygiene on Foodstuffs.
93/43/EEC.

FARKAS, J., 1991. Food Irradiation and Microbiological Safety.
**Institute of Preservation and Lifestock Products Technology
University of Horticulture and Food Industry, Budapest
Hungary:** 1 - 4.

FDA, 1986. Current Good Manufacturing Pratice in Manufacturing,
Processing, Packing and Holding Human Food. **Federal Register**
51: 22475 - 22480.

FDA, 1994. HACCP Models. **Federal Register** 59.

FIELDS, M.L., RICHMOND, B.S., 1968. Food Quality as Determined
by Metabolic By-products of Micro-organisms. **Adv. Food
Research** 16: 161 - 229.

GARRETT, E.S., HUDACK-ROOS, M., 1990. The Use of HACCP in
Seafood Surveillance and Certification. **Food Technol.**
May: 159 - 165.

GARRETT, E.S., HUDACK-ROOS, M., 1991. Microbiology of Marine
Food Processing: 111 - 129.

GEORGALA, D.L. 1957. Quantitative and Qualitative Aspects of the
Skin Flora of North Sea Cod and the Effect thereon of
Processing on Ship and on Shore. **PhD Thesis, Aberdeen
University.**

HANNESSON, G. and DAGBJARTSSON, B. 1972. Radurization of Scampi
Shrimp and Cod. **Isotopes and Radiation Technology.** 9: 468 -
472.

HOBBS, G. and SHEWAN, J.M., 1969. Present Status of Radiation
Preservation of Fish and Fishery Products in Europe. In:
Fishing News Books, Ed. R. Kreuzer, **Freezing and Irradiation
of Fish, Farnham, England:** 488 - 494.

HOBBS, G. and HODGKISS, W. 1982. The Bacteriology of Fish
Handling and Processing. In: **Developments in Food
Microbiology,** Ed. R. Davies, **Applied Science Publishers,
London and New Jersey:** 71 - 117.

HOBBS, G., 1983. Microbial Spoilage in Fish. In: **Food Microbiology: Advances and Prospects,** Ed. T.A. Roberts and F.A. Skinner, **Academic Press, London:** 217 - 229.

HOBBS, G., 1987. Microbiology of Fish. In: **Essays in Agri-cultural and Food Microbiology,** Ed. J.R. Norris and G.L. Pettipher, **John Wiley & Sons, Chichester:** 199 - 226.

HODGKISS, W., 1980. Bacteriology of Raw Materials, Handling and Processing of Fish. In: **Hygienic Design and Operation of Food Plants,** Ed. R. Jowitt, **Ellis Horwood Ltd., Chichester:** 155 - 181.

HORSLEY, R.W., 1977. A Review of the Bacterial Flora of Tele-osts and Elasmobranchs, including Methods for its Analysis. **J. Fish Biol.** 10: 529 - 553.

HUSSAIN, A.M., EHLERMANN, D. and DIEHL, J-F., 1976. Effect of Radurization on Microbial Flora of Vacuum- Packaged Trout *(Salmo gairdneri).* **Archiv fuer Lebensmittelhygiene** 27: 223 - 225.

ICMSF, 1986. Micro-organisms in Food, Vol. 2. **Sampling for Microbiological Analysis** 2nd ed. **Blackwell:** 181 - 193.

JAY, J.M., 1986. In: **Modern Food Microbiology, 3rd ed., Van Nostrand and Reinhold, New York.**

KUMTA, U.S., and MAVINKURVE, S.S., 1971. Radiation Sensitivity and Biochemical Characteristics of Microflora of Bombay Duck *(Harpodon nehereus).* **J. Food Sci.** 36: 63- 66.

LAMPRECHT, E.C., AVERY, K.W.J., HARVEY, R.A. and VERMAAK K., 1983. Preliminary Report on Controlled Atmosphere Packaging and Vacuum Packaging of Hake. **FIRI Annual Report** 37: 33 - 35.

LAMPRECHT, E.C., AVERY, K.W.J., VERMAAK, K., GARRY, D., 1984. Modified Atmosphere Packaging (MAP) and Vacuum Packaging (VP) of Hake Fillets. **FIRI Annual Report** 38: 64 - 67.

LAMPRECHT, E.C., AVERY, K.W.J., HIGGO, D., VERMAAK, K., 1985. Modified Atmosphere Packaging (MAP) of Hake Fillets II. **FIRI Annual Report** 39: 54 - 62.

LEE, J.S. WILLETT, C.L., ROBISON, S.M. and SINNHUBER, R.O., 1967

Comparative Effects of Chlorotetracycline, Freezing and
Gamma radiation on the Microbial Population of Ocean Perch.
Appl. Microbiol. 15: 368 - 372.

LEE, C.S. and PFEIFFER, D.K., 1974. Influence of Recovery Media
and Incubation Temperature on the Types of Micro-organisms
Isolated from Seafoods. **J. Milk Food Technol** 37: 553 - 556.

LISTON, J., 1980. Microbiology in Fishery Science. In: **Advances
in Fish Science & Technology,** Ed. J.J. Connell, **Fishing News
Books, Farnham, England**: 27 - 37.

LISTON, J., 1982. Recent Advances in the Chemistry of Iced Fish
Spoilage. In: **Chemistry and Biochemistry of Marine
Products**, Ed. R.G. Martin, C.J. Flicks, C.E. Bebard and
D. E. Ward, **AVI, Connecticut**: 27 - 37.

LORD, R., 1990. When Good Fish Go Bad. **Seafood Business**
July/August: 87 - 96.

LUPIN, H.M., GIANNINI, D.H. SOULE, C.L. DAVIDOVICH, L.A.
and BOERI, R.L., 1980. Storage Life of Chilled Patagonian
Hake (*Merluccius hubbsi*). **J. Food Technol.** 15: 285 - 300.

MADDEN, J.M., 1994. Concerns regarding the Occurrence of *Listeria
monocytogenes, Campylobacter jejuni* and *Escherichia coli*
Type O157:H7 in Foods regulated by the US Food and Drug
Administration. **Dairy, Food and Env. Sanit.** 14: 262 - 267.

MARTIN, R.E., GRAY, R.J.H., PIERSON, M.D., 1978. Quality
Assessment of Fresh Fish and the Role of the Naturally
Occurring Microflora. **Food Technol.** May: 188 - 198.

MILLER JONES, J., 1992. Food Safety. **Eagan Press, St. Paul
Minnesota.**

MIYAUCHI, D., 1972. Radiation Pasteurization of Pacific Coast
Fishery Products. **Isotopes and Radiation Technol.** 9: 299 -
300.

MOIR, R., 1991. Microbiological Criteria: Minimizing the Risk.
J. Assoc. Off. Anal. Chem. 74: 419 - 420.

MORITA, R.Y., 1975. Psychrophilic Bacteria. **Bacteriol. Rev.**
39: 144 - 167.

MORTIMORE, S. and WALLACE, C., 1994. HACCP - a Practical

Approach. **Chapman & Hall, London**.

MOSSEL, D.A.A., and VAN NETTEN, 1991. Microbiological Reference
    Values for Foods: a European Perspective. **J. Assoc. Off.
    Anal. Chem.** 74: 420 - 432.

NICKELSON, R. and FINNE, G., 1984. Fish, Crustaceans and
    Precooked Seafoods. In: **Compendium of Methods for the
    Micobiological Examination of Foods, APHA.** Ed. M.L. Speck:
    573 - 589.

NIVEN, C.R., JEFFREY, M.B. and CORLETT, D.A., 1981. Differential
    Plating Medium for Quantitative Detection of Histamine-Pro-
    ducing Bacteria. **Appl. and Environmental Microbiology** 41:
    321 - 322.

OLLEY, J. and RATKOWSKY, D.A.,1973. Temperature Function
    Integration and its Importance in the Storage and
    Distribution of Flesh Foods above the Freezing Point.
    Food Technol. in Australia, AIFST/NZIFST Convention Paper:
    66 - 73.

PIERSON, M.D., and CORLETT, D.A., 1992. HACCP - Principles and
    Applications. **van Nostrand Reinhold, New York**.

RHODEHAMEL, E.J., 1992. Overview of Biological, Chemical and
    Physical Hazards. In: **HACCP - Principles and Applications**
    Ed. M.D. Pierson and D.A. Corlett, **van Nostrand Reinhold,
    New York:** 8 - 29.

SABS, 1973. Document No. 35, 1973 Standard Specification on
    South African Rock Lobster Processing.

SABS, 1977. Document No. 585, Standard Specification for the
    Processing of Marine Fish, Crustacea and Molluscs.

SABS, 1987. Document No. 326, Standard Specification for the
    Processing of Shrimps.

SHEWAN, J.M., 1961. The Microbiology of Sea Water Fish. In: **Fish
    as Food,** Ed. G. Borgstrom, **Academic Press, London:** 487 -
    560.

SHEWAN, J.M. and HOBBS, G., 1963. Prepacking Unfrozen Fish.
    Fishing News Int. 2: 103 - 105.

SHEWAN, J.M. and HOBBS, G., 1967. The Bacteriology of Fish

Spoilage and Preservation. **Prog. Indust. Microbiol.** <u>6</u>: 169 - 208.

SHEWAN, J.M., 1977. The Bacteriology of Fresh and Spoiling Fish and the Biochemical Changes Induced by Bacterial Action. In: **Handling, Processing & Marketing of Tropical Fish. tropicl Products Institute, London:** 51 - 66.

SHEWAN, J.M. and MURRAY, C.K., 1979. The Microbiological Spoilage of Fish with Special Reference to Psychrophiles. In: **Cold Tolerant Microbes and the Environment,** Eds. A.D. Russel and R. Fuller, **Academic Press, London.** pp: 117 - 136.

SHIPMAN, D.L. and WYLER, R.L., 1989. Fish Market Guidelines tested in Retail Food Stores. **dairy, food and Env. Sanit.** <u>9</u>: 16 - 20.

SIMIDU, U. and HASUO, K., 1968(a). Salt Dependancy of the Bacterial Flora of Marine Fish. **J. Gen. Microbiol.** <u>52</u>: 347 - 354.

SIMIDU, U. and HASUO, K., 1968(b). An Improved Medium for the Isolation of Bacteria from Marine Fish. **J. Gen. Microbiol.** <u>52</u>: 355 - 360.

SIMMONDS, C.K. and LAMPRECHT, E.C., 1980. Advances in Fish Science and Technology. **Fishing News Books, London.** Ed. J.J. Connell: 297 - 299.

SIMMONDS, C.K., LAMPRECHT, E.C., SEAMAN, P.D., 1981. Preservation of Chilled Hake. **FIRI Annual Report No.** <u>35</u>: 73 - 76.

SIMONDS, C.K. and LAMPRECHT, E.C., 1985. Microbiology of Frozen Fish and Related Products. In: **Microbiol. of Frozen Foods** Ed. R.K. Robinson, **Elsevier Appl. Sci. Publishers, London:** 169 - 208.

SIMMONDS, C.K. and WESSELS, J.P.H., 1986. Irradiation of Hake. **FIRI Technical Report No.** <u>1</u> University of Cape Town, South Africa: 77 - 79.

STRATTON, J.E. and TAYLOR, S.L., 1991. Scombroid Poisoning. In: **Microbiology of Marine Food Products,** ED. D.R. Ward and C.R. Hackney. **Van Nostrand Reinhold, New York:** 331 - 351.

TAYLOR, M.R., 1994. FDA's Plans for Food Safety and HACCP -

Institutionalizing a Philosophy of Prevention. **Dairy, Food and Env. Sanit.** 14: 256 - 258.

TIWARI, N.P. and MAXCY, R.B., 1972. *Moraxella - Acinetobacter* as Contaminants of Beef and Occurrence in Radurized Products **J. Food Sci.** 37: 901 - 903.

VAN DER WATT, H.H. and VAN DER MERWE, G., 1988. Die Rakleef-tydverlenging van Reenboogforel (*Salmo gairdneri*) deur radurisering. **Proceedings of the 9th Biennial SAAFoST Congress, Cape Town, 1987;** Supplement to **Food Review** February/March: 20 - 29.

VAN DER ZANT, C. and SPLITTSTOESSER, D.F.,1992. Compendium of Methods for the Microbiological Examination of Foods.

VAN SCHOTHORST, M., 1994. Microbiological Safety of Foods in the Europe of the Nineties. What does that Imply? **Dairy, Food and Env. Sanit.** 14: 473 - 476.

VENNEMANN, I.H., 1991. Microbial Ecology of *Merluccius capensis, Merluccius paradoxus* (Cape Hake) Processing. **An MSc Thesis, Department of Microbiology and Plant Pathology University of Pretoria, South Africa.**

WARD, D.R. and HACKNEY, C.R., 1991. Microbiology of Marine Food Products. **Van Nostrand Reinhold, New York.**

WESSELS, J.P.H., LAMPRECHT, E.C., RODRIGUES, J. de R. and SIMMONDS, C.K., 1972. Preservation of Hake in Treated Ice. **J. Food Tech.** 7: 303 - 307.

ZOBELL, C.E., 1941. Studies on Marine Bacteria. I. The Cultural Requirements of Heterotrophic Aerobes. **J. Mar. Res.** 4: 42.

ZOBELL, C.E., 1946. Marine Microbiology. **Chronica Botanica, Waltham, Massachusetts:** 240.