

**DEVELOPMENT OF VALUE-ADDED PRODUCTS USING THE  
NECK FLESH OF CAPE HAKE (*MERLUCCIUS CAPENSIS*)**

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### Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

## SUMMARY

The focus of this study was the development of food products produced from the neck flesh of Cape hake (*Merluccius capensis*) as a means of adding value to hake heads. The product prototypes that have been developed include curried fish chowder (packaged in stand-up pouches), fish spread (packaged in plastic casings) and Chakalaka hake (packaged in both cans and retortable pouches). A formula for fish stock, which was used as a base ingredient in the three product prototypes, has also been standardised. Shelf life testing was regarded an essential part of the development processes for the refrigerated product prototypes i.e. the curried fish chowder and the fish spread. Shelf life determinations involved microbiological testing based on set microbiological standards as well as sensory monitoring and pH testing. Proximate chemical- and mineral analyses were performed on freeze-dried samples of the developed product prototypes.

The efficiency of the antimicrobial peptides enterocin 1071A and 1071B, as biological preservatives, versus that of conventional artificial preservatives was evaluated in the fish spread prototype. Three batches of fish spread were prepared: one containing the enterocin crude extract; the second a combination of sodium benzoate and potassium sorbate, while the third batch containing no preservatives served as the control. Microbiological- and histamine tests coupled with organoleptic monitoring and pH testing were carried out over a 21-day period. It was concluded that although enterocins 1071A and 1071B had some preserving effect in the fish spread, the artificial preservative combination was the superior preserving agent. However, neither the biological preservatives nor the artificial preservative combination succeeded in providing a satisfactory shelf life. The preserving agents used in this study were however not necessarily included at optimum levels and higher levels could possibly lead to an improved shelf life.

The influence of two processing temperatures [121°C (249.8°F) and 116°C (240.8°F)] and two food container types (retortable pouch and can) on the sensory quality characteristics of the Chakalaka hake-prototype was investigated. The results indicated that the shorter processing time obtained with a higher processing temperature resulted in a product of better sensory quality. The sensory quality characteristics of Chakalaka hake processed in cans at 121°C were closest to that considered desirable for the product.

## OPSOMMING

Die fokus van hierdie studie was die ontwikkeling van voedselprodukte geproduseer uit die nekvleis van stokvis (*Merluccius capensis*) met die doel om waarde by stokviskoppe te voeg. Die volgende produk-prototipes is ontwikkel: 'n dik vissop met 'n kerriegeur (verpak in regopstaande sakkies), 'n vissmeer (verpak in plastiekomhulsels) en Chakalaka hake (verpak beide in blikkies en retortbestande sakkies). 'n Formule vir visafstreksel, wat as 'n basis-bestanddeel in die drie produk-prototipes gebruik is, is ook gestandaardiseer. Rakleeftydstudies het 'n belangrike deel van die ontwikkelingsprosesse van die verkoelde produk-prototipes, d.i. die dik vissop en die vissmeer, uitgemaak. Rakleeftydbepalings het mikrobiologiese toetsing, gebaseer op vasgestelde mikrobiologiese standarde, sowel as die monitor van sensoriese eienskappe en pH metings behels. Proksimale chemiese- en mineraal analises is uitgevoer op gevriesdroogde monsters van die ontwikkelde produk-prototipes.

Die effektiwiteit van die antimikrobiese peptiede enterosien 1071A en 1071B, as biologiese preserveermiddels, is ondersoek in vergelyking met dit van konvensionele kunsmatige preserveermiddels in die vissmeer-prototipe. Drie mengsels vissmeer is berei waarvan die eerste enterosien kru-ekstrak bevat het; die tweede 'n kombinasie van natrium bensoaat en kalium sorbaat, terwyl 'n derde mengsel geen preserveermiddels bevat het nie en gedien het as kontrole. Mikrobiologiese- en histamien toetse is gelyklopend met organoleptiese monitering en pH metings oor 'n tydperk van 21 dae op monsters van die drie vissmeermengsels uitgevoer. Die ondersoek het getoon dat enterosien 1071A en 1071B wel 'n mate van bederfwing in die vissmeer meegebring het, maar dat die kunsmatige preserveermiddelkombinasie 'n beter preserveringseffek gehad het in die produk. Nie die biologiese preserveermiddels óf die kunsmatige preserveermiddelkombinasie kon 'n bevredigende rakleeftyd teweegbring nie. Die preserveermiddels in hierdie studie is egter nie noodwendig in optimale hoeveelhede gebruik nie en hoër vlakke kan moontlik tot 'n verbeterde rakleeftyd lei.

Die effek van twee prosesseringstemperature [121°C (249.8°F) en 116°C (240.8°F)], sowel as twee verpakkingsstypes (retortbestande sakkie en blik), op die sensoriese kwaliteitseienskappe van die 'Chakalaka hake'-prototipe is ondersoek. Die resultate het aangedui dat die korter prosesseringstyd verkry met 'n hoër prosesseringstemperatuur, geleei het tot 'n produk van beter sensoriese gehalte. Die sensoriese kwaliteitseienskappe van 'Chakalaka hake' geprosesseer in blikke by 121°C was die naaste aan dit wat beskou word as gewens vir die produk.

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## CONTENTS

1. INTRODUCTION	
1.1 Motivation for the study	1
1.2 References	1
2. LITERATURE REVIEW	
2.1 Total utilisation of seafood raw material	2
2.2 Product development	2
2.2.1 The product development process	2
2.2.2 Requirements for new food products	2
2.2.3 Nutritional value of food products and legislation regarding nutritional claims	3
2.3 Sensory evaluation of food products	3
2.3.1 Types of sensory evaluation	3
2.3.2 Tests used in analytical sensory evaluation	4
2.3.3 Execution of an analytical sensory evaluation study	4
2.4 Composition of fish flesh	5
2.4.1 Fish muscle proteins	5
2.4.2 Lipid content of fish	5
2.4.3 Vitamins and minerals in fish	6
2.4.4 Basic cooking properties of fish	6
2.5 Shelf life of food products	6
2.5.1 Mechanisms of deterioration	6
2.5.2 Factors influencing shelf life	7
2.5.3 Role of packaging in shelf life	9
2.5.4 Legislation relating to shelf life	10
2.5.5 Shelf life testing	10
2.5.6 Application of bacteriocins as biological preservatives in food	11
2.6 Thermal processing of foods with the focus on canning	12
2.6.1 Thermal death curves	12
2.6.2 Process determination	13
2.6.3 Containers for canned food: retort pouches as alternative to cans	16
2.7 References	17

3. THE DEVELOPMENT OF VALUE-ADDED FOOD PRODUCTS USING THE NECK FLESH OF CAPE HAKE <i>(MERLUCCIUS CAPENSIS)</i>	21
3.1 Introduction	21
3.2 Raw material	21
3.3 Formula development	21
3.3.1 Fish stock	21
3.3.2 Fish spread	22
3.3.3 Curried fish chowder	23
3.3.4 Chakalaka hake	24
3.4 Shelf life determinations for the refrigerated product prototypes	28
3.4.1 Shelf life determination for the fish spread	29
3.4.2 Shelf life determination for the fish chowder	30
3.5 Proximate and mineral analyses performed on the product prototypes	32
3.6 References	33
4. Characteristics of Chakalaka hake retort-sterilised at two processing temperatures and using two different food container types	39
5. Preservation of fish spread with enterocins 1071A and 1071B, two antimicrobial peptides produced by <i>Enterococcus faecalis</i> BFE 1071	50
6. CONCLUSIONS	57

## 1. INTRODUCTION

### 1.1 Motivation for the study

Cape hake (*Merluccius capensis*) together with pilchards, anchovies and horse mackerel, are the main fish resources in southern Africa and are mainly caught off the West Coast. Supplies of hake and pilchards are assumed to increase in future (Jakobsen, 1997). The fishing industry currently regards hake heads as waste that takes up valuable cold storage space on board the trawlers. The traditional method for disposing of fishery wastes has been to dump it over the shipboard railing into the open sea (Green & Mattick, 1979). The Fisheries Policy Development Committee concerned with environmental issues has however rendered this practice illegal (Fisheries Policy Development Committee, 1996). In addition to this, economic considerations also clearly point to the growing emphasis on improving the total utilisation of seafood raw material. Only utilising the most desirable portion of fish, namely the fillets, which often constitute as little as 20% of the fish, is therefore something of the past (Piggot, 2000).

The Fish Waste Utilisation Program (FWUP) is a project that supports the trend towards total utilisation of raw material by finding different ways for utilising hake heads effectively. Unprocessed, the hake heads are not of substantial monetary worth. Smaller fishing vessels only bring these ashore at the end of less successful fishing trips when storage space have not been filled with dressed (i.e. headed and gutted) fish or fillets. The neck flesh is said to constitute approximately 16 percent of the hake head. The exact percentage of neck flesh, obtained with a standardised cut, will be determined in a study running parallel to the study being reported. If value can be added to the neck flesh by using it as raw material for the production of food products, fishermen may be motivated to obey legislation and bring the hake heads ashore. The aim of this study, which forms one branch of the FWUP, therefore was the development of value-added food products produced from the neckflesh of Cape hake.

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## 2. LITERATURE REVIEW

### 2.1 Total utilisation of seafood raw material

In all of the major fish consuming countries of the world, the main function of fish is to provide high quality protein (Potter & Hotchkiss, 1995). Countries like Japan, which for centuries has had to depend on the sea as the source for most of its animal protein, have also invariably made maximum use of their catch (Conell & Hardy, 1982). However, for many years in the Western world, only the most desirable portion of the fish, namely fillets, was used. The remainder, which generally accounts for the greatest part of the fish, was considered to be waste or raw material for the production of animal feeds. Environmental and economic considerations have however led to a growing emphasis on improving the total utilisation of seafood raw material. The modern attitude is that the portions remaining after the initial edible portion had been removed should be considered secondary raw materials (Piggot, 2000). Feasible ways have to be implemented in which to recover and utilise these secondary raw materials. One such method involves mechanical deboning by means of specialised machinery. Filleting wastes, such as frames, trim waste and tail sections, as well as headed and gutted underutilised species of fish, can be mechanically deboned to produce minced fish (Green & Mattick, 1979). This then can be used in a number of products requiring a protein base or binder such as sausages and cased meats (Piggot, 2000). Other methods for recovering and utilising fishery wastes include the production of fishmeal and fish protein concentrate (FPC). Most of the traditional ways of utilising fish waste, however, involve high capital and operational costs and is not economical for small operators. In addition, little technical information and assistance in waste management has been available to the seafood processor compared to other food industries. The answer lies in new approaches to utilisation that involve either low capital and operational costs or the production of value-added products with a higher profit margin (Green & Mattick, 1979).

### 2.2 Product development

Since this study involved the development of value-added food products using the neck flesh of Cape hake, certain important aspects regarding new food product development had to be considered. Some of these aspects are discussed in #2.2.1 to 2.2.3.

#### 2.2.1 *The product development process*

New product development is a risky, costly and time-consuming process and therefore it is wise to adopt an organised procedure for executing its activities. According to Baker, Hahn and Robbins (1988), the stages in product development include the idea stage, followed by development, taste paneling, consumer sampling, shelf life studies, packaging, production, market testing and finally commercialisation of the product. The order of these stages is not fixed and while some stages may be combined, others can even be omitted. The mentioned stages were tailored to suit this specific study and involved development of the chosen food products on an experimental scale.

#### 2.2.2 *Requirements for new food products*

The requirements for a new product can be seen as a combination of the needs of the consumer, the middleman or retailer and the processor (Williams, 1992). These needs are determined through market research and are illustrated in Table 1. Changing lifestyles and dietary habits of the consumer demand that products offer the essential elements of convenience, health, taste and safety (Linden & Lorient, 1999). For these demands to be met, extensive innovation is required from the food industry.

**Table 1: Market needs analysis**

Requirements for a new food product		
Needs of consumer	Needs of outlet (middleman/retailer/restaurant)	Needs of processor (manufacturer)
<b>Product issues:</b>	<b>Product issues:</b>	<b>Product issues:</b>
Convenience	Specifications	Specifications
Health	Yield	Yield
Colour, taste, texture		
Safety		
Remaining storage life		
Price		
Value (price/quality)		
Availability		
<b>Service issues:</b>	<b>Service issues</b>	<b>Service issues</b>
Reliability (of product)	Reliability (of supply)	Reliability (of supply)
Information	Consistency	Consistency
Guarantees	Stability (e.g. price)	Stability (e.g. price)
Tangibles (assurance)	Communication	Communication

Adapted from: Williams, 1992

### 2.2.3 *Nutritional value of food products and legislation regarding nutritional claims*

All humans require food for growth and maintenance of normal health and as such, the nutritional quality of food is an important aspect in the evaluation thereof. As the knowledge of human nutrition increased and the awareness of educated consumers in this regard grew, demands were made by consumers for nutritional information to be included in food product labels. Methods have been developed for the analysis of different aspects of the nutritional value of foods (Singhal, Kulkarni & Rege, 1997). In this study, proximate chemical analyses were carried out for estimating the moisture, ash, protein and fat contents of the developed products, while the contents of certain minerals were determined through appropriate mineral analyses (#3.5).

Consumers who are aware of the nutritional quality of foods may specifically seek out foods that provide certain nutritional benefits or which comply with certain requirements e.g. being low in fat (Singhal, Kulkarni & Rege, 1997). Legislation governing the labelling and advertising of foodstuffs serves to protect the consumer against misleading claims regarding the characteristics of food products and strives to ensure access to correct information. Regulations relating to the Foodstuffs, Cosmetics and Disinfectants Act (no 54 of 1972) requires that any nutritional claim on a food product label should be substantiated by specified data on the nutrient content of the food. This data should be supplied in a prescribed manner. For a nutritional claim to be made, the food product has to comply with certain set conditions. If, for example, a claim is made that a food product is low in fat, the fat content needs to be less than 3g per 100g product (in the case of solids) or less than 1.5g per 100g product (for liquids). The food product should be labelled with a prescribed nutritional information declaration, which includes nutritional information relevant to the low fat-claim (Government Gazette, 2002).

Another crucial aspect in the evaluation of food quality is its sensory acceptability. The main quality characteristics determining the acceptability of food products are appearance, flavour and texture (Bourne, 1990). These sensory characteristics can be measured by means of a scientific method called sensory evaluation (Penfield & Campbell, 1990).

## 2.3 Sensory evaluation of food products

### 2.3.1 Types of sensory evaluation

Two kinds of sensory evaluation, namely consumer and analytical sensory evaluation, are used for the evaluation of food and both can be applied during product development. Consumer sensory evaluation is usually executed towards the end of formula development or –reformulation and makes use of a panel of consumers representative of the target population (Heymann, 1995). The objective is to determine whether the target consumer likes the product, finds it acceptable or prefers it to another product (Lawless & Heymann, 1998). Analytical sensory evaluation, on the other hand, uses a trained panel to study the variations in specific characteristics of food products for the purpose of product profiling (Heymann, 1995).

### 2.3.2 Tests used in analytical sensory evaluation

Two types of tests are employed in analytical sensory evaluation namely discrimination tests and descriptive tests. Discrimination tests are used to determine whether the judges can detect a difference between the quality characteristics of two samples. It includes paired comparison, triangle, duo-trio and ranking tests. Descriptive techniques, on the other hand, include rating (category scaling and ratio scaling/magnitude estimation), descriptive analysis and time-intensity scaling (Heymann, 1995). Descriptive analysis is used when a detailed description of the sensory attributes of a single product or a comparison between different products is required. This study made use of a generic descriptive analysis technique that involved rating of attributes on unstructured line scales for the purpose of evaluating the sensory characteristics of four Chakalaka hake treatments that each used a different combination of container type and processing temperature (#4). Line marking scales are often used by trained panels in descriptive analysis of multiple attributes: the panelist places a mark on a line of specific length (e.g. 100mm) to indicate the intensity of a specified attribute. Verbal definitions or reference standards may be used as end anchors for these rating scales (Lawless & Heymann, 1998). The anchors typically reflect a continuum from low to high intensity (Stone & Sidle, 1985). The rating scales are assumed to be interval scales (Heymann, 1995). An interval scale has equal divisions between the numbers and has an arbitrary zero point, i.e. no claims are made with regard to the ‘absolute’ magnitude of the attribute measured (Stone & Sidle, 1985). Data from rating scales can be analysed by parametric statistical tests such as *t*-tests, analysis of variance, Pearson correlation, regression analysis and others, depending on the purpose of the study (Heymann, 1995).

### 2.3.3 Execution of an analytical sensory evaluation study

The steps in performing analytical sensory evaluation include training of the panel members, determination of panel member consistency and the evaluation of the samples by the panel members (Lawless & Heymann, 1998). During training, the panelists are exposed to the products to be evaluated and the terms (descriptors) and reference standards needed to describe differences between the products are decided upon. The aim of panel training is to calibrate the panelists and to develop panel consistency (Heymann, 1995). The first sessions of product evaluation can serve as a test for panel consistency or reproducibility. The data from these sessions can be analysed to determine the significance of the interaction effects associated with the panelists. Panel members who are not consistent in their evaluation should either be retrained with regard to the relevant descriptors or be eliminated from the evaluation. Standard sensory practices, such as three-digit coding of products and a randomised serving order, should be employed during the evaluation phase of the study (Lawless & Heymann, 1998). Testing conditions need to be standardised so that the responses reflect only the variable being evaluated (Stone & Sidle, 1985).

The development of food products with desirable nutritional and sensory characteristics requires a good basic knowledge of the nature of the raw material to be utilised. In this study, food products had to be developed utilising hake neck flesh as raw material. The possibility of using other forms of fish flesh, such as mince and fillets as raw material, was however also investigated.

## 2.4 Composition of fish flesh

The main chemical constituents of fish are water (66-84%), proteins (15-25%), lipids (0.1-22%), minerals (0.8-2%), a low quantity of carbohydrates (0.3% of glycogen) as well as vitamins. The structure of fish muscle is similar to that of meat, but the myofibrils are shorter (Freeland-Graves & Peckham, 1996). Fish muscle consists of two main muscle types, namely brown/red and white muscle (Hall & Ahmad, 1997). Brown muscle is well developed in oily fish and is rich in lipids, haem pigments such as myoglobin, and mitochondria (Linden & Lorient, 1999). During storage, oxidation of lipids in this muscle type is catalysed by the haem pigments. This results in faster deterioration in odour and colour in brown muscle than in white muscle. White muscle, which is the prevalent muscle type in fish, is rich in glycolytic enzymes (Freeland-Graves & Peckham, 1996).

### 2.4.1 Fish muscle proteins

Fish muscle contains three groups of protein, namely stroma, sarcoplasmic and myofibrillar proteins (Linden & Lorient, 1999). The stroma proteins make up the connective tissue that surrounds the muscle fibres. These proteins include collagen and elastin and comprise 3-5% of the total protein (Hall & Ahmad, 1997). Since collagen in fish muscle is present in lower proportions than in mammal muscle and also gelatinises at lower temperatures, fish muscle is more tender than that of meat. Thermal degradation of the connective tissue causes the flesh to separate into flakes (Freeland-Graves & Peckham, 1996). The sarcoplasmic proteins are water soluble and are found in the cell plasma where they act as enzymes and oxygen carriers. They comprise 18-20% of the total muscle protein. The largest proportion of muscle proteins, i.e. 65-80% of total protein, consists of miofibrillar proteins such as actin and myosin (Hall & Ahmad, 1997). The myosin proportion of miofibrillar fish proteins is more sensitive to proteolysis and heat denaturation than myosin in skeletal muscle of mammals (Linden & Lorient, 1999). The biological value and content of essential amino acids of fish proteins are comparable to that of meat muscle proteins (Potter & Hotchkiss, 1995).

### 2.4.2 Lipid content of fish

Fish can be categorised according to their extremely variable lipid contents. Low fat fish contain less than 2.5% fat, whereas medium fat and high fat fish contain 2.5-5% and more than 5% fat, respectively (Freeland-Graves & Peckham, 1996). The flesh of Cape hake (*Merluccius capensis*) contains an average of 1.4% fat that is predominantly found in the subcutaneous brown muscle. Cape hake can consequently be classified as a low fat or lean fish (Burt, 1974). Lean fish have a less distinct flavour than high fat fish. The distinct flavour of high fat fish is due to flavours that dissolve and develops from the oils. The flesh near the head of a fish has a higher fat content than the white-muscled tail section (Freeland-Graves & Peckham, 1996). It could therefore be expected that the neck flesh section of Cape hake would contain a higher percentage of fat compared to the rest of its flesh. Since fish lipids need to remain liquid at seawater temperatures, it characteristically contains high levels of unsaturated fatty acids (Hawthorn, 1981). This makes fish lipids desirable from a nutritional viewpoint, but also renders it highly susceptible to oxidation and the development of off flavours and rancidity (Potter & Hotchkiss, 1995).

#### **2.4.3 Vitamins and minerals in fish**

The vitamin content of fish varies according to the fat content. Whereas all fatty fish contain vitamins A and D in the flesh, white or lean fish contains very little of these fat-soluble vitamins except in the liver (Freeland-Graves & Peckham, 1996). Fish liver oils are the richest natural source of vitamin A and also contain high concentrations of vitamin D. Fish muscle is a fairly good source of the B-group vitamins, such as thiamin, nicotinic acid, riboflavin and folic acid, but has a negligible vitamin C content. Fish is a good source of important minerals: it can contribute usefully to intakes of calcium, magnesium and phosphorus and in the case of marine fish, contains about one hundred times the level of iodine than meat. With its lower levels of blood, however, fish contains less iron than lean meat (Hawthorn, 1981).

#### **2.4.4 Basic cooking properties of fish**

The relationship between the constituents in the flesh of a specific fish has a marked effect on the texture and therefore on the cooking properties (Linden & Lorient, 1999). As previously mentioned, fish muscle contains a relatively low proportion of collagen that gelatinises at low temperatures, which makes it more tender than meat. Consequently, the objectives of cooking fish is not to tenderise it, but to apply enough heat to make it safe to eat and to alter the texture without over-coagulating and thereby toughening the proteins. Seafood should be heated for the minimum time required for obtaining doneness. Overcooking causes heat degradation of nutrients, oxidation of vitamins and oils, and leaching of water-soluble minerals and proteins. In addition, it results in excessive moisture loss, which causes the flesh to become tough and dry (Piggot, 2000). Doneness can be determined by the end point temperature, for which a minimum of 60°C is usually recommended. At this point, the appearance of the flesh changes from translucent to opaque (Charley & Weaver, 1998). Pathogenic microorganisms are destroyed at relatively low temperatures and seafood is regarded safe to eat if the internal temperature has been raised above 66°C, where it is actually pasteurised (Piggot, 2000).

### **2.5 Shelf life of food products**

The specification and determination of shelf life is an essential part of the new product development process. The proper determination of shelf life marks the beginning of an effective total quality system aimed at ensuring food products that are safe and of consistent quality (IFST, 1993). A thorough understanding of the various factors influencing shelf life and their control is therefore indispensable to the manufacturer and other groups involved in the food chain. Product shelf life can be defined as the period within which the product, when stored under proper conditions, is safe for human consumption and has an acceptable quality to consumers (Kilcast & Subramaniam, 2000). Changes in food that affect quality and which lead to deterioration and eventually spoilage can be caused by a number of mechanisms such as microbial growth, chemical reactions and physical changes.

#### **2.5.1 Mechanisms of deterioration**

Deteriorative changes in food are caused by microorganisms as well as by chemical reactions and physical changes. The aim of food preservation techniques is to prevent or inhibit the changes that lead to deterioration. In protein rich foods such as fish, changes caused by microorganisms are usually most significant, while foods with a high fat content are more susceptible to chemical and biochemical change. Physical changes occur in all food types (Heber, Löndahl, Persson & Rynnel, 2000). The deteriorative changes limiting the shelf life in a composite food product can however be quite different from those limiting the shelf life of the individual components (Kilcast & Subramaniam, 2000). The

mechanisms for food deterioration may interact and the onset of one mechanism can initiate the onset of the others (IFST, 1993).

In principle, all moist foods can support microbial growth which, given suitable conditions, will lead to spoilage or food poisoning. Microorganisms present in food originate either from the raw materials or from contamination (IFST, 1993). Growth of spoilage organisms can usually be readily identified by sensory changes such as development of off odours and flavours, changes in texture and in the case of mould, by visual growth. The growth of pathogenic organisms such as *Salmonella* species will not necessarily be accompanied by such easily detectable sensory changes (Kilcast & Subramaniam, 2000). A number of factors (see #2.5.2) can influence the growth of the microorganisms significant in food deterioration and need to be controlled in order to inhibit microorganism growth (Potter & Hotchkiss, 1995).

Chemical deteriorative changes arise from reactions within the food or from reactions of food components with external reactors such as oxygen (Kilcast & Subramaniam, 2000). One example of a chemical deteriorative change is rancidity development in fat-containing foods that can occur through different reactions such as hydrolytic reactions, oxidative reactions and flavour reversion reactions (IFST, 1993).

The gain or loss of moisture is a major cause of deteriorative physical changes in food (IFST, 1993). Exchange of moisture between a product and its environment or between the different components of a composite product can lead to changes in texture and flavour and can also encourage growth of microorganisms. ‘Freezer burn’ is an example of the result of moisture migration from the surface of frozen foods. Whereas packaging material often plays a vital role in controlling migration of moisture, it can, however, in certain instances also be the cause of physical deteriorative changes. Migration of chemical components from the packaging material, for example, can produce taints. This could be a problem in products with an extended shelf life such as canned foods (Kilcast & Subramaniam, 2000).

### **2.5.2 Factors influencing shelf life**

A number of factors can influence the shelf life of food products and these can be classified as either extrinsic or intrinsic. Extrinsic factors are those factors that the product encounters while moving through the food chain and, amongst others, include temperature control during manufacturing, storage and distribution and the time-temperature profile during processing. Intrinsic factors depend on the type and quality of raw material and the product formulation and include factors such as the pH value and total acidity, water activity ( $a_w$ ), and use of preservatives. Both types of factors can operate in an interactive way and these interactions can either inhibit or stimulate the reactions that limit shelf life (Kilcast & Subramaniam, 2000). The greatest losses to food supply are due to reactions caused by microorganisms (Potter & Hotchkiss, 2000). The emphasis of the following discussion on the mentioned extrinsic and intrinsic factors will therefore be on how these factors relate to microorganism growth.

#### **2.5.2.1 Temperature control during manufacturing, storage and distribution**

The rate of each of the deterioration reactions mentioned in #2.5.1 is influenced by temperature. Temperature control is therefore crucial during all phases of food production (Heber *et al.*, 2000). The rate of chemical reactions generally increases with a rise in temperature. A rare example of a chemical reaction that is in practice accelerated at low temperatures is the oxidation of fats of which the maximum rate occurs at  $-10^{\circ}\text{C}$ . This increased reaction rate is, however, largely due to an increased concentration of reactants effected by the gradual loss of moisture that occurs during freezing (IFST, 1993). The preserving effect of freezing and refrigeration, on the other hand, is well known.

Freezing can reduce the microbial count in a food, but will rarely eliminate all the microorganisms (Heber *et al.*, 2000). Growth of microorganisms below -12°C is unlikely. Between -12 and 3°C, only psychrophyllics will grow (Michener & Elliot, 1969). These are microorganisms not harmful to health, but which are an important cause of spoilage in protein rich foods (Heber *et al.*, 2000). Pathogenic bacteria are not psychophilic and will not grow below freezing point. However, as temperatures rise above 3°C, growth of pathogens and subsequent food hazards is an increasing possibility (Michener & Elliot, 1969).

#### 2.5.2.2 Heat treatment

Thermal processing refers to the heat treatment of food used to inactivate microorganisms and food enzymes so as to accomplish either commercial sterilisation or pasteurisation. Commercial sterilisation is, for example, achieved in the canning industry to preserve ready-to-eat foods for long periods at room temperature and without the use of preservatives. The principles of commercial sterilisation are discussed in #2.6. Pasteurisation, on the other hand, is used to extend the shelf life of refrigerated fresh foods. The heat treatments applied to the refrigerated products in this study, namely the fish spread and curried fish chowder will therefore be classified as pasteurisation processes. Pasteurisation is a relatively mild heat treatment that eliminates selected vegetative microorganisms (especially pathogens) and enzymes. As not all the vegetative and few of the spore forming microorganisms are destroyed, proper chilling of pasteurised products is still required. Since the heat treatment involved is mild, the nutritional content and sensory characteristics of the product is minimally affected. The severity of the heat treatment (and the resultant shelf life) depends on the nature and sensitivity of the product, resistance of the target microorganism or enzyme, pH conditions as well as the method of heating (Teixeira, 2000). Products can receive in-pack pasteurisation (IPP) or can be pasteurised before packaging and then 'clean filled'. The latter method is often used for soups and sauces. Any pasteurisation method should provide a critical minimum heat treatment as defined by thermal death time experiments and heat penetration analyses. The process is influenced by factors such as number of solid particles, initial temperature of the ingredients, heating rate of equipment and the rate of circulation. The cooking procedure may be considered as being part of the pasteurisation process, but care should be taken to prevent contamination in subsequent processing steps. Products distributed in the chilled state should also be sufficiently cooled in a reasonable time after cooking and processing. The United States Food and Drug Administration (FDA), for instance, requires that chilled foods should be cooled to less than 7.2°C within two hours after processing and kept at or below that temperature during shelf storage (Collins-Thompson & Siddle, 2000).

An example of a pasteurisation process for chilled, ready-to-eat products with an expected shelf life of 10 to 14 days will be a minimal heat treatment of 70°C per two-minute hold. This will eliminate  $10^6$  pathogenic *Listeria monocytogenes* per gram of product. A pasteurisation process is usually defined in terms of a P value, which is the time that a product is kept at a certain processing temperature. The typical P value for *L. monocytogenes* is written as  $P_{70^\circ\text{C}} = 2$ . The z value can also be used to describe a pasteurisation process. It indicates the change in temperature needed to obtain a 10-fold difference in the reduction of microorganisms (Collins-Thompson & Siddle, 2000).

#### 2.5.2.3 Intrinsic product properties

The formulation of a product may have a significant effect on the processing required to obtain a specified shelf life for the particular food. A number of factors in the product formulation can aid in increasing the overall keeping quality and subsequently decrease the severity of processing needed to obtain a particular shelf life (Collins-Thompson & Siddle, 2000). In hurdle technology, which is widely practised in the food industry, intrinsic product factors such as pH,

moisture/water activity ( $a_w$ ) and preservatives are used in combination with extrinsic factors such as heat treatment and cold storage to improve microbial stability (Golden & Arroyo-Gallyoun, 1997). Each of these factors can be seen as a hurdle, which contributes additively, or sometimes synergistically, to microbial inhibition when used in combination with other hurdles (Macdonald & Lanier, 1997). Knowledge about the relationships between these factors allows a decrease in the level of application of each factor, and in effect helps to preserve the sensory properties of foods (Marechal, Martínez de Marnanón, Poirier & Gervais, 1999).

The optimum pH range for growth of foodborne microorganisms is between 5 and 8 (Kilcast & Subramaniam, 2000). At a lower pH, the heat sensitivity of microorganisms tends to increase and the required amount of heat to obtain a certain shelf life is reduced. Using acidic ingredients or adding organic acids such as lactic, citric or acetic acid to lower product pH may also improve keeping quality in combination with chilled storage temperatures (Collins-Thompson & Siddle, 2000). Unlike most bacteria, mould and yeast are however adapted to grow at more acidic (pH 2.5) conditions (Erickson, 2000).

Water activity ( $a_w$ ) is defined as the ratio of the vapour pressure of water in a food to the vapour pressure of pure water. Solutes lower the vapour pressure of water and consequently the  $a_w$  (Charley & Weaver, 1998). Most chilled products have an  $a_w$  higher than 0.9. If the  $a_w$  of chilled products could be decreased to below 0.85, this may prevent or delay outgrowth of microorganisms. A more severe initial heat treatment may however be required for products with lower  $a_w$  (Collins-Thompson & Siddle, 2000). Freezing of products results in a decrease in  $a_w$ , which adds to its preservative effect (Golden & Arroyo-Gallyoun, 1997). While bacteria generally prefer moist conditions, mould is more adapted to growth in dry conditions. Yeast can tolerate the low  $a_w$  that is caused by high sugar concentrations (Erickson, 2000).

Preservatives can be included in product formulations to control the growth of certain microorganisms. The growth of yeast and mould in chilled products can for example be controlled by sodium benzoate or potassium sorbate. The latter can also delay outgrowth of lactic acid bacteria and some spore formers. In meat and fish, sodium chloride and nitrates have been used for protection against *Clostridium botulinum* (Collins-Thompson & Siddle, 2000).

### **2.5.3 Role of packaging in shelf life**

Product packaging acts as a barrier against contamination from outside and as such protects the contents against microbiological, physical and chemical changes during storage (Collins-Thompson & Siddle, 2000). An appropriate choice of packaging system and materials will enable achievement of optimum shelf life (IFST, 1993). The optimal packaging type and materials used for a specific product will be chosen based on factors such as product characteristics, processing considerations, shelf life requirement and the overall cost. Product packaging often plays an integral part in the processing stage (Kilcast & Subramaniam, 2000). The packaging will often complement the processing that has been applied to the product in order to achieve the intended shelf life (IFST, 1993). The containers used for canning of food, for example, are responsible for maintaining commercial sterility of the contents after thermal processing and are discussed in #2.6.3. Commonly used packaging materials include paper, different types of polymers or films, aluminium, or combinations of these as laminates. Metal tins and glass bottles are often used in the canning industry (Collins-Thompson & Siddle, 2000).

#### **2.5.4 Legislation relating to shelf life**

The increasing demand of consumers for consistent high food quality is mirrored in the labelling requirements to which food manufacturers should conform (Kilcast & Subramaniam, 2000). South African labelling legislation requires that an estimated date of durability should be indicated on the label or container of a food product and this is regarded as the responsibility of the manufacturer. The date of durability should be either a ‘Use by’, or a ‘Best before’, or a ‘Sell by’ date (Government Gazette, 2002). The form of date coding used usually relates to the total life of the product. For microbiologically highly perishable food products, a ‘Use by’ date will for example be appropriate, whereas a ‘Best before’ date will be used for other foods, including foods with a shelf life exceeding 18 months (Kilcast & Subramaniam, 2000). A foodstuff may not be sold after the Sell by date has expired. Certain foodstuffs such as fresh unprocessed meat or fish are exempted from a date of durability (Department of Health, 2002). Legislation also protects public safety by means of microbiological standards that are specifically set for a particular type of food (Government Gazette, 1997).

#### **2.5.5 Shelf life testing**

Determination of the shelf life of a new food product is the responsibility of the manufacturer and is routine procedure during product development (IFST, 1993). When shelf life determinations are made during product development, the samples tested should be packaged in a realistic manner, using the same materials likely to be used eventually for the actual packaging (Hersom, 1972). For an existing product, a change in the raw material, formulation, processing or packaging may necessitate re-determination of the shelf life. The objective of any shelf life study is the determination of the endpoint i.e. the point in time when microbiological, chemical, physical or sensory changes have caused the product to fall outside specification (IFST, 1993). Product criteria that are based on the measured numbers of spoilage and pathogenic microorganisms can be defined relatively clearly. Non-microbiological specifications such as sensory criteria are, however, more difficult to define (Kilcast & Subramaniam, 2000).

##### **2.5.5.1 Shelf life tests**

The tests used during shelf life determination are often product specific and are specifically chosen to measure the endpoint in shelf life. These may include sensory evaluation, microbiological testing, chemical as well as physical measurements. Sensory evaluation techniques can be used to measure the changes in sensory qualities such as appearance, odour, flavour and texture throughout storage. Taste tests should however not be included in the evaluation unless the microbiological safety of samples has been assured (IFST, 1993). Microbiological testing entails the enumeration of spoilage microorganisms and/or the detection and identification of indicator organisms and pathogens at set intervals over the shelf life trial period. The time when the microbial count (total count or level of individual organisms) has reached a pre-determined level will be considered to be the end point. Since a built-in ‘safety margin’ should ideally be allowed for when the shelf life of the product is set, the shelf life of the product is usually taken as 70% of the time to spoilage (Kilcast & Subramaniam, 2000). Consequently, if a target shelf life has been requested, the duration of the storage trial should exceed beyond this to enable estimation of a margin of safety (IFST, 1993). Challenge tests may be incorporated in certain shelf life studies. A challenge test involves the deliberate inoculation of a particular food with specific microorganisms to determine the ability of the food to inhibit the growth of the microorganisms in question (Collins-Thompson & Siddle, 2000). Chemical tests can be used to determine the end points of chemical deteriorative reactions in food and to confirm results obtained with sensory testing. Measurement of free fatty acid content and peroxide value can for example indicate the level of rancidity in a product, which can in turn explain the presence of off flavours detected by a sensory panel. Physical tests are most commonly employed to

measure textural changes in food such as loss of crispness. Results from these tests are also usually correlated with the results from sensory testing (Kilcast & Subramaniam, 2000). Specialised instruments are available for the measurement of specific textural attributes, whereas a universal testing machine fitted with the appropriate devices can be used to measure a number of textural parameters (Potter & Hotchkiss, 1996)

#### *2.5.5.2 Indirect methods for determining shelf life: accelerated shelf life testing and predictive modeling*

Food manufacturers are expected to introduce their newly developed products into retail outlets with minimal delay. In the case of long shelf life products, shelf life determinations for the purpose of date marking can however lead to unacceptable delays, as it requires knowledge of the storage characteristics over the intended shelf life period. To circumvent this problem, indirect methods for determining shelf life have been developed, and these are generally based on the principles of accelerated shelf life procedures. Such procedures can only be used if a known and validated relationship exists between the storage properties of the product under an ambient storage condition and under an accelerated condition (Kilcast & Subramaniam, 2000). A common procedure of accelerated shelf life determination is to increase the rate of deteriorative changes by using elevated storage temperatures (IFST, 1993). Finding ways of predicting the rates of deteriorative change resulting from different combinations of extrinsic and intrinsic factors has long been desired by the food industry. With the increasing capabilities of personal computers in recent years, the development of predictive models, particularly for microbiological behaviour, has become an active area of research. Development of predictive models focuses on finding mathematical and statistical relationships between the implicit characteristics of a microorganism and how it behaves in the presence of different combinations of intrinsic (environmental) factors and extrinsic (product related) factors (Kilcast & Subramaniam, 2000). These models can be used to predict the likely change in microbial numbers (growth, death and/or survival) during storage provided that the relevant intrinsic and extrinsic properties of the food product is known (IFST, 1993).

#### *2.5.6 Application of bacteriocins as biological preservatives in food*

Bacteriocins are antimicrobial proteins and peptides that are produced by a number of gram-positive and gram-negative bacteria (Balla, Dicks, Du Toit, Van der Merwe & Holzapfel, 2000). These proteins display a relatively narrow spectrum of activity and are generally only active against bacteria closely related to the producer strain. The producer strain is usually immune to its own bacteriocin. Of the gram-positive bacteria, lactic acid bacteria are known to produce a wide variety of antimicrobial proteins.

Since consumer preferences are turning towards foods that are free from chemical preservatives, increased interest is focused on the use of microbial metabolites such as bacteriocins to inhibit the growth of spoilage and pathogenic microorganisms in food (Ross, Morgan & Hill, 2002). A few of these metabolites (e.g. nisin and pediocin) have already found practical application in food preservation worldwide. Since many bacteriocins are heat stable, they can be used in combination with heat treatment. In addition they are biodegradable, food stable, digestible, safe to health and active at low concentrations (De Vuyst & Vandamme, 1994). Enterocins 1071A and 1071B are two recently isolated bacteriocin-like peptides produced by *Enterococcus faecalis* BFE 1071. These two peptides are always isolated together. A report on the isolation and characterisation of enterocins 1071A and 1071B included the results of an investigation into their inhibitory activity. Enterocins 1071A and 1071B were found to be active against all the strains of *Enterococcus* spp. tested in that study and against six other gram-positive microorganisms (Balla *et al.*, 2000). In this study, the preservative effect of a crude extract containing enterocins 1071A and 1071B was investigated in fish spread and its efficacy compared to that of artificial preservatives.

## 2.6 Thermal processing of foods with the focus on canning

The use of heat to preserve food finds application in various commercially performed processes such as blanching, pasteurisation and canning to achieve different degrees of food preservation (Potter & Hotchkiss, 1995). For canned foods, the objective of thermal processing is to produce a condition of commercial sterility (Lopez, 1987a). This means that the time-temperature combination used in heating is sufficient to achieve a degree of preservation at which all pathogenic and toxin producing organisms have been destroyed, as well as those organisms which could grow and cause spoilage in the product under normal non-refrigerated conditions of handling and storage. As a result, commercially sterilised foods have a shelf life of two years or more (Potter & Hotchkiss, 1995). Principles underlying the destruction of microorganisms can be illustrated by means of thermal death curves (see Figures 1 & 2), which are discussed in #2.6.1.

### 2.6.1 Thermal Death Curves

Under constant heating conditions the same percentage of microorganisms in a bacterial population will be destroyed in a given time interval, regardless of the initial numbers present. This is known as the logarithmic order of death, which can be illustrated by means of a thermal death rate curve (Figure 1).

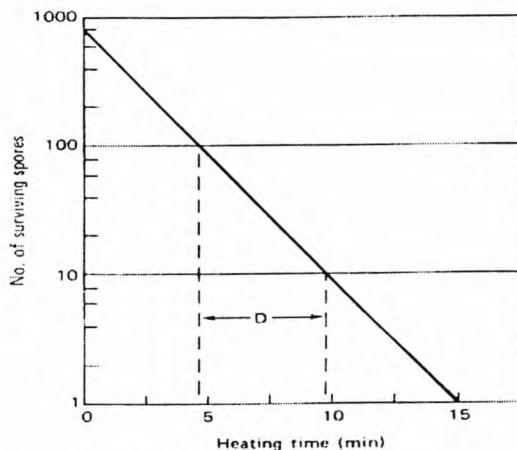


Figure 1: Thermal death rate curve (Fellows, 1988)

The latter provides data on the rate of destruction of a specific organism, in a specific medium and at a specific temperature. An important implication that arises from the logarithmic order of death is that the larger the initial microbial population, the greater the heat treatment required for its destruction (Potter & Hotchkiss, 1995). The time (in minutes) required to destroy 90% of the microorganisms in a population at a given temperature is termed the decimal reduction time or D value (Potter & Hotchkiss, 1995). In thermal death time studies on spore suspensions, the D values of a microorganism determined from the thermal death rate curves at different temperatures can be used to construct a thermal death time (TDT) curve (Figure 2).

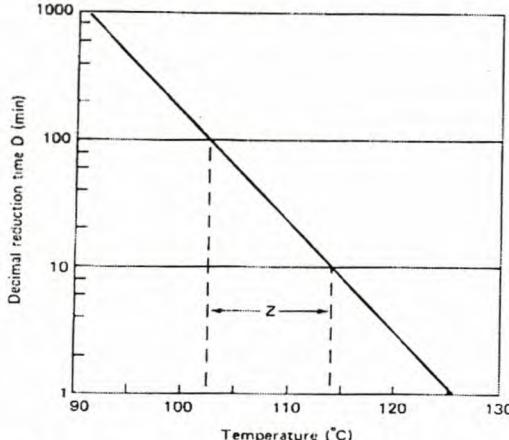


Figure 2: Thermal death time curve (Fellows, 1988)

A TDT curve for a specific microorganism in a specific medium therefore gives data on the destruction times for a given population of that microorganism. TDT curves have been determined for many important pathogens and spoilage organisms (Potter & Hotchkiss, 1995). The slope of the TDT curve is referred to as the z value and is defined as the number of degrees Celsius required to effect a ten-fold change in the D value. The D values and z values give an indication of the heat resistance of a microorganism. Knowledge of the heat resistance of the microorganisms and enzymes associated with a specific food is needed to calculate the thermal process required for their destruction (Fellows, 1988). Calculation of a safe thermal process for canned food is discussed in #2.6.2.

### 2.6.2 Process determination

Data on the heat resistance of significant microorganisms combined with heat penetration data can be employed to calculate a safe heat process for a particular canned food product. The heat resistance of microorganisms and factors that influence the rate of heat penetration into and throughout a food will be discussed in #2.6.2.1 and #2.6.2.2, respectively.

#### 2.6.2.1 Heat resistance of microorganisms

The heat resistance of different microorganisms, and especially of spores, varies widely (Azizi, 1999). Bacterial spores are more heat resistant than vegetative cells. A number of factors may influence the heat resistance of microorganisms when heated in a particular food product. Two such factors are the pH value and the composition of the food product. A lower pH value, i.e. a higher acid intensity, will decrease the heat resistance of microorganisms at a given temperature (Lopez, 1987a). Certain food constituents can protect microorganisms against heat. Sugar in high concentrations, proteins, starch and especially fats can increase the heat resistance of microorganisms (Teixeira, 2000). The great protective effect of fats can be ascribed to their interference with the penetration of moist heat. Moist heat has a greater lethal effect than dry heat at a given temperature. This is due to moisture being a good conductor of heat as it penetrates into microbial cells and spores. If microorganisms are trapped within fat globules, moisture cannot readily penetrate into the cells and the heating becomes more like dry heat (Potter & Hotchkiss, 1995).

The most heat resistant microorganism or enzyme likely to be in a food is used as a base for determining the processing conditions (Fellows, 1988). *Clostridium botulinum* is the most heat resistant pathogen found in foods, especially canned foods in which conditions are anaerobic. This bacterium produces an exotoxin that is the most potent neuroparalytic toxin known (Potter & Hotchkiss, 1995). *C. botulinum* can however not grow at pH levels of less than 4.6 (Teixeira, 2000). This forms the basis on which foods are classified for the purpose of thermal processing as either

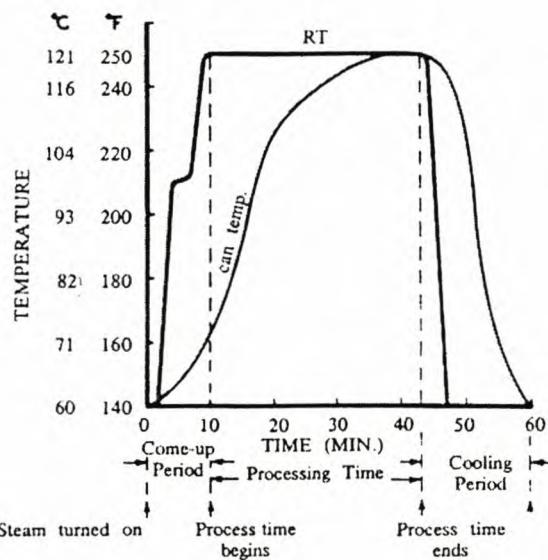
'low acid' (pH above 4.6) or 'high acid' (pH of 4.6 or less). Only the low acid category is of importance with regard to botulism hazard (Lopez, 1987a). High acid foods consequently need milder heat processing compared to low acid foods to ensure commercial sterility (Ababouch, 1999). Thermal processes of low acid canned foods are designed to ensure destruction of *C. botulinum* by using the 12D concept, where D is the decimal reduction time or D value (see #2.6.1). The 12D concept or 'botulinum cook' will accomplish the reduction of any population of the most resistant *C. botulinum* spores to  $10^{-12}$  of its initial count, which represents a probability of survival sufficiently remote to present no significant health risk to consumers (Ababouch, 1999). The destruction of *C. botulinum* is a minimum requirement of thermal processing (Fellows, 1988). Canned foods are in fact processed beyond the minimum botulinum cook because of the occurrence of non-pathogenic, thermophilic spore formers of greater heat resistance than *C. botulinum*. Two such thermophiles capable of spoilage in canned foods are *C. thermosaccharolyticum* and *Bacillus stearothermophilus*. Overprocessing should however be avoided since this will necessarily lead to compromised food quality. Therefore, the accepted rate of survival for the most heat resistant thermophiles is  $10^{-2}$  or  $10^{-3}$ , effected by 2D or 3D processes respectively. This higher risk of survival for thermophiles is regarded as acceptable since these microorganisms have no consequence on health and survivors will remain dormant if retorted cans are rapidly cooled and stored below 35°C. Proper cooling and acceptable handling and storage conditions should therefore be part of the quality assurance system of any canning facility to eliminate conditions that are conducive to the growth of heat resistant microorganisms (Ababouch, 1999).

#### 2.6.2.2 Heat penetration

A heat treatment should ensure that every particle of food within a food container receives sufficient heat for a sufficient length of time to ensure destruction of significant microorganisms. Several factors can affect the penetration of heat into and throughout a container of food. Two such factors are the size and the shape of the container. An external source of heat is used to heat food in hermetically sealed containers within a retort. The larger the container, the longer it will take for the center portion of the contents to be heated to sterilisation temperature. Slim, flexible retortable pouches allow faster heat penetration into the center portion compared to, for example, the cylindrical shape of a can (Potter & Hotchkiss, 1995). Another principle factor influencing the rate of heat penetration is the nature and consistency of the food itself. This determines whether distribution of heat in the food will be via conduction or convection. If the food is liquid or contains liquid of low viscosity, heat transfer will be by means of convection. In solid or highly viscous food, on the other hand, heat will be distributed via conduction (Azizi, 1999). In products containing both free liquid and solids (e.g. Chakalaka hake), heat distribution will be through a combination of conduction and convection. Convection heating is more rapid than conduction heating since convection causes circulation that accelerates temperature rise of the entire contents of the can (Potter & Hotchkiss, 1995). In semi solid foods, agitation can accelerate the rate of heat penetration by increasing the effectiveness of natural convection currents. This can be accomplished with various types of agitating retorts (Fellows, 1988).

The last point in a container or mass of food to reach the final heating temperature is termed the 'cold point'. In a can of solid food in which heat distribution is via conduction, the geometric center is the cold point. However, in cans where the mode of heating is by means of convection, the cold point is located somewhat below the geometric center. If the cold point of a container has received the heat treatment required to eliminate the most heat resistant bacterial spores, it can be assured that every other region within the container has been sufficiently heated and that commercial sterility has indeed been achieved. Heat sensing thermocouples are used to measure temperature at the cold point in containers during the heating process. Containers with thermocouples placed at the cold point are filled with the

appropriate food, sealed and placed in the retort with the other cans that are to be retort processed. Temperature changes during heating and cooling are accurately measured and recorded with time to ensure that the required thermal process (or time-temperature combination) is accomplished (Potter & Hotchkiss, 1995). In a heat penetration test for a specific food, data regarding the heating and/or cooling of the food in its container is obtained so that a sufficient time-temperature combination for product sterilisation can be determined. The result of such a heat penetration test is experimentally derived heating and cooling curves (Lopez, 1987b). A typical heat penetration curve is shown in Figure 3.



**Figure 3: Heat penetration curve (can temp.) and retort temperature (RT) (Lopez, 1987a)**

#### 2.6.2.3 Calculation of a safe heat process

The term process refers to the application of heat to food, either before or after sealing in a hermetically sealed container, for a specific period of time at a particular temperature and under specific conditions. In canning, the objective of processing is to produce a commercially sterile product (Lopez, 1987a). Various equivalent time-temperature combinations can be used to achieve the same degree of lethality (Potter & Hotchkiss, 1995). The F value of a process is a measure of its lethality and is used as a basis for comparing heat sterilisation processes. It represents the total time-temperature combination received by a food during the heat treatment. A reference F value namely  $F_o$ , is used to describe processes that operate at a retort temperature of 121°C (250°F) and which are based on a microorganism with a z value of 10°C (50°F) (Fellows, 1988). The  $F_o$  or 'sterilising value' can be defined as the number of minutes required at 121°C to destroy a specified number of organisms with a z value of 10°C (Potter & Hotchkiss, 1995). If the specified population can be destroyed in 5 minutes at 121°C, the heat treatment has a  $F_o$  value of 5. The same lethality could be achieved with other time-temperature combinations that could therefore also be described as having a  $F_o$  of 5. The  $F_o$  requirements for foods differ and depend on the ease or difficulty with which the specific foods can be heat sterilised (Potter & Hotchkiss, 1995). The minimum  $F_o$  value prescribed for low acid canned food is 3. This prescription is based on the heat treatment required to destroy *C. botulinum* (personal communication, P. Truter, Food Scientist, SABS, Cape Town). In industry,  $F_o$  values higher than this minimum requirement is obtained so as to allow for a built-in safety margin and because of the occurrence of non-pathogenic spoilage microorganisms that are more heat resistant than *C. botulinum* (see #2.6.2.1). The recommended  $F_o$  values for herring in tomato sauce and mackerel in tomato sauce, for example, are 6 and 5 respectively (personal communication, W. Hall, Business Area

Manager, Food Science & Engineering Division at BioChemtek, CSIR, Cape Town). For the canned Chakalaka hake product developed in this study, a final  $F_0$  of 6 was decided upon so as to ensure a safe and shelf stable end product.

A number of methods can be used for the calculation of required process times or process levels. One such method is the ‘general’ or ‘graphical’ method (Lopez, 1987b). When using this method to calculate the total lethality of a process, the concept of ‘unit of lethality’ is applied. One unit of lethality is defined as the heat equivalent to one minute at 121°C against an organism with a given z value. All equally destructive heat treatments will also provide one unit of lethality. The lethality effective in one minute ( $F_0/t$ ) at a temperature other than 121°C is referred to as the ‘lethal rate’ of that temperature and can be calculated with the following equation: lethal rate = antilog  $[(T-121)/z]$ , where T is the temperature (in °C) at the cold point in the container whereas z is the z value of the target microorganism, also in °C (Potter & Hotchkiss, 1995). Each minute during the thermal process will contribute some lethality, the amount depending on the temperature at that time. These lethal rate values can be added to obtain the total lethality or  $F_0$  of the process. The general method can also be used to determine the length of a process necessary to obtain a required process lethality (Lopez, 1987b). In recent years, computers have been employed to monitor process variables such as the temperature of the raw material, the temperature of the steam and cooling water, as well as the processing time and heating and cooling rates. This data is processed by a computer program to calculate the accumulated lethality of the process (Fellows, 1988).

Containers are essential elements in the preservation of foods by canning. They are responsible for maintaining commercial sterility of the contents after thermal processing by means of a hermetic seal that prevents access of microorganisms during the cooling procedure and shelf life of the product (Lopez, 1987b). Retortable pouches, which are hermetically sealed flexible containers, can serve as an alternative to traditionally used metal cans. The seams of these pouches are sealed by heat. The possible advantages and disadvantages of retortable pouches are discussed in #2.6.3.

### ***2.6.3 Containers for canned foods: retortable pouches as alternative to cans***

Retortable pouches are flexible containers that can be heat processed in the same way as traditionally used metal cans. These containers are composed of materials that can withstand high processing temperatures and which provide excellent barrier properties to ensure shelf stability, seal integrity, durability and resistance to puncture. Retortable pouches have a laminate structure and are often composed of polyester film laminated to aluminum foil, in turn laminated to polypropylene. The polyester film contributes high temperature resistance and toughness and enables printing, while the aluminium foil supplies a barrier to light and gases. Polypropylene is compatible with regard to the odour and taste of many foods and provides heat seal integrity, flexibility and strength (Lopez, 1987b).

The slim, rectangular configuration of a retortable pouch allows for faster heat penetration to the cold point during thermal processing than in a cylindrically shaped can with its relatively large cross sectional diameter. As a result, less heat is required for equivalent lethality in pouches and overcooking of the product near the sides of the container is less likely. This means that a higher quality product can be achieved from pouches than cans for food products liable to quality loss due to excessive heat during processing. Not only can reduced exposure to heat result in improved flavour, colour and texture, but also in better retention of heat sensitive nutrients. A further benefit that arises from the faster heat penetration in pouches is a reduced processing time with consequent savings in energy (Potter & Hotchkiss, 1996). Other benefits to the processor include that empty pouches require less storage space and are lighter in weight than

cans. The shelf life of retortable pouch products is comparable to that of foods in metal cans and, as with cans, no refrigeration is required. Heating of the products can be accomplished by immersing the pouch in boiling water for a few minutes. The pouch is easily opened by tearing it across the top at the notch in the side seam, or by using scissors. A disadvantage of flexible retortable pouches, however, is that the filling procedure is slower and more complex than with cans. Due to the flexibility of pouches, detection of leakage is more difficult than with cans and, in addition, pouches can also be punctured (Lopez, 1987b). Pressure problems during cooling of retorted pouches is more likely than with cans and overriding air pressure has to be applied during cooling so that steam pressure on the inside of the pouch does not cause it to burst (Potter & Hotchkiss, 1996).

The focus of this study was the development of value-added food products produced from the neck flesh of Cape hake as a means of adding value to hake heads. The product prototypes developed include curried fish chowder, fish spread and canned Chakalaka hake. A formula for fish stock, which served as a base ingredient in the three prototypes, was also standardised. Information regarding the development of the product prototypes and the fish stock follows in #3.

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### 3. THE DEVELOPMENT OF VALUE-ADDED FOOD PRODUCTS USING THE NECK FLESH OF CAPE HAKE (*MERLUCCIUS CAPENSIS*)

#### 3.1 Introduction

The product prototypes developed in this study are curried fish chowder, fish spread and canned Chakalaka hake. A formula for fish stock, which is used as a base ingredient in the three product prototypes, was also standardised. Formula development was performed at the Department of Consumer Science, University of Stellenbosch and in the case of the Chakalaka hake, the canning procedure was executed at the canning facility of the Cape Technikon. A focus group of food specialists assisted with refinement of the formulae. Different aspects relating to the kind and form of the seafood raw material used during formula development are discussed in #3.2. Information regarding the development of the fish stock and product prototypes as well as the standardised formulae and methods of preparation follow in #3.3. Product briefs are given in Addendum A.

#### 3.2 Raw material

Standardisation of the cutting procedure of the hake head had not been finalised at the time of product development. The exact percentage of neck flesh obtained from the head was therefore still unknown, but was approximated to be about 16%. Hake heads obtained from I&J and Sea Harvest fishing trawlers were cut at Melnyczuk Research & Development (MR & D), Stellenbosch, to provide the required neck flesh. For the purpose of product development, these neck flesh cuts were deboned manually to produce chunks of flesh. Other raw material options such as hake fillets and hake mince, the latter simulating the form of raw material that could possibly be obtained with mechanical deboning of the neck flesh, were however also investigated. The fish stock was prepared either with cuts of neck flesh as is (i.e. without deboning), or with the frames and flesh of hake.

#### 3.3 Formula development

##### 3.3.1 Fish stock

Preparing fish stock is a convenient way of utilising hake neck flesh since the neck flesh can be used as is (without deboning) and the stock can be made in advance and frozen for later use. A fish stock formula was standardised for use as a base ingredient in the developed products. The stock will however also form an ideal base for other fish dishes and the possibility exists for the stock to be sold in bulk to the restaurant and catering industry. The formula and method of preparation for fish stock is given in Table 1.

**Table 1: Formula and preparation method for fish stock**

<b>Ingredients</b>	<b>Mass (g)</b>	<b>Percentage (%)</b>
Water	1500.0	53.35
Hake neck flesh cuts/frames and flesh of hake	560.0	19.92
Carrots	240.0	8.54
Onions	187.5	6.67
Leek with leaves	150.0	5.34
Celery, stalks & leaves	135.0	4.80
Parsley, fresh	22.5	0.80
Salt	15.0	0.53
Black pepper, fine	0.8	0.03
Peppercorns	5 units/0.2	0.007
Bay leave, dried	1 unit/0.1	0.004
<b>Total</b>	<b>2811.1</b>	<b>100</b>

**Method:**

1. Chop vegetables in food processor (Magimix 3500).
2. Bring all ingredients (except salt and pepper) to the boil in a closed saucepan on medium heat setting on stove (Defy 500). Lower heat and simmer (92-98°C) for 20 minutes.
3. Remove saucepan from hot plate and place in cold water to cool.
4. Strain stock through muslin cloth while pressing lightly.
5. Add pepper and salt (15g and 0.8g respectively/1500g) and mix well.
6. Yield: approximately 1500g+. Can be frozen.

**3.3.2 Fish spread**

Meat spreads (e.g. liver spread and chicken and ham spread) in plastic casings are well known to the consumer and popular for use on sandwiches and toasted bread. The aim in developing a fish spread was to simulate these spreads using fish as the base. Anchovy fish paste and fish patés such as smoked snoek paté are the existing fish products closest in concept to the fish spread developed. These products are however each in a class of its own because of their distinctive flavours, different packaging and/or higher price class.

The formula and preparation method used for the fish spread is given in Table 2. Canola oil was chosen as the fat component in the fish spread formula as it has a favourable fatty acid profile, a characteristic that might appeal to the health conscious target consumer. Canola oil is a concentrated source of monounsaturated fatty acids, but contains a low level of saturated fat (Krummel, 1996). To lower the production cost, the canola oil could be substituted with a less expensive sunflower oil without a significant difference in taste. A modified (pre-gelatinised) waxy maize starch, Pettina instant thickener (South Bakels (Pty) Ltd., Cape Town) was used for thickening purposes, since gelatinisation and consequent thickening of other starches such as cornflour require temperatures higher than that achieved during cooking of the fish spread (Charley & Weaver, 1998). Sodium caseinate was used as emulsifier to obtain a creamy texture and good spreadability. Sodium benzoate and potassium sorbate were added as preservatives.

Seafood is regarded as being safe to eat if the internal temperature has been raised above 66°C, where it is actually pasteurised (Piggot, 2000). A core temperature of 68°C (2°C ‘of safety’ above the recommended temperature) was obtained during cooking of the fish spread after which the product was immediately emerged in ice water so as to avoid overcooking.

**Table 2: Formula and preparation method for fish spread.**

Ingredients	Basic formula		Formula with preservatives added	
	Mass (g)	Percentage (%)	Mass (g)	Percentage (%)
Fish flesh, minced/chunks	600.0	61.55	600	61.48
Fish stock, semi-defrosted	144.0	14.78	144	14.76
Canola oil	120.0	12.31	120	12.30
Pre-gelatinised starch Pettina	36.0	3.69	36	3.69
Parsley, fresh	21.6	2.22	21.6	2.21
Sodium caseinate	19.2	1.97	19.2	1.97
Sugar	14.4	1.48	14.4	1.48
Salt	9.6	0.98	9.6	0.98
Lemon juice, fresh	9.6	0.98	9.6	0.98
Black pepper, fine	0.5	0.05	0.5	0.05
Potassium sorbate	-	-	0.584	0.059
Sodium benzoate	-	-	0.438	0.044
<b>Total</b>	<b>974.9</b>	<b>100.01</b>	<b>975.92</b>	<b>100.00</b>

**Method:**

- Cook fish in a saucepan on stove (Defy 500) until just done:  
(Heat stock on setting 5 for 7min, add fish, stir & close with lid. Turn heat down to setting 1. Cook fish for 5min, stirring twice during cooking. Timing is started as lid is closed).
- Place closed saucepan in ice water to cool. (Cooked mass: approximately 720g)
- Mix the dry ingredients.
- Mince together all ingredients (80s) in Magimix 3500 food processor. Stop food processor at t=30s to scrape sides clean.
- Feed mixture into fibre plastic casings (diameter: 6.5cm, thickness: 25µm) and tie open ends with string to form samples. Insert core thermometer in one of the samples.
- Poach the samples in water at a temperature of approximately 78-82°C. Obtain a core temperature of 68°C.
- Immediately cool in ice water.

**3.3.3 Curried fish chowder**

A thick, mildly curried fish soup containing colourful chunks of vegetables, fish and pasta rice was developed and more appropriately named fish ‘chowder’ (Table 3). Fish stock was used as the base of the chowder, as is normally done in the preparation of seafood soups and the product was packaged in stand-up pouches. A major consideration during development of the chowder had a bearing on the texture of the pasta in the final product. One concern was that of the pasta absorbing excessive additional moisture upon standing and becoming too soft. Pasta made from durum wheat is more stable and less likely to disintegrate during cooking than pasta made from bread wheat (Campbell, 1992). In South Africa, pasta is often made from bread wheat, as it is more readily available than durum wheat (personal communication, N. Vorster, R&D manager, Pioneer Food Group). According to P. Wet (Product Manager, Fatti’s & Moni’s (Pty) Ltd), Fatti’s & Moni’s pasta is exclusively made from imported durum wheat. An important factor to bear in mind is that whilst pasta is submerged in the hot liquid cooking medium, it absorbs moisture. To therefore ensure a desirable texture for the pasta in the chowder, Fatti’s & Moni’s pasta was used. In addition, the raw pasta was added to the soup mixture only towards the last five minutes of remaining cooking time so as to limit exposure of the pasta to the hot liquid.

**Table 3: Formula and preparation method for curried fish chowder**

Ingredients	Mass (g)	Percentage(%)
Fish stock	1200	58.77
Fish flesh, minced/chunks	200	9.80
Tomatoes, canned chopped	150	7.34
Onion	120	5.88
Pasta (Fatti's & Moni's Pasta Rice)	100	4.90
Potato	100	4.90
Carrot	100	4.90
Sunflower oil	20	0.97
Parsley, fresh	15	0.73
Flour	14	0.69
Lemon juice, fresh	10	0.49
Sugar	5	0.24
Curry powder (Carthwright's medium strength)	3	0.15
Turmeric	3	0.15
Salt	2	0.10
<b>Total</b>	<b>2042</b>	<b>100.01</b>

Yield: approximately 1750g

#### **Method:**

1. Chop vegetables in food processor (Magimix 3500).
2. Heat oil in a saucepan on stove (Defy 500).
3. Sauté onion in the oil over low heat until transparent.
4. Add carrot, curry, and turmeric and fry lightly.
5. Add flour, sugar, then tomato, potato and fish stock. Bring to the boil.
6. Add fish flesh. Lower heat. Simmer covered at 88-95°C for 15min. Add pasta, adjust heat and simmer for a further 5min. (Frequently check temperature and stir.)
7. Add lemon juice, salt and parsley. Bring chowder to a final temperature of 95°C.
8. Package chowder in stand-up pouches (200g/pouch). Heat sealing of stand up pouch: 140°C for 3s.

#### **3.3.4 Chakalaka hake**

Chakalaka is a spicy vegetable dish popular in South Africa that contains carrots, tomato, onion, peppers, cabbage, chillies and spices. The aim was to develop a canned product in which the traditional chakalaka ingredients are combined with hake chunks and fish stock so as to produce a spicy fish-and-vegetable dish called Chakalaka hake. Development of Chakalaka hake involved the simultaneous development of a suitable standardised formula and thermal process that would ensure a commercially sterile product with desirable sensory quality characteristics. Canning trials were performed at the canning facility of the Cape Technikon, Cape Town. The following procedure was followed during the development of Chakalaka hake.

Before commencement of the canning trials, a formula for a conventionally prepared Chakalaka hake containing domestic ingredients only, was developed. This formula was eventually adapted to make it more suitable for the canning procedure. Cake flour, initially used as the thickening agent, was substituted with a retort stable starch, MAPS 281 (SAARChem, Cape Town). The latter is a modified waxy maize starch able to withstand the high temperatures of retorting whilst also giving an excellent gloss. *Clostridium botulinum*, the most heat resistant pathogen, is unable to grow at pH levels of less than 4.6. High acid foods (pH of 4.6 or less) consequently require less severe processing compared to low acid foods to ensure commercial sterility (Ababouch, 1999). An attempt was made to reduce the pH of the Chakalaka hake to 4.6 or less by adding citric acid. The latter has a greater effect on pH than vinegar (personal communication, N.H. Vlok, Head of Department Food and Agricultural Sciences, Cape Technikon, Cape Town). Part of the vinegar of the original formula was therefore substituted with citric acid in order to reduce the pH. It was however suspected that citric acid may impart an undesirable sour taste when used in high quantities.

The aim of the successive canning trials was the improvement of sensory properties of the product by means of slight adjustments to the formula and/or retorting process. Simultaneously, microbiological safety was ensured throughout. In each trial, 1M-sized multipurpose cans (Foodcan, Paarl) were each filled with 400g Chakalaka hake, prepared according to the formula(e) compiled for the specific trial. Exhausting was performed with a steam-type exhaust box (H.G. Molenaar & Co. (Pty) Ltd., Paarl). This procedure removes excess air from the tins that would otherwise expand during processing, resulting in strain on the container due to the consequent greater pressures within the container than on its outside (Fellows, 2000). After exhausting, the cans were sealed and placed in a batch-type still retort (H.G. Molenaar & Co. (Pty) Ltd., Paarl). Retorting by saturated steam was carried out using a processing temperature of 116°C. One of the cans retorted was fitted with a heat-sensing thermocouple that measured the change in core temperature with time during the retorting process. The thermocouple was linked to a computer that uses data-logging software registering the change in temperature during processing to calculate the accumulated lethality or  $F_o$  value. The  $F_o$  value represents the total time-temperature combination received by a food product during the heat treatment (Fellows, 2000). After retorting, the cans were water-cooled under pressure. The main adjustments made to the formula during development, as well as the retorting data of the successive trials are summarised in Table 4. A discussion of the successive trials follows.

#### Trial 1:

Two different Chakalaka hake formulas were prepared in trial 1. Formula 1 and 2 contained 2g (0.20%) and 4g (0.40%) of citric acid respectively. For both formulas, the vegetables were chopped in a food processor and were fully cooked before canning. A final  $F_o$  value of 8.518 was obtained with the heat treatment. The end product of the first trial was 'overcooked' with regard to taste and texture. In an attempt to improve the texture it was decided to reduce the processing time in the next trial and to experiment with a formula that used raw ingredients only. The pH measurements for both formulas were above 4.6: 5.88 for formula 1 and 5.77 for formula 2. It was consequently decided to add a higher percentage of citric acid in the next trial. For the first trial, product pH was measured directly after the product had been cooled inside the retort. At a later stage however, it was realised that the pH should not be measured directly after cooling since the canned product would then still be warm at the center with the pH not yet stabilised (See Trial 3).

#### Trial 2:

In trial 2, two different formulas were prepared. The vegetables of formula 1 were partly cooked in the sauce before the raw fish was added to the hot vegetable-and-sauce mixture. Formula 2 used raw ingredients only, but gave an unacceptable watery end product. Formula 1 had a better texture than that obtained in the samples of the first trial, most probably due to the shorter processing time applied. 20g (1.95%) citric acid was used in both formulas of this trial in an attempt to reduce the pH to less than 4.6. This high percentage of citric acid however resulted in an overpowering sour and unacceptable taste. The pH measurement was again taken directly after cooling of the product (5.00 at 28.7°C), creating an incorrect impression regarding the effect of citric acid in the product.

After trial 2, it was concluded that a pH of less than 4.6 was unobtainable without compromising taste. However, since the  $F_o$  value achieved up to this point was much higher than that required for ensuring destruction of *C. botulinum* spores, the pH 4.6-margin was of less importance (personal communication, N.H. Vlok, Head of Department Food and Agricultural Sciences, Cape Technikon, Cape Town). The minimum  $F_o$  value prescribed for low-acid canned food is 3, a prescription based on the heat treatment required to destroy *C. botulinum*. A  $F_o$  value of 8.5 and 7 was obtained in

trials 1 and 2, respectively. If a pH of less than 4.6 is unobtainable, a pH of less than 5.2 could still be regarded as beneficial (personal communication, P. Truter, Food Scientist, SABS, Cape Town). Low-acid foods can be further subdivided into medium acid (pH 4.5-5.3) and weakly acid (pH 5.3-7.0) foods and whereas a  $F_o$  value of 7 to 14 is recommended for the latter, a  $F_o$  value of 3 to 6 is sufficient for medium acid foods (Ababouch, 1999). The aim with regard to pH for subsequent trials was therefore a pH of less than 5.2.

#### Trial 3:

The effect of two different quantities of citric acid on the pH was investigated in trial 3. Formula 1 and 2 contained 10g (0.98%) and 5g (0.49%) citric acid respectively. A third formula, with no citric acid, was included as control. Coincidentally, the pH was not measured directly after cooling the retorted product, but after a couple of hours had passed. The pH measurement of the control was 5.64. The pH of formula 1 and 2 was 4.34 and 4.84 respectively which were both lower than the pH measurement obtained in trial 1 when a much higher quantity of citric acid was used. This prompted remeasurement of the pH for trials 1 and 2. The second pH measurements were indeed lower than those recorded first. The conclusion was that, with the first pH measurements that were taken directly after cooling of the retorted product, the pH had not yet stabilised. This was probably due to the temperature of the contents not having yet reached equilibrium. It was consequently decided to measure pH at least 24 hours after retorting, with the product at room temperature. This time lag would allow for the pH to stabilise and would also ensure that the pH is taken at approximately the same temperature each time, making pH measurements more comparable.

The formulas for trial 3 contained increased quantities of sugar (1.46%) and salt (0.39%) and the vegetables were chopped roughly by hand instead of in a food processor. The end product for both formulas had good texture, colour and overall appearance. Both formulas tasted too sour, but formula 2 had the better taste. The sauces of both formulas were slightly thin and could benefit from the addition of more starch. It was also decided that the ratio of fish to vegetables-and-sauce could be increased. Although the retort was switched off after a shorter processing time than in trial 2, a slightly higher final  $F_o$  was obtained. This illustrated the fact that the final  $F_o$  value is not determined by processing time alone, but rather by the time-temperature combinations during the retorting process. Some lethality is effected during each minute of a thermal process, the amount during the interval being dependent on the temperature at the time. The sum of these lethality values gives the total lethality of the process (Lopez, 1987). The aim was however to start drawing a relation between processing time, the  $F_o$  point at which the retort is stopped and the final  $F_o$  value.

**Table 4: Hake chakalaka trials: adjustments to formula and retorting data**

Trial nr.	Main adjustments to formula/main differences between formulas	Core temp.* (start)	Core temp.* (end)	Max.F <sub>0</sub> *	Total retorting time	pH**	Sensory observations
1	<b>Formula 1:</b> 0.20% citric acid <b>Formula 2:</b> 0.40% citric acid <b>Formula 1 &amp; 2:</b> Veg. chopped in food processor, fully cooked before canning	61.9°C	112.8°C	8.5	1h25	1 <sup>st</sup> : Formula 1: 5.88 at 30.5°C Formula 2: 5.77 at 29.8°C  2 <sup>nd</sup> : Formula 1: 4.25 at 23.5°C Formula 2: 4.15 at 23.6°C	<b>Formula 1 &amp; 2:</b> Products overcooked. Veg. too soft (mushy).
2	<b>Formula 1:</b> Veg. semi-cooked, 1.95% citric acid <b>Formula 2:</b> All ingr. raw 1.95% citric acid	73.7°C	112.5°C	7.0	1h20	1 <sup>st</sup> : Formula 1: 5.00 at 28.7°C  2 <sup>nd</sup> : Formula 1: 3.8 at 22.5°C	<b>Formula 1:</b> Slightly better texture than in trial 1. <b>Formula 2:</b> Watery.
3	<b>Formula 1:</b> 0.98% citric acid <b>Formula 2:</b> 0.49% citric acid <b>Control:</b> no citric acid <b>Formula 1, 2 &amp; control:</b> veg. chopped roughly by hand, increased quantities of sugar (1.46%) & salt (0.39%)	62.0°C	112.3°C	7.2 (Switched off at 4.5)	1h11	Formula 1: 4.34 at 21.7°C Formula 2: 4.84 at 22.1°C Formula 3: 5.64 at 21.7°C	<b>Formula 1 &amp; 2:</b> Very sour taste which overpowers rest of taste profile.  <b>Formula 1:</b> Good colour, texture & overall appearance. Too sour. <b>Formula 2:</b> Good colour, texture & overall appearance. Good taste, but too sour.  <b>Formula 1 &amp; 2:</b> Sauce could be thicker.
4	<b>Formula 1:</b> 0.43% citric acid, 3.03% sugar <b>Formula 2:</b> 0.51% citric acid, 4.27% sugar <b>Formula 1 &amp; 2:</b> increased quantities of fish (30.33%), sugar (see above), salt (1.04%) & starch (1.73%)	66.4°C	111.9°C	6.3 (Switched off at 3.8)	1h01	Formula 1: 4.54 at 25.6°C Formula 2: 4.48 at 22.5°C	<b>Formula 1:</b> Acceptable sweet & sour taste, but might be judged too sour by some. Consistency of sauce good. Preferred above Formula 2. <b>Formula 2:</b> Too sour.
5	<b>Formula 1:</b> Fish=35.1%, Starch=1.8%, Citric acid =0.3%, Sugar=3.2%	72.2°C	112.0°C	6.3 (Switched off at 3.7)	1h05	4.69 at 23.6°C	Good sweet & sour taste. Good texture and consistency of sauce.

\*Temperatures and Fo values rounded off to 1 decimal

\*\*pH given is an average of three measurements. The pH measurements of the products produced in trial 1 & 2 were retaken on a later date (see text for explanation). 1<sup>st</sup> refers to 1<sup>st</sup> pH measurement and 2<sup>nd</sup> refers to a 2<sup>nd</sup> pH measurement on a later date. From trial 3 onwards, pH measurements were taken at least 24 hours after canning with the product at room temperature.

#### Trial 4:

The aim for this trial was to decrease the sourness and to improve the viscosity of the sauce and the ratio of the fish to the vegetables-and-sauce. Two formulas were prepared. Formula 1 contained 5g (0.43%) citric acid and 35g (3.03%) sugar, while formula 2 contained 6g (0.51%) citric acid and 50g (4.27%) sugar. Increased quantities of fish (30.33%), starch (1.73%), sugar (3.03% and 4.27% for formula 1 and 2, respectively) and salt (1.04%) were used. Formula 1 gave the best end product. It had an acceptable taste that could be described as 'sweet-and-sour' and the consistency of the sauce was acceptable. In addition, the pH measurement of formula 1 was less than 4.6 namely pH 4.54 (at 25.6°C). However, the pH margin of 4.6 was now considered less important. If adjustments to improve taste in subsequent trials resulted in a slightly higher pH (but preferably less than 5.2), this would have been considered acceptable.

In trial 4, the retort was switched off when the  $F_o$  value was 3.8 and a final  $F_o$  value of 6.3 was obtained. As mentioned, a minimum  $F_o$  value of 3 is required for low acid canned food. In practice, however, a  $F_o$  value higher than 3 is advisable; for the sake of building in a safety margin and because of the occurrence of non-pathogenic spoilage microorganisms more heat resistant than *C. botulinum* (Ababouch, 1999). The  $F_o$  values obtained in industry for other canned fish products were used as a guideline in concluding what the aim with regard to final  $F_o$  value for Chakalaka hake should be. The recommended  $F_o$  values for herring in tomato sauce and mackerel in tomato sauce, for example, are 6 and 5 respectively (personal communication, W. Hall, Business Area Manager, Food Science & Engineering Division, BioChemtek, Cape Town). It was decided to aim for a final  $F_o$  of 6 for the Chakalaka hake, which should be sufficient in ensuring not only a safe, but also a shelf stable end product. As with all canned products, however, it is also important to eliminate conditions that are conducive to the growth of heat resistant microorganisms (Ababouch, 1999). Proper cooling and acceptable handling and storage conditions should therefore be part of the quality assurance system of the canning facility.

#### Trial 5

The focus of trial 5 was the enhancement of the sensory properties of the product and this involved adjustment of the sugar, citric acid and starch levels. The quantity of fish was further increased to 35% so as to adhere to compulsory specifications for canned fish-and-vegetable products (SABS, 1972). The end product had a good sweet-and-sour taste and the consistency of the sauce as well as the overall appearance was judged acceptable by a focus group. The pH measured (4.69 at 23.6°C) was below the targeted pH (see trials 3 and 4). The retort was switched off at a  $F_o$  value of 3.7 and the final  $F_o$  value obtained was 6.3. The percentage formula and the preparation method for this final development trial are given in Table 5.

The development procedure for Chakalaka hake discussed above involved standardisation of the formula, preparation method and thermal process using a processing temperature of 116°C and metal cans as the container type. Stand-up retortable pouches can be used as alternative container type for packaging Chakalaka hake. The possible advantages and disadvantages of using retortable pouches instead of cans have been discussed in #2.6.3. Damage to the sensory properties of foods during thermal sterilisation can be minimised by reducing the processing time through implementing a higher processing temperature (Potter & Hotchkiss, 1996). The effect of the two container types (can versus retortable pouch) as well as two processing temperatures (116°C versus 121°C) on the sensory quality characteristics of Chakalaka hake was investigated (#4).

**Table 5: Percentage formula and method of preparation for Chakalaka Hake**

Ingredients	Mass (g)	Percentage (%)
Hake chunks	880	35.1
Tomatoes; chopped canned	320	12.8
Fish stock	300	12.0
Onion; slices	240	9.6
Carrot; rings	200	8.0
Cabbage; roughly chopped	200	8.0
Green pepper; roughly chopped	140	5.6
Sugar	80	3.2
MAPS 281 modified starch	45	1.8
Sunflower oil	35	1.4
Salt	28	1.1
Brown vinegar	16	0.6
Citric acid powder	8	0.3
Masala for medium curry	8	0.3
Chillies, fresh, seeded and finely chopped	6	0.2
<b>Total</b>	<b>2506</b>	<b>100</b>

Method:

1. Heat oil in saucepan on stove (Defy 242).
2. Sauté onion, green pepper, chillies in the oil over low heat.
3. Add carrot, cabbage and masala and sauté briefly.
4. Add mixture of starch, sugar, salt, citric acid and stir.
5. Add stock, tomato and vinegar. Bring to the boil while stirring. Remove saucepan from heat.
6. Add fish, stir and close lid.
7. Fill containers (400g product/container). Proceed with canning.

### 3.4 Shelf life determinations of the refrigerated product prototypes

Shelf life testing was regarded an essential part of the development processes for the refrigerated product prototypes i.e. the curried fish chowder and the fish spread. The shelf life of these products is seen as the time period during which the product is safe for human consumption and in which acceptable sensory qualities are maintained, under given conditions. Shelf life testing performed in this study involved organoleptic monitoring and pH testing coupled with microbiological testing based on regulations regarding microbiological standards for cooked seawater and freshwater foods (Government Gazette, 1997; Table 6).

Since development of the product prototypes in this study was carried out up to a stage before actual manufacturing in a commercial production plant, the shelf life determinations performed here can be seen as 'preliminary'. In practice, preliminary shelf life determinations are followed up by confirmatory shelf life determinations once final scale-up has taken place, using product samples obtained from pre-launch runs in the actual production plant (IFST, 1993). The preliminary shelf life determinations performed in this study for the curried fish chowder and the fish spread prototypes are discussed in #3.4.1 and #3.4.2, respectively.

**Table 6: Specifications for cooked seawater and freshwater foods (Government Gazette, 1997)**

Test	Growth medium	Specification
Total aerobic count	Plate Count Agar (PCA)	Total aerobic plate count less than 100 000/1g
<i>Salmonella</i>	<i>Salmonella-Shigella</i> Agar (SS-Agar)	No <i>Salmonella</i> present in 20g
<i>Shigella</i>	<i>Salmonella-Shigella</i> Agar (SS-Agar)	No <i>Shigella</i> present in 20g
<i>Escherichiae coli</i>	Violet Red Bile Lactose Agar (VRBL-Agar)	No <i>Escherichiae coli</i> Type 1 present in 20g
Type 1		
<i>Vibrio cholerae</i> and <i>V. parahaemolyticus</i>	<i>Vibrio</i> Selective Agar (TCBS-Agar)	No <i>Vibrio cholerae</i> or <i>V. parahaemolyticus</i> present in 20g
<i>Staphylococcus aureus</i> (coagulase positive)	Baird-Parker Agar Base with Egg Yolk (EY) Tellurite Enrichment	No <i>Staphylococcus aureus</i> (coagulase positive) present in 20g
Coliforms other than <i>E. coli</i> Type 1	Violet Red Bile Lactose Agar (VRBL-Agar)	Number of coliforms, other than <i>E. coli</i> Type 1; less than 1000/100g
Histamine test	Not applicable	Histamine-content: Less than 10 mg/100g (>10mg indicates decomposition, whereas >20mg renders the foodstuff unsafe)

### **3.4.1. Shelf life determination for the fish spread**

The shelf life determination for the fish spread was accomplished as part of an investigation in which the potential of the antimicrobial peptides 1017A and 1017B to be used as biological preservatives was evaluated (#5). The shelf life stability of fish spread samples containing the mentioned peptides was compared to that of samples containing artificial preservatives as well as to that of control samples containing no preservatives. Even in the case of the samples containing preserving agents, the shelf life of the fish spread was relatively short (nine days). This preliminary shelf life determination of the fish spread indicated that additional or more stringent microbiological hurdles would be required for obtaining a more satisfactory shelf life for the fish spread. A factor to be considered is that the preservatives in the experiment were not necessarily used in optimum quantities. The level of artificial preservatives used was the minimum for the type of product investigated (personal communication, Dr. F. Mellett, Food Technologist, Freddy Hirsch Group, Cape Town). The level of enterocin crude extract used was arbitrarily chosen since recommended levels are not available. Higher levels of the preservatives could possibly have an improved preservation effect on the product. A possibility that could be investigated is to use a combination of the peptides and artificial preservatives; each included at various ratios. Another consideration is that this preliminary shelf life determination was performed on samples that had been prepared manually in a test-kitchen. Mechanical production of the samples in a commercial plant will be more streamlined, possibly resulting in less microbial contamination and a reduction in the rate of microbial growth. This points to the importance of implementing an effective system such as HACCP in a food production plant for ensuring safe food products of consistent quality with optimal shelf life (IFST, 1993). The execution of the above-mentioned investigation on antimicrobial peptide use is addressed in #5.

### **3.4.2 Shelf life determination for the fish chowder**

After product scouting, a target shelf life could be set for the fish chowder. Similar existing commercial products have a shelf life of eight to nine days (personal communication, J. Carroll, Food Technologist, Lombardi Foods, Strand). It was expected and also required that the shelf life of the curried fish chowder should be similar to that of the existing products. The shelf life of a food product should ideally include a built-in ‘safety margin’ which would in effect allow tolerance of some product abuse after manufacturing (IFST, 1993). A 12-day shelf-life trial period was consequently decided upon so as to enable estimation of a safety margin. Shelf life tests were performed on product samples at different time intervals over the 12-day period. Two repetitions of the shelf life tests were performed. Microbiological tests were based on specifications set for cooked seawater and freshwater foods (Government Gazette, 1997). These specifications as well as the media used for detection and/or enumeration of the appropriate microorganisms are given in Table 6. If a sample had to fail in conforming to a specification at a certain time of testing during the testing period, the product would have been considered unsafe for human consumption. If spoilage had occurred (i.e. textural or other sensory degradation had become evident during monitoring) before the product was considered unsafe, the shelf life would also have been regarded as expired. Sensory characteristics of the product were monitored by a focus group over the 12-day shelf life period, with fresh product serving as control for comparison purposes. The shelf life of the product was seen as the time period in which the product samples conformed to the specifications set by legislation and during which no signs of spoilage were visible. Specifications for cooked seawater foods also include a specification with regard to maximum histamine content. During shelf life testing of the fish spread, containing a higher percentage of fish than the chowder, histamine tests showed that the histamine content was less than 1/100 of the set maximum limit. It was therefore concluded that execution of histamine tests on the fish chowder would be unnecessary. Product pH was monitored over the 12-day period.

#### *3.4.2.1 Procedure*

Two identical batches of product were prepared as described in Table 3, #3.3.3: one batch for microbiological and pH testing purposes, the other for sensory evaluation. Five 200g samples taken from each batch were sealed in individual stand-up pouches. The samples intended for sensory evaluation were sealed directly after preparation at a temperature of 95°C. The batch intended for microbiological and pH testing was homogenised so as to ensure representative samples for dilution. The homogenised batch was then also heated to 95°C prior to packaging. The samples were stored at 4°C for the duration of the investigation.

Equipment used for microbiological testing was sterilised by either autoclaving or alcohol flaming. Plating of the samples on Plate Count Agar (PCA) was performed every 3<sup>rd</sup> day over a period of 12 days using the following procedure. A dilution series of the sample was prepared. Each dilution was thoroughly mixed with a Vortex mixer VM-300 before a further dilution was prepared. From each dilution, two 100µl volumes were taken and each of these transferred to a petri dish containing plate count agar (PCA). Glass beads were used for spreading purposes. The plates were incubated at 37°C for three days before plate counts were performed. The cell count recorded (total aerobic count) was the average from the two plates representing a given dilution.

Tests for specific microorganisms (see Table 6) were performed on day 12 of storage using the following procedure. A 10<sup>-1</sup> dilution was prepared from the homogenated soup sample. Two 100µl volumes were taken from this 10<sup>-1</sup> dilution, and each transferred to a petri dish containing a selective medium (see Table 6). Glass beads were used for spreading purposes. The plates were then incubated at 37°C. Investigations for presence of suspect colonies were done after the

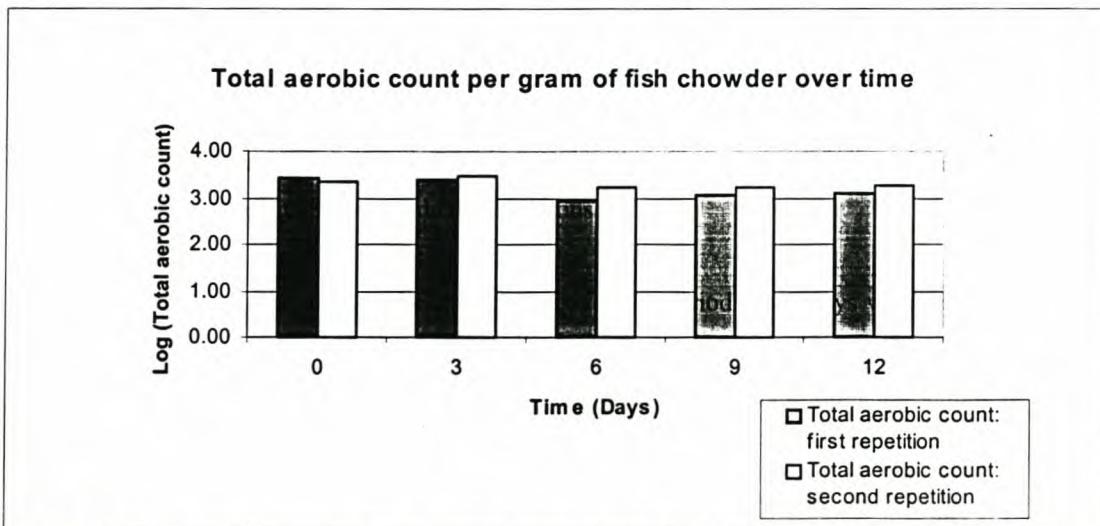
recommended periods of incubation. Presence of suspect colonies, i.e. any cfu that correspond with the description given in the Difco Manual for the pathogen in question, would have prompted further investigation (Difco Laboratories, 1998).

The organoleptic quality of the fish chowder samples was evaluated by a focus group every 3<sup>rd</sup> day over the 12-day period. Freshly prepared product served as reference for comparison purposes. The organoleptic characteristics evaluated included consistency (homogeneity, presence of separation), colour, flavour and odour of the fish chowder. Any changes in the mentioned sensory characteristics were noted on a sensory evaluation sheet used for reference purposes during subsequent sensory evaluation sessions.

The pH of the samples was measured every 3<sup>rd</sup> day over the period of 12 days with a Beckman 100 series ISFET pH meter with a Smart™ ISFET probe. A change in pH was considered to be an indication of microbial spoilage (personal communication, Prof. LMT Dicks, Department Microbiology, University of Stellenbosch, Stellenbosch).

#### 3.4.2.2 Results

In both repetitions of the experiment, large, cream coloured (mostly) and pink cfu grew on the PCA plates. The calculated total viable counts per gram of fish chowder are presented graphically in Figure 1. The total aerobic counts were within the specified limits up to day 12 of storage. No suspect cfu were evident on the selective media and no further tests were performed to determine which organisms grew.



**Figure 1: Total aerobic counts in fish chowder representative of two repetitions**

The focus group discussions on the organoleptic quality of the fish chowder samples can be summarised as follows. The fresh product is a colourful chowder (orange liquid with red, green and white pieces of vegetables, fish and pasta) with a mild curry flavour and -odour. The liquid part of the chowder has a fairly homogenous consistency. The flavour of the chowder was at its best when the product was three- to five days old. This was expected since the flavour of curries is known to improve upon standing (Stobart, 1980). The flavour of the product prior to and after three to five days was however also acceptable. From nine days onward, the curry flavour became slightly blander. On day 12 of storage, despite the flavour being blander, no off-flavours were evident. It was noted that the colour of the chowder is subject to slight variability due to variation in the colour of the ingredients. The orange colour of the product became

slightly more pronounced upon standing. The homogeneity in the consistency of the liquid component of the chowder as well as the texture of the pasta and vegetables remained constant over the testing period.

The pH values measured over the 12-day period are given in Table 7. As can be seen from this table, the pH of the fish chowder remained essentially constant over the testing period, confirming a lack of spoilage in the product.

**Table 7: pH of fish chowder over time**

Time (Days):	First repetition		Second repetition	
	pH	Temperature	pH	Temperature
0	5.94	13.1°C	5.92	12.8°C
3	5.87	12.6°C	5.90	12.3°C
6	5.90	13.1°C	5.93	13.1°C
9	5.88	11.3°C	5.94	13.1°C
12	5.94	12.8°C	5.99	13.2°C

### 3.4.2.3 Conclusion

There was little variation between the results of the two repetitions for the shelf life determination. Since the chowder samples (stored at 4°C) conformed to microbiological specifications (Government Gazette, 1997) and also displayed no signs of spoilage over 12 days, the shelf life of this product can be seen as eight days with a built-in ‘safety margin’ of four days.

### 3.5 Proximate- and mineral analyses performed on the product prototypes

The moisture content of the product prototypes was determined through drying of the product at 100°C for 24h. Samples were freeze dried until constant weight for the determination of total percentages of protein, fat and ash. Protein content was determined by the block digestion method and ashing was done at 500°C for 5h (AOAC, 1997). Fat content was determined by means of chloroform:methanol extraction (Lee, Trevino & Chaiyawat, 1996). Values for the proximate chemical composition of the product prototypes are given in Table 8.

**Table 8: Values for proximate chemical composition of product prototypes**

Product prototype	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Fish spread	66.89	13.45	11.61	2.30
Fish chowder	84.20	3.81	1.26	1.34
Chakalaka hake	80.92	6.46	1.45	2.14

A wet ashing procedure was used to prepare the freeze-dried fish chowder- and fish spread samples for mineral analysis (Pinta, 1982). A dry ashing procedure was used to prepare the freeze-dried Chakalaka hake samples for mineral analysis (Giron, 1973). In the case of the fish chowder and fish spread samples, the calcium (Ca), potassium (K), sodium (Na) and iodine (I) contents were determined using direct current plasma emission spectrometry. Inductively coupled plasma spectrometry (ICP) was used for determining the contents of the specified mineral elements in the Chakalaka hake. The contents of the specified minerals in the product prototypes are given in Table 9.

**Table 9: Mineral content of product prototypes (mg/100g product)**

Product prototype	Calcium	Potassium	Sodium	Iodine
Fish spread	12.2	221.6	359.0	35.6
Fish chowder	18.3	354.9	831.1	80.5
Chakalaka hake	17.17	263.3	395.3	55.6

The proximate chemical composition and mineral content of the product prototypes were found to be comparable to that of similar commercial products with the exception of fat content (Langenhoven, Kruger, Gouws & Faber, 1991; Addendum A: Table 1). The fat content of the fish spread (11.61%) is markedly lower than that of commercially available meat spreads (22-28.5%) and fish paté (19.2%). The fat content of the curried fish chowder (1.26%) is slightly lower than that of commercially available fish chowder (3%). The label of Chakalaka pilchards, a commercially available product similar to Chakalaka hake, does not supply any nutritional information and comparisons between the two products could therefore not be made. Since the fat content of the curried fish chowder and Chakalaka hake is less than 3%, low fat claims are possible for these products (Government Gazette, 2002).

### 3.6 References

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## ADDENDUM A: PRODUCT BRIEFS

### PRODUCT BRIEF: FISH STOCK

**Product description:**

A flavourful and authentic fish stock in frozen form. Produced from hake neck flesh, mirepoix and flavour-giving ingredients. Used as a base ingredient in the developed products.

**Applications:**

Used as the base of dishes such as soups, curries and casseroles containing seafood.

**Target market/distribution:**

Caterers, restaurants, industry

### PRODUCT BRIEF: FISH SPREAD

**Product description:**

The fish version of the well-known meat spreads (liver-, chicken & ham spread etc.) in plastic casings (see Table 1, Addendum A: Market research: product scouting). The spread has hake, a lean white fish with a delicate flavour, as its base and is flavoured with parsley which also adds colour.

**Applications:**

The product can be used in the same way as a meat spread: on toast or sandwiches as part of a meal or as a snack on crackers. More unconventional applications could be investigated such as using the spread as a topping for baked potatoes.

**Competition:**

Anchovy fish paste and fish patés such as smoked snoek paté are the existing *fish* products closest in concept to the fish spread. These products are however each in a class of its own because of their distinctive flavours, different packaging and higher price class. Meat spreads could also be seen as competitive products that could be used as a reference for comparison purposes.

**Unit size:**

125g and/or 200g: like existing meat spreads

**Price class:**

Approximately R4.00-R5.00/200g. Comparable to the price of existing meat spreads (see Table 1, Addendum A).

**Shelf life requirement:**

Should exceed the time needed for distribution by a sufficient number of days to ensure a reasonable remaining shelf life period.

**Legal aspects:**

Labelling and advertising criteria should be met (2002).

**Product properties:****Positive:**

- \* Nutritional benefits: Possibility of claims: fat content lower than similar meat products (11.61% versus 28.5% in liver spread). Contains mainly polyunsaturated fat while liver spread contains mainly saturated fat (Langenhoven, Kruger, Gouws & Faber, 1991).
- \* Fish products have a healthier image than meats.
- \* Convenience
- \* Novelty: product would appeal to the consumer who would like to try something new in place of the 'normal' liver spread on toast.

**Negative:**

- \* South Africans are a meat-consuming nation and extensive marketing might be needed to convince keen meat-eaters to try the product.

**Target consumers:**

A relatively inexpensive product with a wide target market: families from middle to higher income groups.

**Distribution:**

Supermarkets, general food stores

**Consumption location:**

Homes, school or work lunches

**Extra comments:** Other flavouring possibilities: tomato, onion, smoked flavour, pickled fish, curry.

**PRODUCT BRIEF: Curried FISH CHOWDER**

**Product description:**

A mildly curried fish chowder with an authentic fish stock as its base. The coarse vegetable- and fish pieces and the pasta rice in the chowder add to its colourful appearance. Will be sold in a stand-up pouch that can simply be cut open, emptied into a pot or microwavable container, heated and served as a complete meal. Must be kept refrigerated.

**Applications:**

Can be served as a starter or a complete meal with or without bread/cROUTONS/cream/joghurt et cetera.

**Unit size:**

One pouch could contain two servings or 500g.

**Competition:**

Pick & Pay and Woolworths' soup in pouches, canned soup (See Addendum A, Table 1).

**Price class:**

Suggested price: R12.00-R14.00/500g.

**Shelf life requirement:**

Comparable to the shelf life of, for example, Woolworths' soup in pouches that has a shelf life of eight to nine days (personal communication, J.Caroll, Food Technologist, Lombardi Foods). In preliminary shelf life testing, the shelf life was determined to be approximately eight days with a built-in safety margin of four days.

**Legal aspects:**

Labelling and advertising criteria should be met (Government Gazette, 2002).

**Product properties:**

*Positive:*

- \* Nutritional benefits: Possibility of claims: Low fat content (1.26%).
- \* Fish together with vegetables creates a healthy image: will appeal to target consumer.
- \* Convenience

*Negative:*

Some South African consumers might not be familiar with fish chowder/soup.

**Target consumers:**

Medium-high- to higher income groups.

Families

Health-conscious consumers.

**Distribution:**

Supermarkets. Could also be marketed in bulk to caterers, maybe in another, less expensive form of packaging.

**Consumption location:**

Homes

**PRODUCT BRIEF: CHAKALAKA HAKE**

**Product description:**

Chakalaka is a spicy vegetable dish popular in South Africa and contains carrots, tomato, onion, peppers, cabbage, chillies and spices. In canned Chakalaka hake, hake and authentic fish stock are added to the traditional chakalaka ingredients to produce a spicy fish-and-vegetable dish. Chakalaka hake can be made available in both a 'mild' and 'hot' version by varying its chilli-content. May be packaged in both cans and stand-up retortable pouches.

**Applications:**

Very versatile. A complete meal when served with a starch. Heated, it can be served with pasta/bread/rice/samp/mealie ‘pap’ or other grains. Topping for baked potatoes. Can be served cold: mix with mayonnaise and pasta to create a filling salad.

**Unit size:**

400g per container

**Competition:**

Canned Chakalaka pilchards is a similar product (see Table 1, Addendum A)

**Price class:**

Suggested price: R7.00-R8.00 per can, possibly more per pouch.

**Shelf life requirement:**

A long shelf life product: comparable to that of other canned foods.

**Legal aspects:**

Labelling and advertising criteria should be met (Government Gazette, 2002).

**Product properties:**

**Positive:**

- \* Nutritional benefits: Possibility of claims: Low fat content (1.45%).
- \* Fish together with vegetables creates a healthy image: will appeal to target consumer.
- \* Convenience

**Target consumers:**

Medium-high- to higher income groups.

Families

Health-conscious consumers.

**Distribution:**

Supermarkets.

**Consumption location:**

Homes

**Table 1: Market research: product scouting**

Product type	Brand	Packaging	Price	Nutritional information
Meat spreads	Woolworths' liver spread/ham spread etc.	Plastic casing	R3.99/200g-R4.99/200g	-
	Renown liver spread/ham spread etc.	Plastic casing	R3.99/125g (Pick & Pay)	Fat: 27.0%, Protein: 10.4% (liver spread) Fat: 22.0%, Protein: 9.0% (ham & chicken spread)
	Spekenham liver spread/ham spread etc.	Plastic casing	R6.69/250g (Pick & Pay)	-
Anchovy fish paste	Redro	Glass jar	R4.59/125g, R6.89/225g (Checkers)	-
	Peck's Anchovette	Glass jar	R4.69/125g, R6.99/225g (Checkers)	-
Fish paté	Woolworths' smoked snoek paté	Plastic container in carton covering	R8.99/150g	Fat: 19.2% Protein: 15.7% Energy: 1000kJ/100g
	Guy's Gourmet Foods smoked snoek paté	Plastic container	R13.39/150g (Checkers)	-
	Pick & Pay Foodhall Deli smoked snoek paté	Plastic container	R8.95/150g	Fat: 15.8% Protein: 8.9% Carbohydrate: 4.2% Energy: 805.7kJ/100g
Soup	Woolworths soup in stand-up pouches (Variety of flavours, including a fish chowder with hake)	Plastic pouch	R11.99-R16.99 (Fish chowder)/500g (two servings)	Fat: 3.0% Protein: 4.2% Energy: 570kJ/100g
	All Gold Soups (Condensed) (Wide flavour variety)	Can	R4.99/425g (Checkers)	-
Chakalaka pilchards	Tuna Marine Perlemoen Soup	Can	R8.79/425g (Checkers)	-
	Lucky Star	Can	R6.99/410g (Checkers)	-

\*Executed during February, March 2001

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## Characteristics of Chakalaka hake retort-sterilised at two processing temperatures and using two different food container types

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### SUMMARY

The influence of two processing temperatures [121°C (249.8°F) and 116°C (240.8°F)] and two different food container types (retortable pouch and can) on the sensory quality characteristics of 400g canned Chakalaka hake was investigated. A trained panel evaluated sensory characteristics of four treatments of Chakalaka hake that each used a different combination of container type and temperature, namely can at 116°C, retortable pouch at 116°C, can at 121°C and retortable pouch at 121°C.

A higher level of shininess was observed in the treatments processed at 121°C than in those processed at 116°C. The combination of higher temperature and shorter processing time applicable in the former may have had an enhancing effect on the gloss-giving property of the modified starch used in Chakalaka hake, or may have lead to a greater retention in colour, which in turn, resulted in products with more shine. The cans showed less water separation and lower levels of granularity than the pouches. This may have been due to the surface area to volume ratio of the pouches being larger than that of the cans, which may have resulted in a higher proportion of the contents overcooking in the former. The Chakalaka hake processed in cans had more chakalaka flavour than the pouch products. The fish in the treatments processed at 116°C was more juicy than that of the treatments processed at 121°C. This may be due to greater cooking losses at the higher processing temperature. The relatively long processing time of the product processed in cans at 116°C resulted in vegetables that were softer than that of the other treatments.

The results from the sensory evaluation suggested that processing of Chakalaka hake in cans at 121°C resulted in the product with the best sensory quality. Cans proved to be the superior container type and 121°C the superior processing temperature.

### KEYWORDS

Canning, still retort, processing temperature, retortable pouch, flexible packaging, sensory evaluation, fish products

### INTRODUCTION

One of the simplest and most widely used in-container heat treatments of food is sterilisation of cans in a batch-type still (non-agitating) retort (Holdsworth, 1997). The severity of this type of heat treatment can however produce substantial changes in the sensory characteristics of food. Damage to the colour, flavour and texture of foods can be minimised with a reduction in processing time (Fellows, 2000). In agitating retorts, sterilisation of fluid or semi-fluid foods is accelerated by agitation of the containers by means of axial or end-over-end rotation, which increases the effectiveness of natural convection currents. However, canned foods that are more viscous and which heat primarily by

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conduction, will not benefit from agitation during processing (Durance, 1997). Processing time can also be reduced by implementing a higher processing temperature and/or by using a container type with a smaller cross sectional diameter, i.e. a thin profile pack such as a retortable pouch. Higher temperatures permit the use of shorter times for microbial destruction and for achieving the required degree of lethality in canned foods (Potter & Hotchkiss, 1995).

The packaging is an essential element in thermal processing of foods as it is responsible for maintaining commercial sterility of the contents after processing by means of a hermetic seal (Holdsworth, 1997). Commercial sterility is the degree of preservation at which all pathogenic and toxin producing organisms have been destroyed, as well as those organisms which could grow and cause spoilage in the product under normal non-refrigerated conditions of handling and storage (Potter & Hotchkiss, 1995). Flexible retortable pouches are plastic containers with a multilayer structure that can serve as an alternative to the traditionally used metal cans (Holdsworth, 1997). Retort pouches of solid foods such as tuna have attained commercial acceptance and are perceived as having superior quality (Brody, 2002). The shelf life of retortable pouch products is comparable to that of foods in metal cans and as with cans, no refrigeration is required during storage (Lopez, 1987). The slim, rectangular shape of a retortable pouch allows for faster heat penetration to the cold point (i.e. the point that is last to reach the final heating temperature) during thermal processing than in a cylindrically shaped can with its relatively large cross sectional diameter. As a result, shorter processing times is required for equivalent lethality in pouches and overcooking of the product near the sides of the container is less likely. This means that a product of better sensory quality can be achieved from pouches than cans in the case of food products liable to quality loss due to excessive heat (Potter & Hotchkiss, 1995).

The aim of this study was to investigate the influence of two processing temperatures [121°C (249.8°F) and 116°C (240.8°F)] and two container types (retortable pouch and can), used to obtain a  $F_0$  value of 6 during processing, on the sensory quality characteristics of Chakalaka hake by means of analytical sensory evaluation. A minimum  $F_0$  value of 3 is prescribed for low acid canned food based on the heat treatment required for destroying *Clostridium botulinum* spores (personal communication, P. Truter, Food Scientist, SABS, Cape Town). In practice, a  $F_0$  value higher than 3 is advisable for the sake of building in a safety margin and because of the occurrence of non-pathogenic spoilage microorganisms more heat resistant than *C. botulinum* (Ababouch, 1999). Chakalaka is a spicy vegetable dish popular in South Africa which contains carrots, tomato, onion, peppers, cabbage, chillies and spices. In Chakalaka hake, the traditional chakalaka ingredients are combined with hake chunks and fish stock so as to produce a spicy fish-and-vegetable dish.

## MATERIALS AND METHODS

### **Equipment and containers used for canning**

A steam-type exhaust box and a batch-type still retort (H.G. Molenaar & Co. (Pty) Ltd., Paarl) were used during canning trials. 1M-sized multipurpose cans (Foodcan, Paarl) and 140mm x 210mm 'stand-up' retortable pouches (composition: 12µm polyester, 15µm nylon, 9µm aluminium foil, 100µm cast polypropylene) from Kohler flexible packaging (Cape Town) were used. The containers were each filled with 400g product.

### **Canning procedure**

Before commencement of the experiment, a formula, preparation method and thermal process were standardised for Chakalaka hake using a processing temperature of 116°C and metal cans as the container type. The aim was to obtain a commercially sterile product with acceptable sensory quality characteristics.

After the standardisation procedure, the five repetitions of the canning experiment were executed. During each repetition, four identical batches of Chakalaka hake were individually prepared using the standardised formula and preparation method (Table 1).

**Table 1: Formula and preparation method: Chakalaka Hake**

Ingredients:	Mass (g)	Percentage (%)
Hake chunks (Sea Harvest Frozen 'Chunky hake fillets')	880	35.1
Tomatoes, chopped canned	320	12.8
Fish stock	300	12.0
Onion; slices	240	9.6
Carrot; rings	200	8.0
Cabbage; roughly chopped	200	8.0
Green pepper; roughly chopped	140	5.6
Sugar	80	3.2
MAPS 281 modified starch (SAARChem, Cape Town)	45	1.8
Sunflower oil	35	1.4
Salt	28	1.1
Brown vinegar	16	0.6
Citric acid powder	8	0.3
Masala for medium curry	8	0.3
Chillies, fresh, seeded and finely chopped	6	0.2
<b>Total</b>	<b>2506</b>	<b>100</b>

**Method:**

1. Heat oil over low heat.
2. Sauté onion, green pepper and chillies in the oil.
3. Add carrot, cabbage and masala and sauté briefly.
4. Add mixture of starch, sugar, salt, citric acid and stir.
5. Add stock, tomato and vinegar. Bring to the boil while stirring.
6. Add fish, stir and cover.
7. Proceed with filling, sealing and thermal processing.

The batches for each repetition were prepared from ingredients purchased at the same time from the same source. Each batch represented a different treatment that made use of a specific combination of container type and temperature, namely can at 116°C (C116), retortable pouch at 116°C (P116), can at 121°C (C121) or retortable pouch at 121°C (P121). Since the differing processing variables necessitated different thermal processes, each treatment was retorted individually. The order in which the four treatments were prepared and retorted was randomly determined for each of the five repetitions. Directly after preparation of a product batch, containers of the applicable type (either pouches or cans) were filled with 400g product each. The temperature of the Chakalaka hake after filling was a minimum of 70°C. Pouches were immediately heat-sealed after filling, ensuring the minimum amount of headspace. A steam-type exhaust box was used for removal of air from filled cans before sealing. All containers were immediately placed into the retort and heat processed after sealing. To monitor the heat processing, certain containers were fitted with heat sensing thermocouples for measuring the change in temperature at the cold point during the retorting process. The thermocouples were linked to a computer that uses data logging software registering the change in temperature during processing to calculate the accumulated lethality ( $F_0$ ). Retorting by saturated steam was carried out using the applicable processing temperature (116°C or 121°C). A final target  $F_0$  value of 6 was obtained for each of the treatments. To prevent overshooting the specified final  $F_0$  of 6, trials were first executed to establish a smooth operation that ensured repeatable results. For every retorting session, the total retorting time from start to stop was recorded. After retorting, the containers were water-cooled under pressure inside the retort. To ensure repeatability, the handling, transport and storage of the containers prior to and after retorting were performed in a standardised manner similar to that in industry.

### Sensory analysis

A trained panel of six members evaluated samples of the four treatments of Chakalaka hake. During panel training, the panelists were exposed to the products to be evaluated and reference standards were used to illustrate low and high intensities of sensory attributes relevant to the product. An evaluation sheet (Addendum A) was drawn up and refined. The following sensory attributes were included in the evaluation: shininess, presence of water separation, granular appearance, chakalaka flavour, juiciness of fish and texture of vegetables. Table 2 gives definitions of the attributes as decided on by the panel members. An unstructured line scale of 100 mm was used for each attribute, the ends of the scale anchored with descriptive terms. The four treatments of Chakalaka hake were evaluated during five sessions over a period of three days, with each session using the samples from the corresponding block replicate (see Statistical procedures). Samples at room temperature were randomly served in small, white, plastic containers; each coded with a three digit random number. Apple and distilled water were used for cleansing the palate between tastings.

During panel training, the following sensory characteristics were decided upon as being desirable for Chakalaka hake: a shiny appearance, little water separation and granularity, a prominent chakalaka flavour, juicy fish and vegetables that are cooked until tender, but still firm (*Al dente*).

**Table 2: Definition of terms used in the sensory evaluation of Chakalaka hake**

Attribute	Attribute definition
Shininess	Overall shiny appearance of product.
Presence of water separation	Presence of visible free water in the product.
Granular appearance	Presence of visible small particles in the sauce.
Chakalaka flavour	A spicy, sweet-and-sour flavour characteristic of the product.
Juiciness of fish	Degree/amount of moisture inside the piece of fish released upon chewing.
Texture of vegetables	The firmness of the vegetable piece upon first bite.

### Statistical procedures

The experiment consisted of four treatment combinations (C116, P116, C121 and P121) that were replicated five times in a randomised complete block design. The treatment design was a 2×2 factorial experiment with the two processing temperatures (116°C, 121°C) and the two food container types (can, retortable pouch) as factors. Factorial analyses of variance (ANOVA) were performed on the sensory measurements using SAS statistical software (SAS, 1999). The Shapiro-Wilk test was performed to test for non-normality in the distribution curve of the data (Shapiro & Wilk, 1965). Student's t Least Significant Difference (LSD) was calculated at the 5% significance level to compare interaction means. Main effect means were compared using the F-ratio test.

The mean measurements (scores) for the sensory attributes were evaluated against the characteristics considered desirable for Chakalaka hake (see Sensory analysis) so as to determine which combination of container type and temperature resulted in the product with the best sensory quality.

## RESULTS AND DISCUSSION

### Retorting time

The average total retorting times (from retort start to stop) for the five repetitions were: 72 min for C116; 64 min for P116; 58 min for C121 and 53 min for P121.

### Sensory analysis

The results from the test for non-normality and the analyses of variance are summarised in Table 3. In the case of the attribute texture of vegetables, there was significant ( $P<0.05$ ) evidence for non-normality. However, since this deviation from normality was due to kurtosis and not skewness, data interpretation could continue normally (Glass *et al.*, 1972). Interaction between panel member and treatment was significant ( $P<0.05$ ) for all the attributes, except chakalaka flavour. However, analyses that respectively excluded and included a panel member that deviated from the others gave similar results. The full data set was therefore used.

For all the attributes evaluated, treatment ( $2\times 2$  factorial) was a significant ( $p<0.05$ ) source of variation. This required further breakdown of the  $2\times 2$  factorial into interaction effects (Container type  $\times$  Temperature) and main effects (Container type, Temperature). In the case of the attributes shininess, presence of water separation, granular appearance and texture of vegetables, the interaction effect was significant ( $P<0.05$ ) and the respective interaction means were examined. In this case, no inferences can be made regarding the influences of the main effects. In the case of the attributes chakalaka flavour and juiciness of fish, the interaction effect was not significant ( $p>0.05$ ) and the main effects were further examined. Container type, but not processing temperature, had a significant ( $P<0.05$ ) effect on chakalaka flavour. Processing temperature, but not container type, on the other hand, significantly influenced the juiciness of the fish ( $P<0.05$ ). For these two attributes, the main effect means were compared using the F-ratio test. The interaction and main effect means for the measurements of the sensory attributes are given in Table 4.

As can be seen from Table 4, treatments P121 and C121 were significantly ( $P<0.05$ ) more shiny than C116, which were, in turn, significantly ( $P<0.05$ ) more shiny than P116 (63.6). P121 obtained the highest score for shininess. MAPS 281 is a modified waxy maize starch used as thickener in Chakalaka hake. Modified starches are natural starches that have been treated chemically to create a specific change in chemical structure, e.g. crosslinking of molecules, so as to create new physical properties that are desirable in specific applications of food manufacturing (Bennion, 2000). One of the functional properties of MAPS 281 is that it provides a good gloss when cooked (Starch Australasia, 1999). The combination of higher temperature and shorter processing time applicable in both P121 and C121 appeared to have had an enhancing effect on the gloss-giving property of MAPS 281. Another factor that may have influenced the shininess of the product, is the brightness in colour. Time-temperature combinations used in canning have a substantial effect on most naturally occurring pigments in food (Fellows, 2000). The higher degree of shininess in P121 and C121 may be linked to a greater retention in colour obtained with the combination of higher temperature and shorter time applicable in these treatments.

A high presence of water separation in the samples coincided with a high presence of granularity. The cans showed less water separation and lower levels of granularity than the pouches (Table 4). In this investigation, a fixed processing temperature (116°C or 121°C) was used to obtain a final  $F_0$  value of 6, with the time period of heating being the variable factor. However, the surface area to volume ratio of the pouches is larger than that of the cans and could thus have resulted in a higher proportion of the contents overcooking in the former, which may have caused the observed differences.

The Chakalaka hake processed in cans had significantly ( $P<0.05$ ) more chakalaka flavour than the pouch products. The result cannot be explained due to the complex nature of flavour reactions occurring during thermal sterilisation (Fellows, 2000).

The fish in the Chakalaka hake processed at a processing temperature of 116°C was significantly ( $P<0.05$ ) more juicy than that of the product processed at 121°C. Changes in the texture of fish such as loss of juiciness during thermal processing are caused by coagulation of proteins and a decrease in their water holding capacity (Fellows, 2000). The effect of different oven temperatures on properties of baked salmon was investigated in a study by Charley and Goertz (1958) and total cooking losses were found to increase as oven temperature increased. Cooking losses may be related to sensory characteristics, especially juiciness (Bowers & Kropf, 1992). The finding of Charley and Goertz (1958) therefore corresponds with the result that a higher processing temperature leads to loss of juiciness in the flesh of Chakalaka hake.

C121 had the highest score for texture of vegetables (i.e. least soft vegetables). The vegetables from C116 were significantly ( $P<0.05$ ) softer than that of the other treatments. The latter can be attributed to the relatively long processing time of C116 (Potter & Hotchkiss, 1995).

**Table 3: Results of test for non-normality and analyses of variance (ANOVA)**

SOURCE	DF*	Shininess		Water separation		Granular appearance		Chakalaka flavour		Juiciness of fish		Texture of vegetables	
		MS**	P***	MS	P	MS	P	MS	P	MS	P	MS	P
<b>Repetition</b>	4	30.2	0.21	18.8	0.55	166.5	<0.01	51.6	0.59	201.8	0.02	191.3	0.21
<b>Panel member</b>	5	582.7	<0.01	1694.6	<0.01	2965.3	<0.01	92.2	0.29	677.8	<0.01	3153.6	<0.01
<b>Panel member × Repetition</b>	20	21.1	0.43	40.1	0.06	32.00	0.46	99.9	0.17	189.8	<0.01	190.4	0.11
<b>Treatment (2x2 factorial)</b>	3	2685.7	<0.01	4012.6	<0.01	3973.0	<0.01	316.7	<0.01	323.0	<0.01	768.4	<0.01
Container-type	1	681.6	<0.01	8433.6	<0.01	9937.2	<0.01	700.8	<0.01	2.1	0.86	53.3	0.52
Temperature	1	6307.5	<0.01	1763.3	<0.01	1128.5	<0.01	140.8	0.17	940.8	<0.01	1333.3	<0.01
Cont. type × Temp.	1	1068.0	<0.01	1840.8	<0.01	853.3	<0.01	108.3	0.23	26.1	0.53	918.5	<0.01
<b>Panel member × Treatment</b>	15	178.6	<0.01	312.4	<0.01	193.8	<0.01	98.3	0.20	268.1	<0.01	497.1	<0.01
Panel member × Container type	5	86.4	<0.01	581.2	<0.01	482.5	<0.01	34.2	0.80	127.2	0.10	78.3	0.7
Panel member × Temperature	5	339.4	<0.01	179.2	<0.01	19.1	0.70	233.3	0.01	618.2	<0.01	1375.3	<0.01
Panel m. × Cont. type × Temp.	5	109.9	<0.01	176.7	<0.01	79.7	0.04	27.6	0.86	58.9	0.49	37.8	0.91
<b>Error</b>	72	20.3		24.3		31.7		72.8		65.6		126.3	
<b>Corrected Total</b>	<b>119</b>												
<b>Non-normality</b>	P=0.1266		P=0.1246		P=0.6846		P=0.4263		P=0.9789		P=0.0201		

\*DF=degrees of freedom, \*\*MS=mean square, \*\*\*P=probability of F ratio (An effect with P≤0.05 is considered significant).

**Table 4: Sensory analysis of four treatments of Chakalaka hake (interaction and main effect means)**

FACTORS		SENSORY ATTRIBUTES					
		Shininess	Water separation	Granular appearance	Chakalaka flavour	Juiciness of fish	Texture of vegetables
Inter-action effects	<b>Can</b>	<b>116°C</b>	74.3 <sup>b</sup>	48.7 <sup>a</sup>	48.5 <sup>a</sup>	73.6 <sup>a</sup>	68.9 <sup>a</sup>
	<b>Can</b>	<b>121°C</b>	82.9 <sup>a</sup>	48.8 <sup>a</sup>	47.7 <sup>a</sup>	73.9 <sup>a</sup>	64.2 <sup>b</sup>
	<b>Pouch</b>	<b>116°C</b>	63.6 <sup>c</sup>	73.3 <sup>c</sup>	72.0 <sup>c</sup>	66.9 <sup>b</sup>	69.6 <sup>a</sup>
	<b>Pouch</b>	<b>121°C</b>	84.1 <sup>a</sup>	57.8 <sup>b</sup>	60.5 <sup>b</sup>	70.9 <sup>ab</sup>	63.0 <sup>b</sup>
<b>*LSD (P=0.05)</b>		2.3174	2.5354	2.8983	4.3928	4.1673	5.7849
Main effects	<b>Can</b>		78.6	48.8	48.1	73.7	66.6
	<b>Pouch</b>		73.8	65.5	66.3	68.9	66.3
	<b>P</b>	<0.01	<0.01	<0.01	<0.01	0.86	<0.01
	<b>116°C</b>	69.0	61.0	60.2	70.2	69.2	42.2
	<b>121°C</b>	83.5	53.3	54.1	72.4	63.6	48.8
	<b>P</b>	<0.01	<0.01	<0.01	0.17	<0.01	<0.01

a-c: Values with different superscripts differ significantly ( $P \leq 0.05$ ). Scores assigned 'a' considered more desirable than scores assigned 'b', which are, in turn, considered more desirable than scores assigned 'c'.

\*LSD=Least significant difference

## CONCLUSIONS

By evaluating the sensory results against the characteristics considered desirable for Chakalaka hake, it was concluded that the Chakalaka hake processed in cans at 121°C (C121) had the best overall sensory quality. For C121, all of its attributes evaluated, except juiciness of fish, were assigned ‘a’ superscripts (see Table 4). This indicated that the sensory quality of C121 were closest in nature to that considered desirable for Chakalaka hake. The results also suggested that cans were the superior container type and 121°C the superior processing temperature. The results indicated that the shorter processing time obtained with a higher processing temperature resulted in a product of better sensory quality. Contrary to all expectations, using retortable pouches instead of cans did not result in better sensory quality despite a shorter processing time.

## ACKNOWLEDGEMENTS

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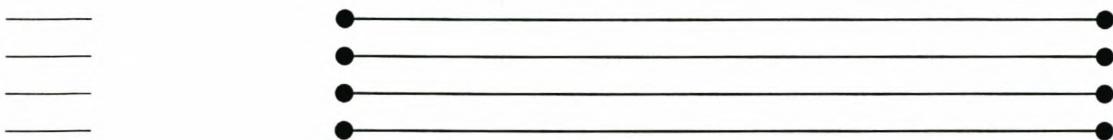
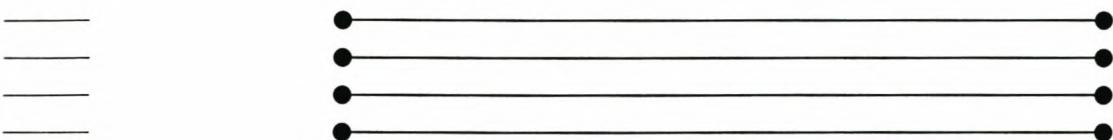
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**ADDENDUM A****Evaluation of Chakalaka Hake***Date:* \_\_\_\_\_*Set no:* \_\_\_\_\_ *Name of panel member:* \_\_\_\_\_**Instructions**

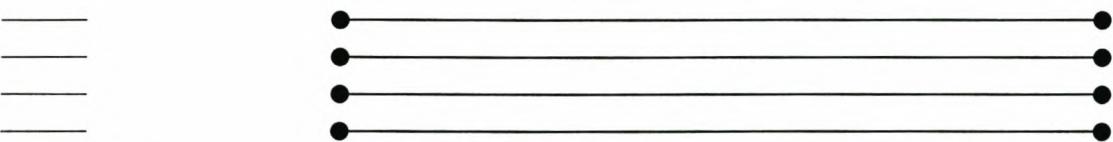
- Evaluate from left to right
- Refresh mouth with apple and water between each sample

**Appearance: View samples****Shininess:**

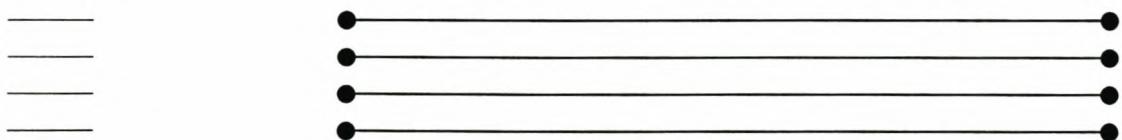
Not shiny 0 \_\_\_\_\_ 100 Very shiny

**Presence of water separation** No water separation present 0 \_\_\_\_\_ 100 Water separation present**Granular appearance of sauce:**

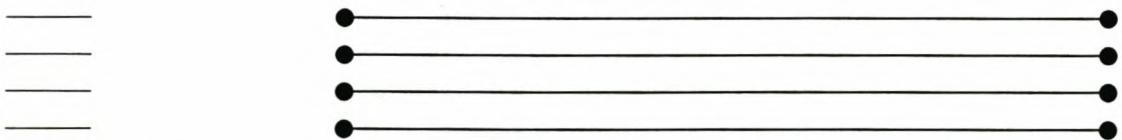
No granularity 0 \_\_\_\_\_ 100 Granularity present

**Flavour and Texture: Taste samples and refresh mouth between samples****Chakalaka flavour:**

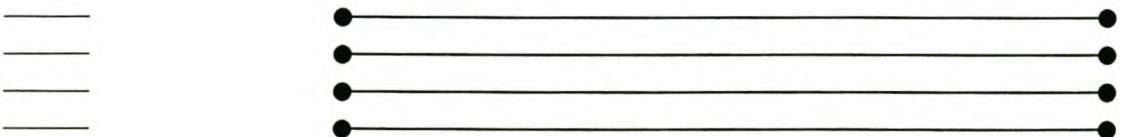
Less prominent chakalaka flavour 0 \_\_\_\_\_ 100 Prominent chakalaka flavour

**Texture of fish:**

No juiciness 0 \_\_\_\_\_ 100 Juicy

**Texture of vegetables:**

Excessively soft texture 0 \_\_\_\_\_ 100 'Al dente' texture



# Preservation of fish spread with enterocins 1071A and 1071B, two antimicrobial peptides produced by *Enterococcus faecalis* BFE 1071

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## KEYWORDS

Fish spread, enterocins, preservatives, shelf life studies

## SUMMARY

Enterocins 1071A and 1071B, two bacteriocins produced by *Enterococcus faecalis* BFE 1071, were evaluated as preservatives in fish spread. Changes in microbial cell numbers, pH and histamine content were determined over 21 days of storage at 4°C. The total aerobic cell numbers recorded for a batch of fish spread preserved with enterocins 1071A and 1071B, added at  $1.2 \times 10^5$  antimicrobial units (AU)/g fish spread, remained between  $8 \times 10^4$  and  $2 \times 10^5$  cfu/g for the first six days of storage. During the next 12 days of storage, the cell numbers increased to  $5 \times 10^6$  cfu/g in the same batch. The total aerobic cell numbers in a batch preserved with a combination of sodium benzoate (0.045%, m/m) and potassium sorbate (0.059%, m/m) remained between  $8 \times 10^4$  and  $2 \times 10^5$  cfu/g during the first 18 days of storage. Samples tested at day 21 yielded total aerobic cell numbers of  $1 \times 10^7$  cfu/g and  $5 \times 10^6$  cfu/g for batches preserved with enterocins and a combination of sodium benzoate and potassium sorbate, respectively. In a batch containing no preservatives, cell numbers gradually increased from  $1 \times 10^5$  cfu/g to  $1 \times 10^8$  cfu/g over 21 days. Colonies selected from Baird-Parker Agar plates were identified as being coagulase negative staphylococci, most probably *Staphylococcus epidermidis*. The histamine content was less than 0.1mg/100g fish spread, independent of the preservative used. The pH of the three batches remained constant over the 21-day storage period. Minimal variations were recorded in the textural and organoleptic qualities of the three batches.

## INTRODUCTION

Bacteriocins are defined as proteins or protein complexes antagonistic against bacteria genetically closely related to the producer organism (Tagg *et al.*, 1976; Klaenhammer, 1988). Many papers have been published on the production, isolation and characterisation of bacteriocins from lactic acid bacteria, mainly *Lactobacillus*, *Lactococcus* and *Pediococcus* spp. (reviewed by De Vuyst & Vandamme, 1994; Jack *et al.*, 1995). Although the spectra of antimicrobial activity of a variety of bacteriocins, especially from the genera *Lactobacillus* and *Lactococcus* (reviewed by De Vuyst & Vandamme, 1994) are well described, little data has been published on the incorporation of these peptides in food and the *in situ* evaluation of their antimicrobial potential.

A strain of *Lactobacillus sakei*, which produces sakacin A, was evaluated with regard to its ability to control *Listeria monocytogenes* in minced meat stored at 8°C (Schillinger *et al.*, 1991). Although sakacin inhibited *L. monocytogenes* in the minced meat, the activity was less than recorded in MRS (De Man *et al.*, 1960) broth. A possible explanation for the lower activity could be the binding of sakacin to lipids, similar to what has been observed for nisin, a lantibiotic

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produced by *Lactococcus lactis* subsp. *lactis* (Scott & Taylor, 1981). Although the *in situ* activity of sakacin decreased after nine days at 8°C, the cells did not gain resistance to the bacteriocin and remained sensitive. Similar results were recorded for sakacin-producing strains incorporated in spiced and cured raw pork sausages (Schillinger *et al.*, 1991), as well as vacuum packed bologna type sausages (Schmidt & Kaya, 1990).

To date, seven bacteriocins of *Enterococcus faecalis* have been described, of which four have been biochemically and genetically characterised (reviewed by Balla *et al.*, 2000). None of these bacteriocins have been evaluated as a preservative in any food product. Enterocins 1071A and 1071B, two bacteriocin-like peptides produced by *Enterococcus faecalis* BFE 1071 (Balla *et al.*, 2000), differ from Type 1 enterocins (Ike *et al.*, 1990, 1992) in that they are not hemolytic, have an activity spectrum narrower than that recorded for the cyclic peptide antibiotic AS-48 (Martínez-Bueno *et al.*, 1994) and Type 2 enterocins (Fujimoto *et al.*, 1995; Tomita *et al.*, 1997), but broader than that recorded for Type 3 enterocins (Tomita *et al.*, 1996). Enterocins 1071A and 1071B are active against *Enterococcus* spp., *Lactobacillus salivarius* subsp. *salivarius*, *Listeria innocua*, *Microcooccus* sp., *Peptostreptococcus aerogenes*, *Propionibacterium freudenreichii* subsp. *shermanii* and *Streptococcus agalactiae*. Unlike sakacin and many other group II bacteriocins, enterocins 1071A and 1071B causes cell lysis, most likely due to destabilisation of the plasma membrane as proposed by the 'barrel stave' mechanism (Balla *et al.*, 2000; Ojcius *et al.*, 1991).

In this paper, we evaluated enterocins 1071A and 1071B as preservatives of fish spread.

## MATERIALS AND METHODS

### **Isolation and partial purification of enterocins 1071A and 1071B**

*Enterococcus faecalis* BFE 1071 was grown in MRS broth (Biolab, Biolab Diagnostics, Midrand, South Africa) at 37°C until mid-logarithmic growth ( $OD_{600} = 1.8$ ). The cells were harvested (8000 x g, 10 min, 4°C) and the bacteriocins isolated from the supernatant by ammonium sulfate precipitation (75% wt/vol, final conc.). The precipitate was collected by centrifugation (14000 x g, 20 min), resuspended in MilliQ water and desalted (overnight at 8°C) by using a 1 kDa cut-off dialysis membrane (Spectrum, Los Angeles, USA). The dialyzed sample was freeze-dried and stored at room temperature. The antimicrobial activity of the partially purified (crude extract) enterocins was determined according to the methods described by Balla *et al.* (2000).

### **Preparation of the fish spread and preservation**

A base mixture for the fish spread consisted of 61.55% (m/m) fresh minced hake flesh, obtained from a local dealer, 14.78% (m/m) fish stock (boiled and cooled), 12.31% (m/m) Canola oil, 3.69% (m/m) Pettina pregelatinised starch (South Bakels (Pty) Ltd., Cape Town), 2.22% (m/m) fresh parsley, 1.97% (m/m) sodium caseinate, 1.48% (m/m) sugar, 0.98% (m/m) salt, 0.98% (m/m) lemon juice and 0.05% (m/m) black pepper. The hake flesh was cooked in the fish stock and cooled before it was minced with the other ingredients to form a fish spread. The fish spread was divided into three equal batches. To the first batch, 1.0% (m/m) of the crude extract of enterocins 1071A and 1071B was added (i.e.  $1.2 \times 10^5$  AU/g fish spread) and the batch was labelled 'enterocin batch'. The second batch received a combination of sodium benzoate (0.045%, m/m) and potassium sorbate (0.059%, m/m) and was labelled 'artificial preservative batch'. The third batch received no preservatives and was labelled 'control batch'. The batches of fish spread were then stuffed into plastic casings (6.5cm in diameter) and the open ends tied with string. The stuffed casings were pasteurised in water at 78-82°C. At a core temperature of 68°C, measured with a SAFOMA TTX 290 SKW core thermometer

(Geiger & Klotzbücher, Cape Town, South Africa), the samples were removed and cooled in ice water. The samples were packed in an airtight plastic container and stored at 4°C for the duration of the 21-day investigation period.

#### Evaluation of shelf life

Microbiological and histamine tests were based on specifications for cooked seawater food set by the South African Department of Health (Government Gazette, 1997).

#### Microbiological analyses

Every third day, over a period of 21 days, a sample was aseptically taken from each batch of fish spread, serially diluted in sterile peptone water and plated onto Plate Count Agar (PCA, Merck). The plates were incubated at 37°C for three days. Colonies from two plates were counted and the average cell count determined. On days 3, 6, 15 and 18 of storage, samples were analysed for the presence of *Salmonella* spp., *Shigella* spp., *Escherichia coli* Type 1, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* (coagulase positive) and coliforms other than *E. coli*. The specifications and the growth media used in the microbiological analyses are listed in Table 1.

**Table 1: Specifications and growth media used in microbiological analysis of fish spread**

Test	Growth medium	Specification (Government Gazette, 1997)
Viable cell count (aerobic)	Plate Count Agar	Less than $1 \times 10^5$ cfu/g
<i>Salmonella</i> spp.	<i>Salmonella-Shigella</i> Agar	No <i>Salmonella</i> spp. in 20g
<i>Shigella</i> spp.	<i>Salmonella-Shigella</i> Agar	No <i>Shigella</i> spp. in 20g
<i>Escherichiae coli</i> Type 1	Violet Red Bile Lactose (VRBL) Agar)	No <i>E. coli</i> Type 1 in 20g
<i>Vibrio cholerae</i> and <i>Vibrio parahaemolyticus</i>	<i>Vibrio</i> Selective (TCBS) Agar	No <i>V. cholerae</i> or <i>V. parahaemolyticus</i> in 20g
<i>Staphylococcus aureus</i> (coagulase positive)	Baird-Parker Agar Base with Egg Yolk (EY) and Tellurite	No <i>S. aureus</i> (coagulase positive) in 20g
Coliforms other than <i>E. coli</i>	Violet Red Bile Lactose Agar	Less than $1 \times 10^3$ cfu/100g

#### Histamine detection

The presence of histamine was tested for on day 22 of storage. Histamine was extracted with trichloroacetic acid (TCA) and the concentration determined by using high pressure liquid chromatography, according to the method described by Pozo & Saitua (1988).

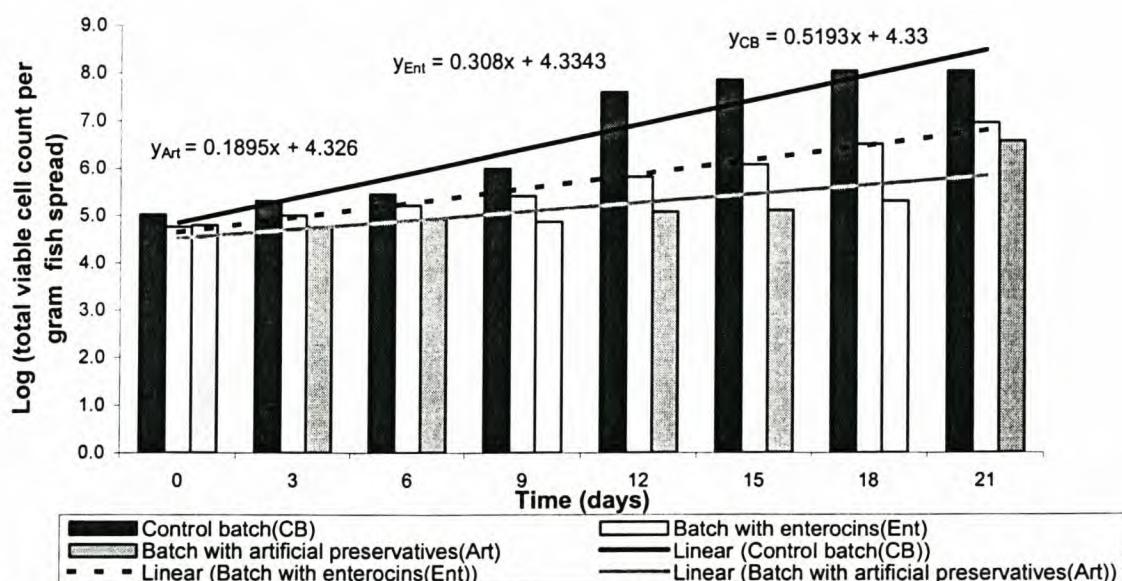
#### pH tests

The pH of the samples was measured with a Beckman 100 series ISFET pH meter (Beckman Coulter, Cape Town, South Africa) with a Smart™ ISFET probe. A change in pH was considered to be an indication of microbial spoilage.

## RESULTS AND DISCUSSION

The enterocin content in the ammonium sulfate precipitated fraction (protein crude extract) was calculated at 12 000 AU mg<sup>-1</sup> protein. At 1% (m/m) crude extract added to the fish spread, it resembled  $1.2 \times 10^5$  AU/g fish spread.

The total number of aerobic microbial cells recorded per gram fish spread, over the 21-day storage period, is presented in Fig. 1. The cell numbers recorded for the enterocin batch remained between  $8 \times 10^4$  and  $2 \times 10^5$  cfu/g for the first six days of storage, followed by an increase to  $5 \times 10^6$  cfu/g during the next 12 days of storage. The artificial preservative batch yielded between  $8 \times 10^4$  and  $2 \times 10^5$  cfu/g during the first 18 days of storage. At 21 days of storage cell numbers of  $1 \times 10^7$  cfu/g and  $5 \times 10^6$  cfu/g were recorded for the enterocin batch and the artificial preservative batch, respectively. In the control batch, cell numbers gradually increased from  $1 \times 10^5$  cfu/g to  $1 \times 10^8$  cfu/g over 21 days. Linear regressions were fitted to the data (Fig. 1), to quantify the increase in bacterial counts (log of the total viable cell count per gram of fish spread) over time. The rate of increase in the artificial preservative batch (0.1895) was nearly half of that in the enterocin batch (0.308), which was also lower than that in the control batch (0.5193).



**Fig. 1.** Total viable cell counts recorded during cold storage of fish spread batches containing enterocins 1071A and 1071B, artificial preservatives and no preservatives, respectively.

Colonies selected from Baird-Parker Agar plates were black and shiny. Pure cultures were Gram-positive, thus representing *Staphylococcus* spp. At each time of testing, the highest viable cell count of staphylococci was recorded in the control batch, the second highest cell count in the enterocin batch, while the artificial preservative batch yielded the lowest cell count of staphylococci. The cell numbers were less than  $3 \times 10^4$ /g of fish spread on day 3 of storage for all three batches. In the artificial preservative batch, the cell numbers remained constant. Cell numbers increased to  $1.2 \times 10^5$ , and then decreased slightly, while it increased to more than  $3 \times 10^5$  on days 15 and 18 of storage, in the enterocin and control batch, respectively. None of the colonies on Baird-Parker Agar produced clear zones, suggesting that the isolates were not coagulase positive and therefore not members of *S. aureus* (Kloos & Schleifer, 1986, Lancette & Tatini, 1992). Further tests indicated that the isolates were non-haemolytic (personal communication, L. de Bruyn, Western Cape Veterinarian Laboratory, Stellenbosch) staphylococci and most probably members of the species *Staphylococcus epidermidis*.

Growth on TCBS Agar was only recorded for the control batch and the enterocin batch. The number of viable cells recorded on days 3 and 6 of storage was less than  $3 \times 10^4$  for both of these batches. In the enterocin batch, the cell counts increased to  $6.1 \times 10^4$  and then to  $1 \times 10^5$  on days 15 and 18 of storage, respectively, while an increase to  $3 \times 10^5$  was recorded in the control batch. The colonies were opaque and yellow with surrounding yellow zones, typical of *Vibrio* spp. (Baron, Peterson & Finegold, 1994). Isolates from these colonies were Gram-positive, non-motile and oval

cocci. Since *Vibrio* spp. are Gram-negative, motile and curved rods (Desmarchelier, 1999), these isolates did not belong to the genus *Vibrio*. It may very well have been sucrose fermenting *Proteus* spp., which produce yellow colonies on TCBS agar (Macfaddin, 1985).

The histamine content on day 22 of storage was less than 0.1mg/100g for all three batches, i.e. less than 1% of the cut-off value of 10mg/100g. Fish with a histamine content of more than 10mg/100g food is considered spoiled, whilst a concentration of higher than 20mg/100g renders it unsafe for human consumption (Government Gazette, 1997).

The pH of the three batches remained essentially constant over the 21-day period.

## **CONCLUSIONS AND RECOMMENDATIONS**

Based on results obtained in this study, enterocins 1071A and 1071B did preserve the fish spread, but to a lesser extent than a combination of sodium benzoate and potassium sorbate. The level of sodium benzoate and potassium sorbate used was the minimum for this type of product (personal communication, Dr. F. Mellett, Food Technologist, Freddy Hirsch Group, Cape Town). The level of enterocin crude extract used was chosen arbitrarily, since recommended levels are not available. The preserving agents used in this study were therefore not necessarily included at optimum levels. An improved preservative effect could be possible with higher levels of the preserving agents. Another possibility that could be investigated is to use a combination of the peptides and artificial preservatives, and at various ratios.

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## 6. CONCLUSIONS

The focus of this study, as one branch of the Fish Waste Utilisation Program (FWUP), was the development of value-added food products produced from the neck flesh of Cape hake as a means of adding value to hake heads. Three product prototypes, namely curried fish chowder, fish spread and canned Chakalaka hake, were developed on an experimental scale. A formula for fish stock, which was used as a base ingredient in the three product prototypes, was also standardised. For the purpose of product development, neck flesh cuts were deboned manually to produce chunks of flesh and the fish stock was prepared with cuts of neck flesh as is (i.e. without deboning). The neck flesh was however also substituted for other raw materials (hake fillets and -mince in the product prototypes and hake frames in the stock) with very satisfactory results. The option therefore exists for the products to be produced using raw materials other than neck flesh, depending on factors such as availability and cost. A possibility that has not been investigated, but which will also be in support of the main aim of improving the total utilisation of fish resources, is to also make use of other fish species (e.g. by-catch) in the production of the developed products and fish stock.

Canned foods have a long history and are likely to remain popular for the foreseeable future due to their convenience, long shelf life and economy. Since food canning is such a mature technology, it might be assumed that the potential for further development in this field is very limited. However, the technology of thermal sterilisation of food proceeds to evolve and recent developments have focused on aspects such as improvement in sensory quality and alternative packaging (Brody, 2002). In this study, the investigation into the influence of two container types and two processing temperatures on the sensory characteristics of canned Chakalaka hake indicated that a higher processing temperature lead to an improvement in the sensory quality characteristics of the product. Contrary to all expectations, using retortable pouches instead of cans did not result in an overall improvement in sensory quality, despite a reduction in processing time. The conclusion was that the delicate nature of the ingredients used in Chakalaka hake renders this product less suitable for flexible packaging and that the physical protection supplied by a rigid container type is required. The finding indicates the need for developments in thermal processing to be product-specific. Further improvement in the sensory quality of Chakalaka hake may be possible with technologies such as variable retort temperature (VRT) processing. The latter is specifically applicable to conduction-heated foods in hermetically sealed containers (Durance, 1997).

As mentioned, consumer preferences are turning towards food that are free from chemical preservatives and increased interest is focused on the use of microbial metabolites such as bacteriocins to inhibit the growth of spoilage- and pathogenic micro-organisms in food (Ross, Morgan & Hill, 2002). In this study, enterocins 1071A and 1071B, two bacteriocin-like peptides produced by *Enterococcus faecalis* BFE 1071, were evaluated as biological preservatives in the fish spread prototype. Enterocins 1071A and 1071B did preserve the fish spread, but to a lesser extent than a combination of sodium benzoate and potassium sorbate. The enterocin crude extract used in the investigation was however not necessarily included at an optimum level and it was proposed that higher levels could possibly lead to an improved preservative effect. Another suggestion was to investigate the use of a combination of the peptides and artificial preservatives, at various ratios. The use of enterocins as biological preservatives has not been evaluated in any other food product. A possibility would be to investigate their preservative effect in red meat products. The enterocin crude extract used in the fish spread has a 'barbecue-like' smell and flavour, which could be well suited to red meat products.

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