ANALYSES OF MICROBIAL HAZARDS OF LAKE VICTORIAN NILE PERCH (*Lates niloticus*) PROCESSING AT INDUSTRIAL LEVEL IN KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for the Master of Science degree in Microbiology with our approval.

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DEDICATION

I dedicate this thesis to my beloved wife, Ann Wangechi and my beloved mum, Josephine Waithira Mungai whose support; encouragement and understanding made completion of this work possible.

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ABSTRACT

The quality of processed seafood produced for international market should conform to international standards. Over the last few decades, major seafood importing countries have introduced hygiene regulations and legislation, including definitive standards for fishery products. In 1991, European Union (EU), which is the largest single market for Kenya's Nile perch products established regulations laying down health conditions for production and the placing on the market of fishery products. The Kenyan Niie perch industry has been caught unaware and is not geared to meet such requirements. This has led to a substantial rejection of consignments and subsequent economic losses. Decomposition and the presence of pathogenic organisms have been indicated as the main reasons for most of these rejections.

To meet the EU requirements, Nile perch processing establishments have to implement a quality control system based on the Principles of Hazard Analysis Critical Control Point (HACCP). Implementation of such a system requires an assessment of microbial hazards likely to occur during the processing of Nile perch.

To address this requirement, analyses of microbial hazards during the processing of Nile perch were conducted in two establishments over a period of eight months. The analyses consisted of observing all operations of fish processing, measuring temperature of fish throughout the processing line and collecting samples and testing them for common fish-borne pathogens and indicator microorganisms. Among the procedures followed for microbiological examination of samples are those

recommended by the International Commission on Microbiological Specifications for Foods (ICMSF).

Results showed that the fish received at the processing establishments had aerobic mesophilic colony count (AMCC) of less than 10^6 CFU /g with hydrogen sulphide (H_2S) producing bacteria accounting for less than 3 % of AMCC. Enterobacteriaceae, coliforms, faecal coliforms and *Staphylococcus aureus* counts of raw fish were within the limits recommended by ICMSF. *Vibrio cholerae* and *Clostridium perfringens* were isolated only from the incoming whole fish and their incidences in both establishments were 1.4 % and 0.5 % respectively. *Salmonella* was present in 5% of incoming whole fish and in 16.7 % of fish samples collected after the fish was filleted in one establishment. *Shigella* spp. was not recovered from any fish sample.

The processing operations in both establishments resulted in significant reduction of all the microbial parameters tested. However, it was observed that certain microbial hazards existed during the fish processing operations in both establishments, particularly under circumstances of poor handling of fish. Whole fish washing, filleting, beheading and gutting, fillet skinning, control of fish temperature and sanitation and establishment hygiene were identified as Critical Control Points (CCPs) at or by which the identified microbial hazards could be controlled. The study recommended that Nile perch processing establishments should put in place control measures aimed at eliminating, preventing or minimising the microbial hazards.

CHAPTER ONE

1.0 INTRODUCTION

Lake Victoria is the second largest lake in the world and lies across the equator (1^0 00' S, 33^0 00' E) about 350 km West of Nairobi (Jansen, 1996). The lake is shared between Kenya (6 %), Uganda (45 %) and Tanzania (49 %). The surface area is some 68,000 Km^2 and the shoreline length is 3400 Km. The Lake is estimated to provide a living for about 50,000 to 70,000 fishermen; Kenyan fishermen alone numbering about 20,000 (Fisheries Department, 1998).

Nile perch (*Lates niloticus* (Linn.)), which is a predatory fish of the Centropomidae family, was introduced in Lake Victoria near Jinja, Uganda in 1959 (Payne, 1987; Ogunja *et al.*, 1992). The Nile perch spread around the lake in a clockwise direction, probably correlated with the pelagic habit of the larvae and the movement of surface currents (Hughes, 1983). The rapid proliferation of this large fish started in the Kenyan part of the lake after a lag-phase of 15-20 years and is today the major fish catch from Lake Victoria in all the three riparian states (Reyholds and Greboval, 1988). Thus, out of a total catch in the Kenyan waters of Lake Victoria of 16,581 metric tonnes (MT) in 1975, only 51 MT were Nile perch (0.3 %). In 1978, 1,000 MT of Nile perch were landed, in 1981 23,000 MT and in 1985 the production increased to 50,000 MT. The Nile perch landings rose to a peak of 123,000 MT in 1991, and have since been on a generally declining trend (Othina and Odera, 1996). In 1997, the landings of perch were estimated at 97,145 MT corresponding to 58 % of the total catches (Fisheries Department, 1998).

There was a faster increase in Nile perch production in Uganda and Tanzania. In 1981, less than 1,000 MT of Nile perch was landed in each of these countries (Greboval and Mannini, 1992). In 1986, approximately 41,000 MT of Nile perch was caught in Uganda and about three times that amount in Tanzania. The total production of Nile perch in the three countries in 1993 was close to 363,000 MT, with 29 % landed in Kenya, 27 % in Uganda and the rest in Tanzania (Anonymous, 1997a).

Initially, some reluctance towards the consumption of the perch was noticed, but the local population became used to the "oily and fatty" fish as new forms of fish processing at artisanal level developed. Moreover, the Nile perch became even more popular and spread to new markets all over East Africa (Greboval and Mannini, 1992). There emerged a huge demand for Nile perch, which expanded beyond the three countries sharing the lake. A market for the perch developed quickly in the industrialised countries.

In order to satisfy this market, small enterprises filleting and exporting fish were set up along the shoreline of Lake Victoria (Tetty, 1988). The first Nile perch processing establishments in Kenya were set up in the early and mid 1980s and they proved to be so profitable that more establishments were set up in all the three countries. Today there are about 35 establishments spread around Lake Victoria (Jansen, 1996; Anonymous, 1997a). In Kenya, there are about 20 Nile perch processing establishments located in urban centres like Kisumu, Migori, Homabay, Nairobi and Mombasa (Fisheries Department, 1998).

The main product of Nile perch is its fillet. Ideally the fillet should comprise of 30-35 % of the body weight of a medium size Nile perch. It is estimated that 14,531 MT of Nile perch products were exported from Kenya in 1997 earning Ksh. 2,615 million (Ogunja, 1998; Fisheries Department, 1998). The export markets include European Union (EU) (56.8 %), Israel (32 %), United States (6.7 %), Japan (3.4 %) and Australia (1 %). Besides fillet, other Nile perch products of economic value are Nile perch swim bladders used to manufacture isinglass, oils utilised in formulating meals and skin used in the leather industry (Ogunja *et al.*, 1992).

The quality of processed seafood produced for international market should conform to international standards. Over the last few decades, major seafood importing countries have introduced hygiene regulations and legislation, including definitive standards for fishery products. Periodically, these countries, with a view to improve the quality of imports have laid down new requirements. In order to ensure safety of seafood products entering the E.U, the Council of the European Communities has established the Council Directive of 22 July 1991 (EEC, 1991). The directive spells out the health conditions for production and the placing on the market of fishery products. Article 6 of this directive refers to the implementation of a quality control system based on the principles of Hazard Analysis Critical Control Point (HACCP). The United States Food and Drug Administration (FDA) has also introduced regulations that mandate the application of HACCP to the processing of seafood (FDA, 1995). This regulation, announced in 1995, became effective on December 18, 1997 and is applicable to all fish and fishery products sold in the United States, be they domestic or imported.

The East African Nile perch industry has been caught unaware and is not geared to meet such requirements. This has led to a substantial rejection of consignments and subsequent economic losses. Decomposition and the presence of pathogenic organisms have been indicated as the main reasons for most of these rejections (Lima dos Santos *et al.*, 1993). For example, in March 1997, *Salmonella* spp. was isolated from chilled and frozen Nile perch fillets (Anonymous, 1997b). As a result of these findings, drastic measures were taken by EU sanitary authorities to submit all lots of imported products from this region to bacteriological checks. In January 1998, the EU banned fresh fish imports from East Africa and Mozambique because of a cholera epidemic in these countries (EEC, 1998). The EU has been the main market for these countries and their fish exports totalled about 55,000 MT worth US$ 250 million in 1996 (Anonymous, 1998).

To meet the stringent EU requirements, Nile perch processing establishments have to provide comprehensive and effective systems for ensuring the safety of their products. This means that Good Manufacturing Practices (GMP), which includes hygienic practices and cleaning and disinfecting regimes have to be introduced and maintained. In addition, the concept of GMP has to be extended by the HACCP system.

The HACCP approach to food safety was developed in the early 1970's by Pillsbury company to provide means of assuring the safety of foods produced for the US space exploration program (Bauman, 1992). The system, which anticipates and prevents hazards before they happen, is divided into hazard analysis (HA) and determination of critical control points (CCPS) (Lee, 1991; Huss, 1992; Corlett and Pierson, 1992).

The hazard analysis involves identification and evaluation of hazards likely to arise from raw materials, processing and handling procedures. The hazards may be microbiological, chemical or physical (Rhodehamel, 1992). Hazard analysis is followed by determination of the CCPS-points, steps or procedures in the production process at which the identified hazards can be prevented, eliminated or reduced to acceptable levels. Once the system is established, the main effort of the quality assurance is directed towards the CCPS and away from final product testing. It is currently the best system available for improving the microbiological safety of foods including seafood (Huss, 1994a; Anandavally, 1995; FAO/WHO, 1997).

1.1 Working null hypotheses

"There are no microbial hazards during the processing of Nile perch at industrial level in Kenya".

1.2 Aims and objectives of the study

Development of a HACCP system for Nile perch processing establishments requires an assessment of hazards associated with all stages of fish processing. The general objective of the present study was to carry out an assessment of microbial hazards likely to occur during the processing of Nile perch at industrial level in Kenya. The specific objectives were: -

(a) To determine the microbiological quality of whole Nile perch on arrival at the processing plants from Lake Victoria;

(b) To determine changes in microbiological quality of Nile perch during the processing operations at the establishments;

(c) To identify the likely sources of microbial hazards and measures for their prevention and control;

(d) To identify the critical control points at which the microbial hazards can be controlled.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The microflora of freshly caught fish

The microflora on newly caught fish depends on the microbial content of the waters in which they live (Shewan, 1977; Reay and Shewan, 1949). However, in the same water, different species of fish may harbour different microflora, because of their ingestion of different foods. The numbers of microorganisms can also be influenced by the method of catching. Thus trawled fish usually carry bacterial loads 10 to 100-fold higher than those of lined fish, because fish are dragged for long periods of time along the sea bottom prior to landing (Chen, 1995; Shewan, 1949).

The microflora on fish from cold or temperate waters which has been studied in details is mostly dominated by psychrophilic, Gram-negative, rod shaped bacteria of the genera *Moraxella, Acinetobacter, Pseudomonas, Shewanella putrefaciens, Flavobacterium* and *Vibrio* (Shewan, 1977). Gram-positive organisms such as *Bacillus*, *Micrococcus*, *Lactobacillus*, *Corynebacterium* and *Clostridium* can also be found in varying proportions. The ratio of Gram-positive to Gram-negative strains varies, but the Gram-positive range from 0 to 30 % of the total flora (Shewan *et al.*, 1960; Liston, 1960). The total number of these organisms vary enormously and Liston (1980) states a normal range from 10^2 to 10^7 Colony Forming Units per gram (CFU /g).

Less agreement has been reached as to the microflora on warm or tropical water fish. Predominance of Gram-positive mesophilic bacteria such as *Bacillus*, *Micrococcus, Corynebacterium*, and sometimes enteric bacteria has been reported (Shewan, 1977,

Gillespie and MacRae, 1975). However, Lima dos Santos (1978) found that tropical fish carried a slightly lower number of *Pseudomonas* spp. than temperate water fish. He concluded that except for a slightly higher incidence of Gram-positive and enteric types, the bacterial flora on tropical fish was very similar to that reported for temperate fish. Similar findings were reported by Gram (1989) who found that the flora on newly caught Nile perch were similar to those on temperate water fish. Gram-positive organisms accounted for 39 % while Gram-negative strains constituted 61 %. The total bacterial load on tropical fish vary between 10^2 and 10^7 CFU /g with a mean of 10^3-10^4 CFU /g (Liston, 1980). This is not significantly higher than for temperate water fish.

The flora of freshwater fish is significantly different from that of marine species. Liston (1980) found a high proportion of Gram-positive bacteria such as *Streptococcus*, *Micrococcus*, *Bacillus* and *Coryneforms* while Shewan (1977) reported the presence of the genus *Aeromonas* in all freshwater fish and its absence in marine fish.

Moulds, yeast and viruses do not play a large part in the microbiology of fish in so far as its quality as food is concerned, but are associated with some of the fish diseases (Liston, 1980). However, some halophilic moulds (*Sporendonema* and *Oospora*) have been classified as spoilers. The yeast, *Rhodotorula* is occasionally responsible for pink discolouration in oysters (Huss, 1994b).

2.2 The spoilage of fish

Fish and other seafood are considered to be among the most perishable of foodstuffs; even when held under chilled conditions the quality quickly deteriorates (Garthwaite, 1992). The fish muscle is characterised by a high content of non-protein nitrogen (NPN), but a low content of glycogen resulting in a high post mortem pH (> 6.0) that favours microbial growth (Frazier and Westhoff, 1988; Love, 1992). In addition, the muscle has a high content of polyunsaturated fatty acids which are susceptible to oxidative deterioration leading to rancidity, which is most pronounced in fatty fish (Morrison, 1993).

Spoilage of fish has been demonstrated to be caused mainly by bacterial activity (Liston, 1980; Gram, 1989). However, the initial loss of quality is brought about by autolytic deterioration due to the action of enzymes that are present in the gut and in the flesh of the fish (Shewan and Murray, 1979; Bremner *et al.*, 1988). The enzymes decompose macromolecules such as proteins, glycogen and nucleic acids into small molecular substances, which are available for microbial growth. The spoilage bacteria develop most rapidly in the gills, where the earliest signs of decomposition, such as off-odours, can be detected (Shewan, 1977). They also grow in the surface slime on the skin and eventually penetrate into the flesh. The mechanism of penetration is not yet elucidated, and some controversy exists as to its nature (Shewan and Murray, 1979; Huss, 1995).

The typical pattern of bacterial growth during the spoilage of fish follows the classical population curve (Reay and Shewan, 1949; Shewan, 1977). In the first stage, there is no marked spoilage, odours are absent and the flesh is firm. This corresponds to the

lag phase and the initial part of logarithmic growth phase. During the second stage the odour strengthens and becomes musty and mousy, and the flesh becomes softer. The logarithmic phase is now well under way. The third stage is marked by sweet and fruity odours. During this stage the bacterial growth begins to slow down. In the last stage, the odours become stronger and more offensive and include ammonia, hydrogen sulphide (H_2S), methyl mercaptan, dimethylsulphide, trimethylamine (TMA), indole etc. This stage parallels the resting phase and the fish flesh is soft and slimy.

The odours that develop during the last phases of spoilage are products of the bacterial decomposition of the non-protein nitrogen fraction of the fish flesh. This has been shown in a large number of experiments where quality changes of sterile fish during chill storage has been compared to the changes observed in contaminated blocks (Lerke *et al.*, 1967; Shaw and Shewan, 1968; Herbert and Shewan, 1975; Herbert *et al.*, 1975; Gram, 1989). In the sterile samples, off-odours and off-flavours as detected in contaminated samples do not develop and, correspondingly, compounds like TMA and volatile sulphides are not detected.

The spoilage rate of fish and hence the shelf life depends on the temperature and conditions of storage. Thus deteriorative processes are quicker at higher temperatures. At 4^0C the rate is twice that of fish stored in ice (Spencer and Baines, 1964; Lima dos Santos, 1981; Simmonds and Lamprecht, 1985). In addition, the extension of the shelf life of chilled fish held in carbon dioxide (CO_2) enriched atmosphere over that held in air is significant (Barnett *et al.*, 1982; Villemure *et al.*, 1986).

The spoilage rate also varies with the species. Shewan (1977) suggested that differences in post mortem pH, trimethylamine oxide (TMAO) content and surface slime properties (such as amounts of lysozyme and number of strains of spoilage bacteria) could be of importance. Liston (1980) found that longer shelf lives were related to slower bacteria growth rates.

2.3 Specific spoilage bacteria

Even though the microflora of freshly caught fish is very diverse, only a part of this flora contributes to spoilage (Tarr, 1954; Shewan, 1971; Huss, 1995). The spoilage flora become dominant during storage and is characterised by the ability to produce metabolites responsible for off-odours and off-flavours associated with spoilage.

Several methods are employed in order to evaluate the spoilage potential of various microorganisms. A technique where sterile fish muscle is inoculated with pure cultures of bacteria has been used in United Kingdom (Herbert *et al.*, 1971; Shewan, 1977) while others have used heat-treated fish broths (Castel and Greenough, 1957; Kobataka *et al.*, 1987). Van Spreekens (1977) utilised irradiated muscle blocks and Lerke *et al.* (1965) a sterile filtered raw fish juice. From these studies, *S. putrefaciens* has been identified as specific spoilage bacteria due to its ability to reduce TMAO to TMA (Laycock and Regier, 1971; Van Spreekens, 1977). This organism also produces volatile sulphides such as hydrogen sulphide, methyl mercaptan and dimethyl sulphide when decomposing sulphur-containing amino acids like cystine, cysteine and methionine (Shaw and Shewan, 1968; Herbert *et al.*, 1975; Shewan, 1977).

The ability of *S. putrefaciens* to produce H_2S has been used in the development of iron containing bacteriological media for the detection of this specific spoiler, where bacteria forming H_2S from thiosulphate or amino acids appear as black colonies due to precipitation of iron sulphide (Levin, 1968; Jensen and Schutz, 1980; Gram *et al.*, 1987). Van Spreekens (1977) and Gillespie (1981) have classified a non-H_2S producing group of bacteria similar to *S. putrefaciens* as spoilage bacteria due to their ability to reduce TMAO. Certain species of *Pseudomonas* such as *P. perolens*, *P. fragi*, *P. fluorescens* and *P. putida* have also been classified as fish spoilage bacteria (Castell and Greenough, 1957; Herbert *et al.*, 1971; Van Spreekens, 1977; Hobbs and Hodgekiss, 1982). These bacteria do not produce H_2S but several other volatile sulphides (such as methyl mercaptan, dimethyl sulphide and dimethyl disulphide), ketones, esters and aldehydes.

Flavobacterium are also able to produce offensive off-odours (Castel and Mapplebeck, 1952). However, they are slow growing bacteria and whenever they appear in a mixed culture with the *Pseudomonas*, the latter group dominates spoilage. Similar conclusions were drawn on spoilage by *Aeromonas, Moraxella, Acinetobacter* and *Vibrio* (Lerke *et al.*, 1965).

Most species of Enterobacteriaceae have the capacity to reduce TMAO to TMA (Sikorski *et al.*, 1990). However, they are seldom encountered in spoiling fish although some may be found in fish caught in polluted waters.

The predominant kinds of bacteria causing spoilage of fish vary with the temperature at which the fish are held. The spoilage in iced fish is dominated by the genera

Shewanella, Pseudomonas and, to a lesser extent *Moraxella* and *Acinetobacter* (Shewan, 1977; Hobbs, 1983). By the end of spoilage life (when total counts reach 10^7-10^{10} CFU /cm^2), *Pseudomonas* and *Shewanella* represent 80–100 % of the final flora. This is mainly due to their relatively short generation time at low temperatures (Morita, 1975; Davaraju and Setty, 1985). Ambient storage of fish leads to an increase in the numbers of mesophilic bacteria of which Enterobacteriaceae and Vibrionaceae dominate (Gorczyca *et al.*, 1985; Gram, 1989).

The spoilage bacteria also depend on the storage conditions of fish. For instance, CO_2-enriched atmosphere selectively inhibits the growth of the typical spoilage bacteria such as *Pseudomonas* spp., *Moraxella* and *Acinetobacter* (Finne, 1982; Wilhelm, 1982; Wang and Ogrydzlak, 1986). During refrigerated storage in CO_2 atmosphere, Gram-positive organisms such as *Lactobacillus* spp. and *Alteromonas* dominate in the microflora of fish. These organisms do not normally participate significantly in spoiling of fish muscle. They grow, however, under refrigeration and modified atmosphere much more slowly than in air at higher temperatures and produce other metabolites than the typical spoilage microflora. Salted fish are spoiled by salt-tolerant or halophilic bacteria of the genera *Serratia, Micrococcus*, *Bacillus*, *Alcaligenes, Halobacterium* and *Sarcina* (Horner, 1992). These bacteria are responsible for pink spoilage, so-called because of the colour of their colonies and consequent appearance of the cured fish.

2.4 Pathogenic bacteria

The importance of fish and shellfish as vehicles of human infection and intoxication, and the ability of pathogens to survive long periods of storage in frozen fishery

products is well documented (Huss, 1994b; Bryan, 1980; Brown and Dorn, 1977; Raj and Liston, 1961). The principal diseases transmitted to man by seafood are bacterial infections. Bacterial infections constitute the largest proportion of seafood-borne diseases. Microbial evaluations of in-line and finished products indicate that bacterial contamination occurs primarily through exposure to polluted environments or cross-contamination during landing, transport, processing, storage, or during preparation for consumption (Sikorski *et al.*, 1990; Ridley and Slabyj, 1978; Harrison and Lee, 1968).

Seafood-borne pathogenic bacteria may conveniently be divided into two groups, indigenous bacteria and non-indigenous bacteria (Huss, 1994b). Indigenous bacteria are common and widely distributed in the aquatic environments in various parts of the world with the water temperature having a selective effect. Thus, the more psychrotrophic organisms (*Clostridium botulinum* types E, B and F and *Listeria)* are common in arctic and colder climates, while the more mesophilic types (*Vibrio cholerae, Vibrio parahaemolyticus*) represent the natural flora on fish from coastal and estuarine environments of temperate or warm tropical zones. Other members of this group are *Aeromonas hydrophila* and *Plesiomonas shigelloides.* Non-indigenous bacteria consist of *Salmonella* spp., *Shigella* spp., *Escherichia coli, Staphylococcus aureus* and *Clostridium perfringens*. Fish and shellfish caught from unpolluted waters are generally free of these pathogenic bacteria but may become contaminated during subsequent handling and processing (Huss, 1995; Bryan, 1980; Shewan and Hobbs, 1967).

2.4.1 Indigenous bacteria

2.4.1.1 *Clostridium botulinum*

C. botulinum is a Gram-positive, obligately anaerobic, endospore-forming bacillus. The organism is widely distributed in soil, aquatic sediments and may be found in the intestines of man and other animals including fish (Shewan, 1977). *C. botulinum* produces toxins, which cause a neuroparalytic illness, commonly referred to as botulism. Seven distinct types of botulinum toxin (types A-G) are recognised, although botulism in man is usually caused by types A, B and E, and more rarely F and G (Eley, 1994).

In food-borne botulism, the food becomes contaminated with spores from the environment, which are not destroyed by the initial cooking or processing. If the food is then kept in conditions appropriate for growth, the spores may germinate, leading to production of toxin (Frazier and Westhoff, 1988). All fresh fish products are excellent media for growth and toxin production by the psychrotrophic, non-proteolytic strains of *C. botulinum* (Types E, B and F) (Huss, 1981). Increased speed of toxin production is noted at temperatures above 3^0C until an optimum temperature of 25-30^0C is reached. However, the rate of toxin production is also greatly dependent on the actual sporeload.

Huss (1981) showed that out of 165 outbreaks of botulism caused by fish products, lightly preserved products (smoked, fermented) represented by far the most dangerous group. In contrast fresh and frozen fish have never been shown to cause human botulism. This is probably due to the fact that fresh fish normally spoil before reaching the temperature at which the organism produces toxins. In addition,

botulinum toxin is very heat-labile (Huss, 1981, Hauschild, 1989; Eley, 1994) which means that normal household cooking will destroy any pre-formed toxin. Thus the risk is clearly associated with foods that do not require cooking immediately before consumption.

2.4.1.2 *Vibrio* species

Most are of marine origin and they require sodium ions for growth (Hoover, 1985). The genus contains a number of species, which are pathogenic to man such as *V. parahaemolyticus, V. cholerae, V. fluvialis, V. furnissii, V. hollisae, V. mimicus, V. vulnificus, V. alginolyticus* and *V. damsela* (Karunasagar and Karunasagar, 1994). The pathogenic species are mostly mesophilic, generally occurring in tropical waters and in highest numbers in temperate waters during summer months (Huss, 1994b; Eley, 1994).

V. parahaemolyticus usually causes gastroenteritis or diarrhoea of sudden onset and varying severity (Taylor *et al.*, 1982). Food poisoning associated with this organism is almost always associated with consumption of raw or lightly cooked contaminated marine fish (Sakazaki, 1979; Huss, 1994b). There is a pronounced seasonal incidence, with outbreaks occurring mostly during the summer months, when *V. parahaemolyticus* is most prevalent in the aquatic environments from which seafood is harvested (Eley, 1994). Most outbreaks occur because the raw or uncooked seafood is allowed to contaminate and reinoculate cooked seafood (Frazier and Westhoff, 1988). Holding recontaminated cooked seafood at high temperatures permit rapid growth allowing bacterial counts to rise above the infective dose of 10^6 organisms per gram of food.

Proper cooking of seafood is the only method currently available to inactivate *V. parahaemolyticus*, though it will not affect any pre-formed thermostable Kanagawa haemolysin which would remain stable in the cooked food (Eley, 1994). Cooking is obviously not effective, however, if cross-contamination with raw product is allowed to take place following the cooking process (Bryan, 1980). Strict hygiene measures are also essential.

V. cholerae occurs in two serotypes, the 01 and the non-01 (WHO, 1984). The serovar 0 group 1 contains two serological groups based on variations in the O or somatic antigen factors, namely Ogawa (factors A and B) and Inaba (factors A and C). These serotypes may exist in either the classical or El tor biotype. *V. cholerae* serogroup 01 is the causative agent of cholera, an epidemic disease (Huss, 1994b; Attridge and Rowley, 1990). Other serovars, non-01, do not represent the same health threat as does *V. cholerae* 01 but can be responsible for intestinal infection or gastroenteritis.

Cholera is usually a waterborne disease characterised by gastroenteritic symptoms. However, food-borne and nosocomial outbreaks are also important, and person-to-person transmission may occur under conditions of extreme crowding and poor hygiene (Miller *et al.*, 1985). Following ingestion of *V. cholerae* and passage through the stomach, the bacteria multiply and produce an enterotoxin that either stimulates the mucosal cells to secrete large quantities of isotonic fluid, or increases the permeability of vascular endothelium (Attridge and Rowley, 1990).

V. cholerae has been found as a natural resident of aquatic environments in cholera-free areas and its presence is not necessarily associated with feacal contamination or

sporadic human infections (Rogers *et al.*, 1980; Feacham *et al.*, 1981). It is therefore to be expected that freshly harvested seafood might be harbouring this pathogenic *Vibrio* spp. In cholera-endemic areas, seafood could be contaminated with *V. cholerae* reaching the marine environment through sewage discharge (Karunasagar and Karunasagar, 1994; Bryan, 1980). Postharvest contamination could occur due to poor sanitary conditions in processing units and carriers of *V. cholerae* handling seafood.

There is no evidence that contaminated fish cause cholera infection in man (Anonymous, 1991). However, epidemiological investigations have incriminated shellfish in outbreaks of the disease (Morris and Black, 1985). Fish and shellfish form a suitable substrate for survival of *V. cholerae* and studies have indicated that the organism persists in fish and shellfish at room temperature for 2 to 5 days and under refrigeration for 1 to 2 weeks (Ganowiak, 1990). This allows relatively low initial numbers to increase dramatically under improper conditions of harvesting, processing, distribution and storage.

2.4.1.3 *Aeromonas* species

These are Gram-negative, facultatively anaerobic, non-spore-forming bacilli which are oxidase positive. The genus contains species (*A. hydrophila*, *A. sobria*, A. *caviae*) pathogenic for frogs, fish and mammals including humans (Krieg, 1984; Huss, 1994b). In recent years the motile *Aeromonas* spp., particularly *A. hydrophila* has received increasing attention as a possible agent of food-borne diarrhoea (Eley, 1994). However, the role of *Aeromonas* as an enteric pathogen is not fully clarified.

Aeromonas is ubiquitous in freshwater environments, but may also be isolated from saline and estuarine waters (Knochel, 1989). These organisms can also be found in untreated sewage excreted from both symptomatic and asymptomatic persons and from farm animals. Isolations of these organisms have been made from meat, fish and shellfish, ice cream and many other low-acid foods of high water activity. Indeed the organism has been identified as the main spoilage organism of raw salmon (Huss, 1994b), packed in vacuum or modified atmospheres and fish from warm, tropical waters (Gram *et al.*, 1990).

Species of *Aeromonas* produce a wide range of toxins such as cytotoxins, enterotoxins, haemolysins and a tetrodotoxin-like sodium channel inhibitor (Varnam and Evan, 1991; Eley, 1994). However, the role of these toxins in producing disease in man is unresolved and currently no method is available for differentiating between apathogenic environmental strains and pathogenic strains. Thus there is no evidence that toxins pre-formed in food play any role, and the association between eating fish and shellfish and *Aeromonas* infection is at best circumstantial (Ahmed, 1991).

There are two major problems associated with the control of *Aeromonas* species, their frequent presence in food and the fact that many strains are psychrotrophic (Eley, 1994; Harrigan and Park, 1991). This means that they are capable of reaching high numbers even in refrigerated foods. Fortunately, these are relatively heat-sensitive organisms and heat processing is usually effective in killing them (Condon *et al.*, 1992).

2.4.1.4 *Plesiomonas shigelloides*

These Gram-negative, facultatively anaerobic, non-spore-forming bacilli, which are oxidase positive, are widespread in nature but are mostly associated with water, both freshwater and seawater (Arai *et al.*, 1980). Due to its mesophilic nature, there is a marked seasonal variation in the numbers isolated from waters being much higher during warmer periods. Transmission by animals and intestines of fish is common, and it is likely that fish and shellfish is the primary reservoir of *Plesiomonas shigelloides* (Koburger, 1989).

As in the case of *Aeromonas* species, there is some controversy regarding the enteropathogenicity of *P. shigelloides* (Eley, 1994; Frazier and Westhoff, 1988). *P. shigelloides* may cause gastroenteritis with symptoms varying from mild illness of short duration to severe diarrhoea. However, it is possible that only a few strains carry virulent characteristics since volunteers ingesting the organism do not always become ill (Herrington *et al.*, 1987). Very few foods have been implicated with any degree of certainty in *P. shigelloides* food poisoning; those that have include fish, crabs and oysters (Eley, 1994).

2.4.1.5 *Listeria* species

Seven species of *Listeria* are currently recognised, but only 3 species *L. monocytogenes*, *L. ivanovii* and *L. seeligeri* are associated with disease in human and/or animals (Huss, 1994b). However, human cases involving *L. ivanovii* and *L. seeligeri* are extremely rare with only four reported cases.

L. monocytogenes causes listeriosis, a severe and often fatal illness, to which certain populations (e.g., pregnant mothers, new-borns, immunocomprised individuals, transplant recipients) may be susceptible (Rhodehamel, 1992). The organism is ubiquitous and is widespread in the environment, where it may survive for long periods. Its presence has been reported in animal and human faeces, and consequently in sewage; it has also been isolated from soil, surface water, vegetation, foods including fish and fish products and domestic kitchens (Ryser and Marth, 1991; Fuchs and Reilly, 1992). Most of these environment strains are probably non-pathogenic. Other *Listeria* spp. than *L. monocytogenes* appear to be more common in tropical areas and are of less significance in this part of the world compared to temperate environments (Fuchs and Reilly, 1992; Karunasagar *et al.,* 1992).

L. monocytogenes is primarily transmitted to humans through foodstuffs contaminated during production and processing (Ryser and Marth, 1991). Frequent isolations from seafood (Gecan *et al.*, 1994; Berry, 1994; Rorvik and Yndestad, 1991) and the demonstration of growth potential in chilled (4^0C) smoked salmon (Ben Embarek and Huss, 1992; Fuchs and Reilly, 1992) are evidence that seafood may be important in the transmission of *L. monocytogenes*. However, so far there have only been a few documented cases of seafood involvement and three cases where seafood involvement is suspected (Ben Embarek, 1994).

L. monocytogenes is particularly difficult to control, since it is widespread in environment, and because it possesses physiological characteristics (e.g. multiplication at refrigeration temperatures) that allow growth under conditions that are usually adverse for most other pathogenic bacteria. However, the quantitative

level of *L. monocytogenes* contamination on fish and fishery products can be maintained at very low level (< 10/g) by proper GMP and factory hygiene (Huss, 1994b).

2.4.2 Non-indigenous bacteria

2.4.2.1 *Salmonella* species

These are Gram-negative, facultatively anaerobic, non-spore forming bacilli that can be split into more than 2000 serotypes according to a system based on somatic (0), capsular (vi) and flagella (H) antigens, known as the Kauffmann - white scheme (Frazier and Westhoff, 1988). These mesophilic organisms are widely distributed in nature and are commonly found in the intestinal tracts of animals and human beings and in environments polluted with animal or human excreta (Huss, 1994b). Survival in water depends on many parameters such as ecological (interaction with other bacteria) and physical factors (temperature). It has been demonstrated by Rhodes and Kator (1988) that *Salmonella* spp. can multiply and survive in the estuarine environment for weeks. Jimenez *et al.* (1989) has presented similar results on survival in tropical freshwater environment.

The principal symptoms of salmonellosis are non-bloody diarrhoea, abdominal pain, fever, nausea and vomiting which generally appear 12-36 hours after ingestion. However, symptoms may vary considerably from grave typhoid like illness to asymptomatic infection. The disease may also proceed to more serious complications. The infective dose in healthy individual varies according to serovars, foods involved and susceptibility of the individuals. There is evidence for a minimum infection dose

of as little as 20 cells (Varnam and Evans, 1991), while other studies have consistently indicated $>10^6$ cells.

There is a great risk of *Salmonella* infection from fish and shellfish gathered in polluted waters (Reilly and Twiddy, 1992). Contamination occurs also through improper plant sanitation or more probably through improper personal sanitation of workers, who having had salmonellosis often become carriers of the organism for a period of time after symptoms have gone (Ganowiak, 1990; Huss, 1994b). However, seafood is a much less common vehicle for *Salmonella* than other foods, and fish and shellfish are responsible for only a small proportion of *Salmonella* cases (Ahmed, 1991). Most seafood is cooked prior to consumption and these products pose minimal health risks to the consumer except by cross-contamination in kitchens. This is borne out by epidemiological evidence presented by Ahmed (1991), reporting on 7 outbreaks of seafood-borne salmonellosis in USA in the period 1978-1987. Three of these outbreaks were due to contaminated shellfish including 2 outbreaks after consumption of raw oysters harvested from sewage polluted waters.

2.4.2.2 *Shigella* species

The genus *Shigella* is composed of Gram-negative, facultatively anaerobic, non-spore-forming organisms that do not ferment lactose and are non-motile. This genus consists of 4 distinct species i.e. *Sh. dysenteriae, Sh. flexneri, Sh. boydii* and *Sh. Sonnei*. The organism is host-adapted to humans and higher primates, and its presence in the environment is associated with feacal contamination (Eley, 1994). *Shigella* strains have been reported to survive in water for up to six months (Wachsmuth and Morris, 1989).

Shigella food poisoning (shigellosis), which is an infection of the gut may vary in severity from asymptomatic infection to fulminating dysentery (Guerrant, 1985). The severity of symptoms depends on the species of *Shigella* implicated. When the causative organism is *Sh. dyenteriae*, there is often abdominal pain, fever, frequent passage of bloody/fluid stools, and the patient may also have headaches, nausea and undergo prostration. The incubation period may vary from 1-7 days and symptoms may persist for 10-14 days or longer. *Shigella* spp. is highly infectious due to low infective dose: 100 bacteria or less can produce disease. Death in adults is rare, but the disease in children can be severe (Huss, 1994b). In tropical countries with low standards of nutrition, shigellosis accounts for the death of at least 500,000 children per annum (Guerrant, 1985).

The majority of outbreaks of shigellosis worldwide are associated with drinking contaminated water and person to person transmission may also spread the disease by the feacal-oral route (Eley, 1994). However, fish and shellfish salads are sometimes implicated as vehicles of outbreaks of shigellosis (Bryan, 1980; Huss, 1994b). This happens only when a human carrier of *Shigella*, who does not practice adequate personal hygiene and whose hands consequently become contaminated with faeces, prepares fish or shellfish products that are not subsequently heated to temperatures that kill Shigellae. Preventive procedures should emphasise personal hygiene of workers, thorough cooking or reheating (where applicable) and rapid cooling and proper refrigeration (Eley, 1994).

2.4.2.3 *Escherichia coli*

E. coli is part of the normal flora of the intestinal tract of man and warm-blooded animals (Bryan, 1979). Generally the *E. coli* strains are intestinal commensal organisms which on occasions can become opportunistic pathogens when transferred to other sites in the body. However, within the species there are at least 4 types of pathogenic strains, enteropathogenic *E. coli.* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC) and Enterohaemorrhagic *E. coli* (EHEC)/ Verocytotoxic *E. coli* (VTEC or *E. coli* 0157: H7) (Eley, 1994). Pathogenic strains of *E. coli* produce diseases of the gut which vary in severity depending on a number of factors such as type of strain, susceptibility of victim and degree of exposure.

Serotyping as well as phage typing and genetic methods are used in epidemiological studies to separate among the various *E. coli* types, but there are no specific phenotypic markers to separate between pathogenic and non-pathogenic strains (Huss, 1994b). However, some atypical properties such as being lactose-negative or failure to produce indole at 44^0C are more common between pathogenic strains (Varham and Evans, 1991). VTEC do not grow at all on selective media at 44^0C (Tarr, 1994). EIEC serotypes closely resemble *Shigella* in many respects (Harrigan and Park, 1991). DNA-DNA hybridisation experiments have shown there is essentially a taxonomic concordance and a considerable serological relatedness.

E. coli may be isolated in environments polluted by feacal material or sewage, and the organism can multiply and survive for a long time in this environment (Rhodes and Kator, 1988). However, it has been demonstrated that *E. coli* can also be found in

unpolluted warm tropical waters, where it can survive indefinitely (Jimenez *et al.*, 1989).

There is no indication that seafood is an important source of *E. coli* infection (Ahmed, 1991). Most infections appear to be related to contamination of water or products during processing and subsequent handling.

2.4.2.4 *Staphylococcus aureus*

These are Gram-positive, facultatively anaerobic, non-spore-forming cocci which are coagulase and deoxyribonuclease (DNA'se) positive. The organisms can be found in air, water, dust, milk and in sewage (Huss, 1994b). However, the main reservoir is the human nose, throat and skin. The human carrier rate may be up to 60 % of healthy individuals with an average of 25-30 % of the population being positive for enterotoxin-producing strains (Ahmed, 1991).

Staphylococcal food poisoning is caused by the ingestion of a pre-formed enterotoxin produced by growth of the bacteria in food (Frazier and Westhoff, 1988). The enterotoxin is very stable being resistant to proteolytic enzymes and heat. Eight serologically distinct types (A, B, C_1, C_2, C_3, D, E and F) have so far been recognised (Eley, 1994). However Staphylococcal enterotoxin A (SEA) is frequently involved and is found in the food associated with approximately 75 % of outbreaks due to this organism.

Fish may be contaminated with *S. aureus* via infected food handler or from another source previously contaminated by humans (Ganowiak, 1990). To avoid

contamination and growth, good sanitary conditions and temperature control are necessary. *S. aureus* is mesophilic with a minimum growth temperature of 10^0C, but higher temperatures (>15^0C) are required for toxin production (Huss, 1994b).

S. aureus competes poorly with other food-spoilage microorganisms and raw, naturally contaminated food is unlikely to be toxic unless severely spoiled (White and Hall). Exceptions to this are foods containing fairly high levels of salt, such as seafood, which may select growth of salt tolerant *Staphylococci*, thus enabling toxic levels to be reached before obvious spoilage has occurred (Bryan, 1980).

S. aureus is particularly resistant to freezing (White and Hall, 1985) and moderately resistant to frozen storage (Raj and Liston, 1961). Therefore, freezing cannot be relied upon to give a significant reduction in the level of contamination from *S. aureus*, and prolonged frozen storage will not reduce the potential for growth during subsequent thawing (White and Hall, 1985).

Canned seafood have been implicated in several outbreaks of *Staphylococcus* enterotoxin poisoning (Ababouch, 1992). This has prompted research to assess the validity of thermal processes used in the fish canning industry and it has been demonstrated that *S. aureus* can survive in thermally processed fish packed in oils.

2.4.2.5 *Clostridium perfringens*

C. perfringens is a Gram-positive, anaerobic, spore-forming bacillus. The organism is widely distributed occurring in soil, dust and among the intestinal microflora of warm-blooded animals (Mead *et al.*, 1982). Consequently, the organism is a common contaminant of raw foods and food ingredients and, because of its ability to produce resistant spores, may persist in food products.

C. perfringens produces four major lethal toxins namely ∝, ß, γ and δ (Eley, 1994). The relative production of these toxins is used as a basis for dividing the species into five toxin types, A to E. Only the type A (which can be further subdivided into a number of serotypes) are responsible for food poisoning (Stringer *et al.*, 1982).

In food poisoning outbreaks caused by *C. perfringens* large numbers of the organism (10^6 or more /g) are normally found in the suspected food (Bryan, 1980; Eley, 1994). The organisms multiply when they reach the gastro-intestinal tract followed by sporulation with subsequent release of enterotoxin (White and Hall, 1985). The toxin damages epithelial cells and inhibits the absorption of glucose, which causes an influx of sodium and chloride ions and water (Eley, 1994). This results in excess fluid movement into the lumen of the gut, leading to diarrhoea.

C. perfringens can contaminate seafood from water, soil, mud or through contact surfaces (Ganowiak, 1990). The organism has received more attention in recent years as a food-borne poisoning in which cooked fish have been implicated.

2.5 Statistics on seafood-borne diseases

The true incidence of diseases transmitted by foods worldwide is not known (WHO, 1989). There are several reasons for this: Epidemiological data is only available from countries with surveillance programmes, that is, developed countries and no data is available from developing countries. In the countries that have a reporting system there is severe underreporting. It has been estimated that as few as 1 % of the actual cases of food-borne diseases are recorded (Mossel, 1982). This is because neither the victim nor the physician is aware of the etiological role of foods. Furthermore, the food responsible is often not available for analysis and the true vehicle for the disease agent is not identified.

Between 1973 and 1987, a total of 7,458 food-borne disease outbreaks involving 237,545 cases were reported in the United States by the Centre for Disease Control (Ahmed, 1991). A specific food vehicle was identified in only 3699 (50 %) of the outbreaks. Of these food items seafood was the food most frequently associated with disease and accounted for approximately 10 %. The pathogenic bacteria responsible for fish-associated outbreaks were *C. botulinum, V. parahaemolyticus, Shigella, Salmonella, V. cholerae, C. perfringens,* and *S. aureus.*

Available data for the EU member states indicate that disease outbreaks from fish and shellfish between 1983 and 1992 ranged from 1.9 % of the total food-borne outbreaks in United Kingdom to 12.4 % in Denmark (FAO/WHO, 1990, 1992, 1995). When the known food source was identified the range of fish and shellfish outbreaks was from 4.4 % in the United Kingdom to 16.1 % in Finland.

The food and veterinary office of the European Union has also initiated a Rapid Alert System for food (Cato, 1998). This system is used to notify all member countries of the EU when food products are detected that are a source of danger to health. The source of danger and the country of origin are reported. Between 1992 and November 1997, seventy-one seafood alerts were reported and this represented 42.5 % of all food alerts. Fish and fishery products from East Africa were among the seafood products implicated. The principal sources of danger to health, which were of bacterial origin and the number of times each was reported were *Salmonella* (27), *V. parahaemolyticus* (7), *V. cholerae* (4), *Listeria monocytogenes* (3), *Staphylococcus* (2) and *C. perfringens* (1).

From 1987 to 1996, a total of 1475 seafood-borne disease outbreaks resulting in 33,253 cases were recorded in Japan (Japan Ministry of Health and Welfare, 1997). Fish and shellfish accounted for 93 cases. *V. parahaemolyticus* caused 75 % of bacterial disease cases while *Salmonella, S. aureus, E. coli* and *C. perfringens* were the other major causes.

2.6 HACCP in the seafood industry

The last three decades have witnessed an increase in the number and frequency of food-borne diseases (Quevedo, 1992). Moreover, the traditional approaches of ensuring quality and safety of foods, which include end-product testing and inspection are not giving the expected result for the prevention of food-borne diseases (Huss, 1994b). This therefore underscores the need for comprehensive and effective systems for ensuring food safety.

The Hazard Analysis Critical Control Point (HACCP) system provides a more specific and critical approach to the control of microbiological hazards in foods than that provided by traditional inspection and quality control approaches (WHO/ICMSF, 1992; Huss, 1994b). The system identifies specific hazards and measures for their prevention and control to ensure food safety. In addition to enhanced food safety, the benefit of applying HACCP, include better use of resources and more timely response to production problems (WHO, 1993). The application of the HACCP system can also aid inspection by regulatory authorities by focusing on matters of high health risk and promote domestic and international trade by increasing confidence in food safety. The system is currently recognised as the language of safety all over the world and has become the basic tool to international seafood quality assurance (Lima dos Santos and Sophonphong, 1998; Anandavally, 1996). The use of HACCP in the seafood industry has been endorsed by Codex Alimentarius, the European Union, United States, Canada, Australia, New Zealand and Japan (Cato, 1998).

Seven principal activities are employed in the development of HACCP plan (Huss, 1994b; Anandavally, 1995). These principal activities are:

- Hazard analysis and determination of measures necessary to control them;
- Identification of critical control points (CCPS) required to prevent or control hazards;
- Establishment of critical limits for each critical control point;
- Establishment of monitoring and checking procedures;
- Establishment of corrective action to be taken when monitoring indicates that a particular CCP is not under control;

- Establishment of verification procedures to confirm that the HACCP system is working effectively;
- Establishment of documentation concerning all procedures and records appropriate to these principles and their application.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Facilities evaluated

Microbial hazards likely to occur during processing of Nile perch at industrial level in Kenya were evaluated in two establishments between November 1997 and June 1998. The establishments (hereafter designated A and B) were selected randomly and represented the two types of Nile perch products mainly exported from Kenya. The two establishments had provided adequate space for equipment and other installations that are required for fish processing, and the Kenyan government had approved them to export Nile perch products to European Union (EU) market.

Establishment A was located in Nairobi's Industrial area and specialised in processing of whole fish into chilled fillets. Processing operations in this establishment consisted of whole fish washing, fish filleting, fish skinning and trimming, fish packing and chilling of the processed product. Establishment B was located in Nairobi's Ruaraka area and produced frozen whole fish (headless and gutted). Its processing operations comprised whole fish washing, fish beheading and gutting, fish trimming, fish packing and freezing of the processed product. The processing capacity for establishment A was about 28 metric tonnes (MT) of whole fish per day while that of establishment B was about 21 MT per day.

3.2 Source of fish sampled

The fish received by the establishments was caught in L.Victoria by artisanal fishermen using mainly gill nets and sometimes also long lines. The vessels of these fishermen were small canoes that had capacity to carry 1 ton of fish from the fishing

grounds to the designated landing sites along the Kenya shores of L. Victoria. There were no cooling facilities on-board and the only preservation method used was covering the fish with wet water weeds to keep it moist and relatively cool. Sail usually powered the boats and the trip from fishing grounds to landing beaches took several hours. Transportation of iced fish from landing beaches to the establishments was in insulated trucks.

3.3 Microbial hazard analyses

Hazard analyses consisted of (a) monitoring fish processing and handling practices to identify sources and modes of contamination; (b) measuring temperatures in internal regions of fish throughout the processing line; and (c) collecting samples and testing them microbiologically (Bryan *et al.*, 1991). Samples were tested for aerobic mesophilic colony count (AMCC), *Salmonella*, *Shigella*, *S. aureus*, *C. perfringens*, *E. coli* biotype 1, as applicable, depending on the nature of the sample and the organisms that would be likely contaminants. Microbiological analysis was carried out at National Public Health Laboratory Services (NPHLS), Nairobi.

Temperatures were measured by inserting a thermocouple (type T, capable of measuring temperatures from -40^{0}C to 150^{0}C and having a needle-type sensor) into the approximate geometric centre of fish. The thermocouple leads were plugged into a battery powered Barnant hand-held thermometer (Model 115, Sigma chemical Co., USA). Before use in fish, thermocouples were washed in soapy water, rinsed, wiped dry with a paper towel, and immersed three times in 95 % alcohol and flamed after each immersion.

3.4 Sampling procedures

A total of 318 fish samples were collected from establishment B during the study. However, less number of samples (108) were collected from establishment A as fish processing operations in this establishment were suspended in January 1998 following EU ban on fresh fish imports from East Africa because of a cholera epidemic in the region.

Samples of fish that were collected were not the same as those having thermocouples attached, but the sampling points were identical. The samples were drawn in triplicate at six key points along the processing line during normal commercial operations. Thus, representative samples of approximately 300 g were drawn from whole fish immediately after arrival at the factory, whole fish after initial washing, fish after filleting/beheading and gutting, fish after skinning and/or trimming, fish after packing and fish after chilling/freezing. These field samples were collected aseptically and put into sterile stomacher bags, which were fastened at the top with rubber bands.

Water samples from the various outlets within the establishments were collected aseptically into sterile Erlenmeyer flasks (1000 ml) with dust-proof ground glass stoppers. In case of chlorinated water samples, the sample bottles contained 0.1 ml of a 2 % solution of sterile sodium thiosulphate for every 100 ml of water sample collected (WHO, 1984).

On all sampling occasions, fish-contact surfaces e.g. tables, trays, knives, cutting boards, personnel hands (with or without gloves) were sampled by rubbing a sterile swab over a 100 sq. cm of surfaces touched by fish. If the surface was dry, the swab

was first moistened in sterile 0.1 % peptone water (Difco) and replaced immediately after sampling.

All samples were put in an insulated container with ice, and taken to the laboratory on the day of collection. Examination started on that day or samples were kept refrigerated (at 4^0C) overnight and tested the next morning.

3.5 Laboratory procedures

3.5.1 Microbial analysis of fish

Ten grams of fish sample (skin together with underlying tissues or skinned fillet) was excised aseptically from each of the triplicate field samples. The analytical samples (30 g) were transferred into 270 ml of 0.1 % buffered peptone water in stomacher bags and homogenised using a Colworth Stomacher 400 (Seward Laboratory, London, England, UK). The homogenate was thoroughly mixed before decimal dilution(s) were made according to International Commission on Microbiological Specification for Foods (ICMSF, 1978). The resulting homogenate was used for all microbiological tests except for detection of *V. cholerae, C. perfringens*, *Salmonella* and *Shigella.*

3.5.1.1 Aerobic mesophilic colony count and hydrogen sulphide producers count

Aerobic mesophilic colony count and hydrogen sulphide (H_2S) producers were carried out on Iron Agar Lyngby (Oxoid) (Jensen and Schultz, 1980; Gram *et al.*, 1987) by surface plating 0.1 ml of appropriate dilutions. Incubation was at 30^0C for 3 days. Black colonies (H_2S-producers) were enumerated separately from non-black colonies, both components on the plate going to make the aerobic mesophilic colony count.

3.5.1.2 Enumeration of Enterobacteriaceae

Enterobacteriaceae were enumerated by surface plating 0.1 ml of serial dilutions of homogenate to the surface of violet red bile glucose agar (VRBGA, Oxoid). The spread plates were incubated at 37^0C for 48 h. Organisms of this group form purple/red colonies (Harrigan and Pack, 1991).

3.5.1.3 Enumeration of coliforms and feacal coliforms

Coliforms and feacal coliforms were enumerated by the Most Probable Number (MPN) technique according to ICMSF (1978). 1 ml of appropriate decimal dilutions was pipetted into each of 3 tubes of Lauryl sulphate tryptose broth (Oxoid). The tubes were incubated at 35^0C and examined after 24 and 48 h for acid and gas production. Presumptive positive tubes were confirmed as coliforms by subculturing 3 loopful from each tube into a tube of brilliant green lactose bile broth (Oxoid). These were incubated at 35^0C and examined for gas production after 48 h. For feacal coliforms, presumptive positive tubes were subcultured into tubes of E.C broth (Difco) and incubated at 44.5^0C in a water bath for 24 h. The incubated broth was examined for gas production. Samples without gas were incubated for an additional 24 h.

3.5.1.4 Enumeration of *Staphylococcus aureus*

Pathogenic *S. aureus* was enumerated by spreading 0.25 ml of the homogenates and their dilutions on each of four petri-dishes with Baird-Parker (BP) agar (Oxoid) supplemented with tellurite and egg yolk emulsion (Oxoid). These were incubated at 37^0C and examined for typical pitch-black colonies after 24 h and 48 h (ICMSF, 1978). Presumptive colonies were grown overnight at 35^0C in brain heart infusion

broth (prepared from ingredients) and tested for coagulase activity with rabbit plasma containing 0.1 % (w/v) EDTA (Difco) (ICMSF, 1978; ISO, 1983).

3.5.1.5 Enumeration of *Escherichia coli* biotype 1

E. coli biotype 1 was enumerated by the Anderson Baird-Parker method (Anderson and Baird-Parker, 1975) in Tryptone Bile Agar (TBA, Oxoid), by using cellulose acetate filter membranes (Pore size 0.45 μm; 85 mm diameter, Millipore). 0.1 ml of 1: 10 dilution was inoculated onto the surface of the membrane, which had previously been placed on top of the same medium for 4 h at 37^{0}C. Incubation was at 44 ± 0.5^{0}C for 18-24 h. Colonies were then tested for indole production using Vracko and Sherris reagent. Indole producing colonies are stained pink within 5 min.

3.5.1.6 Isolation of *Salmonella*

For isolation and confirmation of *Salmonella*, procedures recommended by ICMSF (1978) were followed. Analytical samples of 25 g were homogenised in 225 ml of buffered peptone water and incubated for 16 h at 37^{0}C. Tetrathionate brilliant green broth (Difco) and selenite broth (Difco) were used as selective enrichments. All media were incubated at 43^{0}C and isolations made at 24 and 48 h by streaking a plate of brilliant green agar (BGA, Difco) and xyline lysine desoxycholate (XLD) agar (Oxoid). The plates were incubated at 37^{0}C for 20-24 h. Colonies of *Salmonella* spp. on BGA are pink or red (occasionally colourless) surrounded by a zone of bright red medium and are red with black centres on XLD agar (Harrigan and McCance, 1976). Suspect *Salmonella* colonies were picked and inoculated into triple-sugar iron agar (Oxoid) and lysine-iron agar (Oxoid) for identification (ICMSF, 1978). The

identification of suspect colonies was confirmed by serological tests using polyvalent antisera (Fisher Diagnostics).

3.5.1.7 Isolation of *Shigella*

For isolation of *Shigella*, procedures of ICMSF (1978) were used. Samples of 25 g were homogenised in 225 ml of buffered peptone water and incubated for 16 h at 37^{0}C. 10 ml of pre-enrichment resuscitation culture was added to 100 ml of Gram-negative (GN) broth for enrichment and incubated at 37^{0}C for 24 h. XLD agar (Oxoid) and *Salmonella-Shigella* agar (Difco) were used for selective isolation. Typical *Shigella* colonies on XLD agar appear uniformly red and are pale yellow or nearly colourless on *Salmonella-Shigella* agar. Biochemical and serological tests as outlined in procedures of ICMSF (1978) confirmed typical colonies of *Shigella.*

3.5.1.8 Isolation of *Vibrio cholerae*

V. cholerae was isolated by direct plating and enrichment as outlined by Karunasagar and Karunasagar (1994). Samples of 25 g were homogenised with 225 ml sterile salt-free alkaline peptone water. Homogenates were transferred to sterile 1 litre Erlenmeyer flasks and incubated at 37^{0}C for 6 h. A loopful was then streaked on Thiosulphate-Citrate-Bile-Salts-Sucrose (TCBS) agar (Oxoid) and incubated for 24 h at 37^{0}C. Typical yellow colonies of *V. cholerae* were picked and confirmed by biochemical and serological tests (Madden *et al.*, 1984).

3.5.1.9 Isolation of *Clostridium perfringens*

Tryptose-sulphite-cycloseine (TSC) agar (Merck) was used for the isolation of *C. perfringens* (ICMSF, 1978). Samples of 25 g were homogenised with 225 ml of

Reinforced Clostridial Medium (RCM, Oxoid). 1 ml of appropriate decimal dilutions was added to petri dishes in duplicates. 15-20 ml of TSC agar was added. The plates were placed in anaerobic jar and incubated at 37^0C for 24 h. Gelatin-lactose and motility-nitrate tests were used for confirmation.

3.5.2 Microbial analysis of surface swabs

Swabs taken from fish contact surfaces were prepared by adding 10 ml of 0.1 % peptone water followed by agitation. The swab washings were examined for the presence of coliforms, feacal coliforms, *E. coli* biotype 1, *Salmonella* and *V. cholerae*. Swabs from personnel hands were in addition examined for the presence of *S. aureus*. The procedures followed for examination of these organisms were similar to those outlined for fish samples.

For examination of *C. perfringens*, swabs were prepared by adding 10 ml of Reinforced Clostridial Medium (RCM, Oxoid) (Harrigan and McCance, 1976). 2 ml of the swab washings was added into each of 5 tubes containing Differential Reinforced Clostridial Medium (DRCM, Oxoid) and incubated at 30^0 C for 7 days. Tubes showing blackening were confirmed for *C. perfringens* by Gelatin-lactose and motility- nitrate tests.

3.5.3 Microbial analysis of water

Bacteriological examination of water for sanitary quality followed the procedures recommended by the World Health Organisation (1984). Enumeration of aerobic plate count (APC) was made by the pour plate method through use of plate count agar

(Difco). Incubation was at 37^{0}C for 48 h and at 22^{0}C for 72 h. Coliforms, feacal coliforms and *C. perfringens* were enumerated by the most probable number methods.

For isolation of *Salmonella*, 100 ml amounts of water were passed through membrane filters (Pore size 0.45 µm, Millipore). The filters were placed in bottles containing buffered peptone water and incubated for 18 h at 37^{0}C for resuscitation. Selenite broth (Difco) was used as selective enrichment medium. The medium was incubated at 37^{0}C and sub-cultured every 12 h for 4 days on Bismuth sulphite (BS) and Desoxycholate citrate (DC) agars (Oxoid). Typical colonies of *Salmonella* on BS agar appear black with or without a metallic sheen. On DC agar, they are pale, nearly colourless, smooth and translucent (Harrigan and McCance, 1976). Presumptive colonies of *Salmonella* were picked and confirmed by biochemical and serological tests as described above for fish samples.

V. cholerae was isolated by passing 100 ml amounts of water through membrane filters (pore size 0.22 µm, Millipore). The filters were placed in bottles containing salt-free alkaline peptone water and incubated at 37^{0}C for 6 h. The filters were then placed on TCBS agar and incubated for 24 h at 37^{0}C. Typical yellow colonies were picked and confirmed by biochemical and serological tests as described above for fish samples.

For isolation of pathogenic staphylococci, 100 ml amounts of water were passed through membrane filters (Pore size 0.45 µm, Millipore). The filters were placed on plates with Baird-Parker agar. These were incubated at 35^{0}C for 48 h. Further confirmation was on the basis of coagulase production (rabbit plasma, EDTA, Difco).

3.6 Statistical analysis

The data obtained was analysed and where necessary appropriate transformation of data was carried out. Data analysis was carried out using Analysis of Variance (ANOVA) to determine if differences in counts by sampling point in each establishment were significant. Where significant differences were indicated, Tukey's honestly significant difference method was used to determine where the differences existed. The t-test was used to compare the differences in bacteria counts on the raw fish and finished products sampled from the two establishments. In cases where total bacteria numbers were not enumerated, the number of positive samples were expressed as a percentage of total number of samples examined.

CHAPTER FOUR

4.0 RESULTS

4.1 Aerobic mesophilic colony count (AMCC) at 30^0C

The results of AMCC of Nile perch along the processing line in both establishments are shown in Table 1.

Table 1. Aerobic mesophilic colony count (AMCC) at 30^0C of Nile perch (*Lates niloticus*) along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Geometric mean of AMCC (CFU /g)
A	Fish as received	2.3×10^{5a}
	Fish after washing	6.3×10^{4ab}
	Fish after filleting	3.3×10^{4b}
	Fish after skinning and trimming	5.9×10^{3c}
	Fish after packing	4.0×10^{3c}
	Fish after chilling	1.6×10^{3c}
B	Fish as received	2.4×10^{5a}
	Fish after washing	7.4×10^{4b}
	Fish after beheading and gutting	6.0×10^{4b}
	Fish after trimming	1.8×10^{4c}
	Fish after packing	6.2×10^{3c}
	Fish after freezing	3.5×10^{3d}

[abcd] Within each establishment, counts having a common letter in their superscripts are not significantly different at the experimentwise error rate of $\alpha = 0.05$ with Tukey's honestly significant difference method.

The results indicate that the AMCC of the received fish in establishment A was 2.3 x 10^5 CFU /g. This reduced to 6.3 x 10^4 CFU /g after washing, 3.3 x 10^4 CFU /g after filleting, 5.9 x 10^3 CFU /g after skinning and trimming, 4.0 x 10^3 CFU /g after packing and 1.6 x 10^3 CFU /g after chilling. The processing operations in this establishment caused a significant reduction in the AMCC ($P < 0.001$, Appendix 1a).

In establishment B, the AMCC was 2.4 x 10^5 CFU /g when fish arrived at the factory. This reduced to 7.4 x 10^4 CFU /g after washing, 6.0 x 10^4 CFU /g after beheading and gutting, 1.8 x 10^4 CFU /g after trimming, 6.2 x 10^3 CFU /g after packing and 3.5 x 10^3 CFU /g after freezing (Table 1). The reduction in AMCC of fish along the processing line was significant ($P < 0.001$, Appendix 1b).

The whole fish received in both establishments had AMCC that was not significantly different (t-test, $P > 0.05$). However, the AMCC of the finished chilled product and that of frozen product were significantly different (t-test, $P < 0.05$), with the chilled product from establishment A having a lower AMCC value.

4.2 Hydrogen sulphide (H_2S) producing bacteria count at 30^0C

Table 2 shows H_2S-producing bacteria count and H_2S-producing bacteria count expressed as percentage of AMCC. In establishment A, the count on the received whole fish was 5.6 x 10^3 CFU /g corresponding to 2.4 % of the AMCC. This initial level of H_2S-producing bacteria reduced to 1.9 x 10^3 CFU /g, 7.2 x 10^2 CFU /g, 8.7 x 10^1 CFU /g, 3.2 x 10^1 CFU /g and 1.4 x 10^1 CFU /g after washing, after filleting, after skinning and trimming, after packing and after chilling respectively.

Table 2. Hydrogen sulphide producers count at 30^0C of Nile perch (*Lates niloticus*) along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Geometric mean of H_2S-producers count (CFU /g)	H_2S-producers count as % of AMCC
A	Fish as received	5.6×10^{3a}	2.4
	Fish after washing	1.9×10^{3ab}	3
	Fish after filleting	7.2×10^{2b}	2.2
	Fish after skinning and trimming	8.7×10^{1c}	1.5
	Fish after packing	3.2×10^{1cd}	0.8
	Fish after chilling	1.4×10^{1d}	0.9
B	Fish as received	6.2×10^{3a}	2.6
	Fish after washing	1.3×10^{3b}	1.8
	Fish after beheading and gutting	1.0×10^{3b}	1.7
	Fish after trimming	2.3×10^{2c}	1.3
	Fish after packing	1.2×10^{2c}	1.9
	Fish after freezing	4.1×10^{1d}	1.2

[abcd] Within each establishment, counts having a common letter in their superscripts are not significantly different at experimentwise error rate of $\propto = 0.05$ with Tukey's honestly significant difference method.

The number of H_2S–producing bacteria on fish received in establishment B was 6.2 x 10^3 CFU /g and accounted for 2.6 % of the AMCC (Table 2). The counts decreased to 1.3 x 10^3 CFU /g after washing, 1.0 x 10^3 CFU /g after beheading and gutting. The number of H_2S-producing bacteria reduced further to 2.3 x 10^2 CFU /g after trimming, 1.2 x 10^2 CFU /g after packing and reached a value of 4.1 x 10^1 CFU /g after freezing.

Results showed that during the processing operations in both establishments, there was a significant reduction ($P < 0.001$, Appendix 2a) in H_2S-producing organisms. The counts on received whole fish in the two establishments were not significantly different (t-test, $P > 0.05$). However, the counts on the final chilled product and the frozen product were significantly different (t-test, $P < 0.05$), with the chilled product from establishment A having a lower value of H_2S-producing bacteria. In both establishments, the number of H_2S-producing bacteria did not exceed 3 % of AMCC.

4.3 Enterobacteriaceae and coliforms counts

The results of Enterobacteriaceae and coliforms counts on Nile perch are depicted in Table 3. Results showed that the whole fish received in establishment A had Enterobacteriaceae count of 5.2 x 10^2 CFU /g. Subsequent washing of fish reduced the counts to 1.7 x 10^2 CFU /g. This reduced further to 1.0 x 10^2 CFU /g, 3.6 x 10^1 CFU /g, 2.2 x 10^1 CFU /g and 1.2 x 10^1 CFU /g after filleting, after skinning and trimming, after packing and after chilling respectively. The Most Probable Number (MPN) of coliforms when fish arrived at the establishment was 2.8 x 10^2 /g. This reduced to 1.3 x 10^2 /g after washing, 7.8 x 10^1 /g after filleting, 2.6 x 10^1 /g after skinning and trimming, 1.4 x 10^1 /g after packing and 5 /g after chilling.

Table 3. Enterobacteriaceae and coliforms counts of Nile perch (*Lates niloticus*) along the processing line in two fish establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Enterobacteriaceae (CFU /g)	Coliforms (MPN /g)
A	Fish as received	5.2×10^{2a}	2.8×10^{2a}
	Fish after washing	1.7×10^{2b}	1.3×10^{2ab}
	Fish after filleting	1.0×10^{2b}	7.8×10^{1bc}
	Fish after skinning and trimming	3.6×10^{1c}	2.6×10^{1cd}
	Fish after packing	2.2×10^{1cd}	1.4×10^{1de}
	Fish after chilling	1.2×10^{1d}	5^{e}
B	Fish as received	3.5×10^{2a}	2.0×10^{2a}
	Fish after washing	1.7×10^{2b}	1.1×10^{2b}
	Fish after beheading and gutting	1.2×10^{2b}	9.3×10^{1b}
	Fish after trimming	5.6×10^{1c}	4.2×10^{1c}
	Fish after packing	3.2×10^{1d}	2.0×10^{1d}
	Fish after freezing	1.4×10^{1e}	5^{e}

[abcde] For each column within each establishment, counts having a common letter in their superscript are not significantly different at experimentwise error rate of $\propto$ = 0.05 with Tukey's honestly significant difference method.

In establishment B, Enterobacteriaceae count on received whole fish was 3.5 x 10^2 CFU /g while the coliforms count was 2.0 x 10^2 MPN /g. Enterobacteriaceae count reduced to 1.7 x 10^2 CFU /g after washing, 1.2 x 10^2 CFU /g after beheading and gutting, and 5.6 x 10^1 CFU /g after trimming. The counts reduced further to 3.2 x 10^1 CFU /g and 1.4 x 10^1 CFU /g after packing and after freezing respectively. The number of coliforms also reduced during the processing operations. The counts were 1.1 x 10^2 MPN /g after washing, 9.3 x 10 MPN /g after beheading and gutting, 4.2 x 10 MPN /g after trimming, 2.0 x 10 MPN /g after packing and 5 MPN /g after freezing.

In the two establishments, the reduction along the processing line of both Enterobacteriaceae and coliforms count was significant ($P < 0.001$, Appendix 3a, 3b, 4a, 4b). The counts on whole fish received in both establishments were not significantly different ($P > 0.05$, Appendix 10a). Similarly, counts on the final products sampled in the two establishments were not significantly different (t-test, $P > 0.05$).

4.4 Faecal coliforms and *E. coli* biotype 1 counts

Table 4 shows the number of feacal coliforms and *E. coli* biotype 1 on Nile perch along the processing line. In establishment A, feacal coliforms count on received whole fish was 38 MPN /g while *E. coli* biotype 1 count was 29 CFU /g. The feacal coliforms count reduced to 29 MPN /g after washing, 20 MPN /g after filleting and 8 MPN /g after skinning and trimming. The count after packing was 4 MPN /g which decreased to less than 3 MPN /g after chilling. Similarly, there was a decrease in *E. coli* biotype 1 count along the processing line. The initial *E. coli* biotype 1 count

reduced to 22 CFU /g, 13 CFU /g, 5 CFU /g, 3 CFU /g and less than 3 CFU /g after washing, after filleting, after skinning and trimming, after packing and after chilling respectively.

The number of feacal coliforms on whole fish received in establishment B was 37 MPN /g. Washing of fish reduced the count to 30 MPN /g while beheading and gutting reduced the count to 26 MPN /g. This reduced further to 12 MPN /g, 6 MPN /g and 3 MPN /g after trimming, after packing and after freezing respectively. *E. coli* biotype 1 count of 30 CFU /g when fish was received at the establishment reduced to 23 CFU /g after washing, 19 CFU /g after beheading and gutting, 9 CFU /g after trimming, 4 CFU /g after packing and less than 3 CFU /g after freezing.

The reduction in feacal coliforms and *E. coli* biotype 1 numbers along the processing line in each establishment was significant ($P < 0.001$, Appendix 5a, 5b, 6a, 6b). The counts on whole fish received in both establishments were not significantly different (t-test, $P > 0.05$). Faecal coliforms and *E. coli* biotype 1 counts on the finished products were also not significantly different (t-test, $P > 0.05$).

4.5 *Staphylococcus aureus* count

Results of *S. aureus* counts on Nile perch collected at the various stages of processing in both establishments are listed in Table 5. The results showed that *S. aureus* count on fish received in establishment A was 3.5×10^2 CFU /g. This decreased to 1.6×10^2 CFU /g after washing, 9.3 x 10 CFU /g after filleting and 4.0 x 10 CFU /g after skinning and trimming. *S. aureus* count on fish after packing and after chilling was less than 10 CFU /g.

Table 4. Faecal coliforms and *E. coli* biotype 1 counts on Nile perch (*Lates niloticus*) along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Faecal coliforms (MPN /g)	*E. coli* biotype 1 (CFU /g)
A	Fish as received	38^{a}	29^{a}
	Fish after washing	29^{ab}	22^{ab}
	Fish after filleting	20^{b}	13^{b}
	Fish after skinning and trimming	8^{c}	5^{c}
	Fish after packing	4^{d}	3^{d}
	Fish after chilling	$<3^{d}$	$<3^{d}$
B	Fish as received	37^{a}	30^{a}
	Fish after washing	30^{ab}	23^{ab}
	Fish after beheading and gutting	26^{b}	19^{b}
	Fish after trimming	12^{c}	9^{c}
	Fish after packing	6^{d}	4^{d}
	Fish after freezing	3^{e}	$<3^{d}$

< 3 means no visible colonies appeared on 10^{-1} dilution.

^{abcde} For each column within each establishment, counts having a common letter in their superscript are not significantly different at experimentwise error rate of α = 0.05 with Tukey's honestly significant difference method.

Table 5: *Staphylococcus aureus* count of Nile perch *(Lates niloticus)* along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Geometric mean of *S. aureus* count (CFU /g)
A	Fish as received	3.5×10^{2a}
	Fish after washing	1.6×10^{2b}
	Fish after filleting	9.3×10^{1b}
	Fish after skinning and trimming	4.0×10^{1c}
	Fish after packing	$<10^{d}$
	Fish after chilling	$<10^{d}$
B	Fish as received	3.2×10^{2a}
	Fish after washing	2.5×10^{2ab}
	Fish after beheading and gutting	2.1×10^{2b}
	Fish after trimming	8.6×10^{1c}
	Fish after packing	$<10^{d}$
	Fish after freezing	$<10^{d}$

<10 means no visible colonies appeared on 10^{-1} dilution.

[abcd] Within each establishment, counts having a common letter in their superscripts are not significantly different at the experimentwise error rate of $\propto = 0.05$ with Tukey's honestly significant difference method.

In establishment B, *S. aureus* count on raw fish was 3.2 x 10^2 CFU /g. This reduced to 2.5 x 10^2 CFU /g after washing, 2.1 x 10^2 CFU /g after beheading and gutting, 8.6 x 10 CFU /g after trimming and less than 10 CFU /g after packing and after freezing.

During the processing operations in both establishments, there was a significant reduction ($P < 0.001$, Appendix 7a, 7b) in *S. aureus* count. Counts on whole fish received in the two establishments were not significantly different (t-test, $P > 0.05$).

4.6 Incidence of *Salmonella, Shigella, V. cholerae* and *C. perfringens*

Results for testing for *Salmonella* spp., *Shigella* spp., *V. cholerae* and *C. perfringens* in the fish samples collected at various stages of processing in both establishments are summarised in Table 6. *Salmonella* was recovered from 5.6 % of incoming whole fish in establishment A. Isolations were also made from 16.7 % of samples collected after the fish was filleted. No *Salmonella* was recovered from fish samples collected from other stages of processing. *V. cholerae* was isolated from 22.2 % of incoming whole fish in establishment A. However, this organism was not isolated from fish samples collected from subsequent stages of processing. *C. perfringens* was detected in 5.6 % of samples of incoming raw fish. No isolations were made from samples collected from the other stages of processing.

In establishment B, *Salmonella* was not isolated from any of the samples collected while *V. cholerae* was isolated from 3.8 % of incoming whole fish (Table 6). *C. perfringens* was recovered from 1.9 % of samples collected from whole fish as received. However, this organism was not recovered in any sample collected from the subsequent processing steps.

Table 6. Incidence of *Salmonella*, *Shigella*, *V. cholerae* and *C. perfringens* in Nile perch (*Lates niloticus*) along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	No. of samples examined	Percentage positive for [a]		
			Sal.	V.C	C.P
A	Fish as received	18	5.6	22.2 **b**	5.6
	Fish after washing	18	0	0	0
	Fish after filleting	18	16.7	0	0
	Fish after skinning and trimming	18	0	0	0
	Fish after packing	18	0	0	0
	Fish after chilling	18	0	0	0
B	Fish as received	53	0	3.8 **b**	1.9
	Fish after washing	53	0	0	0
	Fish after beheading and gutting	53	0	0	0
	Fish after trimming	53	0	0	0
	Fish after packing	53	0	0	0
	Fish after freezing	53	0	0	0
	Total all sampling stages	**426**	**1**	**1.4**	**0.5**

[a] *Shigella* was not isolated from any fish sample.
b *V. cholerae* isolated from fish samples when there was a cholera outbreak in area around Lake Victoria.
Sal. = *Salmonella*; V.C = *V. cholerae;* C.P = *C. perfringens*.

Results from this study showed that *Salmonella* spp., *C. perfringens* and *V. cholerae* O1 were present in 1 %, 0.5 % and 1.4 % of fish samples tested respectively. No *Shigella* spp. was recovered from any of the fish samples collected from both establishments.

4.7 Bacteriological examination of fish-contact surfaces

Results of laboratory analyses of swabs from fish contact surfaces in establishment A and B are shown in Tables 7 and 8 respectively. Results for swab samples collected from establishment A showed that 27.4 % were positive for coliforms, 7.5 % positive for faecal coliforms, and 4.8 % positive for *E. coli* biotype 1. For each of these bacteria, the highest incidence (55 %) was from fish holding crates at the fish receiving section followed by raw fish washing brushes with an incidence of 44.4 %. Both *Salmonella* and *V. cholerae* were present in 5.6 % of swab samples collected from the surfaces of washing brushes at the fish receiving section. *C. perfringens* was not isolated from any fish contact-surface swab.

In establishment B, coliforms, faecal coliforms and *E. coli* biotype 1 were detected in 23.6 %, 7.8 % and 4.8 % of swab samples respectively. Fish holding crates at the fish receiving section had the highest number of positive samples for these bacteria followed by beheading and gutting table. *Salmonella* was isolated from swabs collected from cutting table, cutting knife and trimming board, and the incidence was 5.6 %, 1.9 % and 3.7 % respectively. No isolations of *V. cholerae* and *C. perfringens* were made from swab samples collected from this establishment.

Table 7. Results of bacteriological examination of fish-contact surface swabs collected along Nile perch (*Lates niloticus*) processing line in establishment A, 1997/98.

Sampling stage	Description of contact surface	No. of samples examined	Percentage of samples examined positive for [a]				
			Coliforms	Faecal coliforms	*E. coli*	*Salmonella*	*V. cholerae*
Fish receiving	Washing brush	18	44.4	16.7	11	5.6	5.6 **b**
	Holding crate	20	55	25	15	10	0
Filleting	Filleting table	18	39	11	5.6	5.6	0
	Filleting knife	18	22.2	5.6	5.6	5.6	0
	Holding crate	18	27.8	0	0	0	0
Skinning and trimming	Cutting board	18	11	0	0	0	0
	Cutting knife	18	5.6	0	0	0	0
	Holding crate	18	5.6	0	0	0	0
Packing	Packing table	20	5	0	0	0	0
	Polythene sheet	20	0	0	0	0	0
Total all sampling stages		**146**	**27.4**	**7.5**	**4.8**	**3.4**	**0.7**

[a] *C. perfringens* was not isolated from any fish-contact surface swab.

b *V. cholerae* isolated from a swab sampled when there was a cholera outbreak in the Lake Victoria region.

Table 8. Results of bacteriological examination of fish-contact surface swabs collected along Nile perch (*Lates niloticus*) processing line in establishment B, 1997/98.

Sampling stage	Description of contact surface	No. of samples examined	Percentage of samples examined positive for [a]			
			Coliforms	Faecal coliforms	*E. coli*	*Salmonella*
Fish receiving	Washing brush	54	42.6	12.9	7.4	0
	Holding crate	53	51	22.6	19	0
Beheading and gutting	Cutting table	54	48	14.8	5.6	5.6
	Cutting knife	54	29.6	11	7.4	1.9
	Holding crate	54	20.3	7.4	5.6	0
Trimming	Trimming board	54	18.5	5.6	3.7	3.7
	Trimming knife	54	11.1	1.9	0	0
	Holding crate	54	7.4	1.9	0	0
Packing	Packing table	54	7.4	0	0	0
	Polythene bag	54	0	0	0	0
Total all sampling stages		**539**	**23.6**	**7.8**	**4.8**	**1.1**

[a] *V. cholerae* and *C. perfringens* were not isolated from any fish-contact surface swab.

4.8 Bacteriological examination of swabs from personnel handling fish

Table 9 depicts the results of bacteriological examination of swabs from hands of personnel handling fish in both establishments. In establishment A, coliforms, faecal coliforms and *E. coli* biotype 1 were present in 22.5 %, 5 % and 3.8 % of samples respectively. The incidence of these organisms was relatively lower in establishment B where 20.8 % were positive for coliforms, 3.2 % were positive for faecal coliforms and 0.9 % positive for *E. coli* biotype 1. *Salmonella* was detected in establishment A in 3.8 % of the samples. However, no isolations of *Salmonella* were made from establishment B. *S. aureus* was present in 28.8 % and 29.6 % of the workers' hands in establishment A and B respectively. *C. perfringens* was not isolated from personnel hands in both establishments.

4.9 Bacteriological quality of water and ice

The bacteriological quality of water and ice used during the processing of fish in establishment A and B is given in Table 10. In establishment A, the Aerobic Plate Count (APC) at 22^0C was 320 CFU /ml for untreated water, 40 CFU /ml for treated water and 53 CFU /ml for ice. The APC at 37^0C was 56 CFU /ml, 6 CFU /ml and 8 CFU /ml for untreated water, treated water and ice respectively. In establishment B, the APC at 22^0C was 260 CFU /ml for untreated water, 22 CFU /ml for treated water and 28 CFU /ml for ice. At 37^0C, untreated water had APC of 38 CFU /ml while treated water had APC of 4 CFU /ml. Ice samples collected from this establishment had APC of 5 CFU /ml at 37^0C. Results revealed that counts at 22^0C were slightly higher than counts at 37^0C.

Table 9. Results of bacteriological examination of swabs from hands of personnel handling fish in two Nile perch (*Lates niloticus*) processing establishments in Kenya, 1997/ 98.

Establishment code	Sampling stage	No. of samples examined	Percentage of samples examined positive for [a]				
			Coliforms	Faecal coliforms	*E. coli*	*Salmonella*	*S. aureus*
A	Fish receiving	20	40	10	5	5	30
	Filleting	20	35	10	10	10	40
	Skinning and trimming	20	10	0	0	0	25
	Packing	20	5	0	0	0	20
Total all sampling stages		**80**	**22.5**	**5**	**3.8**	**3.8**	**28.8**
B	Fish receiving	54	31.5	5.6	3.7	0	40.7
	Beheading and gutting	54	33.3	7.4	0	0	25.9
	Trimming	54	9.3	0	0	0	33.3
	Packing	54	9.3	0	0	0	18.5
Total all sampling stages		**216**	**20.8**	**3.2**	**0.9**	**0**	**29.6**

[a] *C. perfringens* was not isolated from any of swabs from personnel hands.

Table 10. Results of bacteriological examination of water and ice used during the processing of Nile perch (*Lates niloticus*) in two processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	No. of samples examined	APC per ml at		No. positive for		Mean coliforms count for positive samples
			22^{0}C	37^{0}C	Coliforms	F. coliforms	
A	Untreated water [a]	6	320	56	1	0	3 /100ml
	Treated water [a]	6	40	6	0	0	Nil
	Ice [a]	6	53	8	0	0	Nil
B	Untreated water [a]	18	260	38	2	0	2 /100ml
	Treated water [a]	18	22	4	0	0	Nil
	Ice [a]	18	28	5	0	0	Nil

F. coliforms = faecal coliforms; APC = Aerobic plate count.

[a] *Salmonella, V. cholerae, S. aureus* and *C. perfringens* were not isolated in 100mls of any of the water sample.

Coliform bacteria were detected in one sample of untreated water in establishment A and two samples of untreated water in establishment B. Faecal coliforms, *Salmonella, V. cholerae*, *S. aureus* and *C. perfringens* were not isolated in 100 mls of any of the water samples collected from both establishments.

4.10 Core temperature (^{0}C) of fish

Table 11 shows the core temperature (^{0}C) of Nile perch along the processing line in the two establishments. The temperature of the raw fish received in both establishments was found to be below 2^{0}C. During the processing operations in both establishments, there was a gradual increase in fish temperature with the highest temperature being recorded after packing but before chilling / freezing. The temperature range of chilled product was -0.1^{0}C to –3.8^{0}C while that of the frozen product was –15.5^{0}C to –19.8^{0}C.

Table 11. Core temperature (^{0}C) of Nile perch (*Lates niloticus*) along the processing line in two fish processing establishments in Kenya, 1997/98.

Establishment code	Sampling stage	Temperature range (^{0}C)
A	Fish as received	-0.3 to 1.3^{0}C
	Fish after washing	0.9 to 1.8^{0}C
	Fish after filleting	2.1 to 3.4^{0}C
	Fish after skinning and trimming	3.8 to 5.4^{0}C
	Fish after packing	5.2 to 7.2^{0}C
	Fish after chilling	-1.0 to –3.8^{0}C
B	Fish as received	-0.5 to 1.9^{0}C
	Fish after washing	0.8 to 2.4^{0}C
	Fish after beheading and gutting	2.5 to 4.2^{0}C
	Fish after trimming	4.1 to 8.4^{0}C
	Fish after packing	4.8 to 12.0^{0}C
	Fish after freezing	-15.5 to –19.8^{0}C

CHAPTER FIVE

5.0 DISCUSSION

The Aerobic Mesophilic Colony Count (AMCC) at 30°C of Nile perch received in the two fish processing establishments was less than 10^6 CFU /g (Table 1). This was in the range of 10^4-10^6 CFU /g obtained on freshly caught Nile perch by Gram *et al.* (1990). The results obtained were also consistent with the counts recorded by many other workers for different species of fish (Shewan, 1971; Shewan, 1977; Liston, 1980; Davaraju and Setty, 1985). The AMCC values obtained during this study would not necessarily correlate with food safety since many pathogens would not grow under the cultural conditions often used. However, they represented the quality of fish and gave information on the general standard of hygiene and fish temperature control at the landing beaches and during transportation. The International Commission of Microbiological Specifications of Foods (ICMSF) recommended that raw fish having AMCC of less than 10^6 CFU /g should be considered good quality while those having AMCC in excess of 10^7 CFU /g be considered unacceptable (ICMSF, 1986).

On the basis of this guideline, it appears that the raw fish received in both processing establishments was of acceptable quality. This was probably due to adequate icing of fish at the landing beaches and during transportation to the processing establishments. Gram *et al.* (1990) reported that fish from tropical waters such as Nile perch tend to have mesophilic strains of bacteria. The minimum growth temperature for most mesophilic organisms is about 10^0C (Lowry and Gill, 1985). Therefore, the organisms would not grow at temperatures below 2^0C (Table 11) recorded when fish were received at the establishments. The lower AMCC values may also be attributed to

efficiency of the compartments in which fish were transported. The compartments were insulated and provided with drains that allowed a continuous trickle of melting ice, thus helping to carry away slime, blood and microorganisms.

In the present study, hydrogen sulphide (H_2S) producing bacteria identified, as black colonies on iron agar did not exceed 3 % of AMCC at 30^0C (Table 2). Although no specific H_2S-producing bacteria were determined during the present study, Gram (1989) identified *Pseudomonas* spp., *Shewanella putrefaciens* and *Aeromonas* spp. as the bacteria associated with H_2S production in Nile perch preserved in ice. According to Barile *et al.* (1985), H_2S-producing bacteria are very resistant to chilling and their presence in the iced fish received at the establishments would not be surprising. The bacteriological production of hydrogen sulphide is a very common cause of spoilage in a variety of foods such as chilled fish and its presence at high levels signifies spoilage (Chai *et al.*, 1968; Herbert *et al.*, 1971; Gram *et al.*, 1987). The low counts of these organisms indicated that artisanal fishermen landed fish of good quality and that there was proper handling and sufficient chilling of fish during transportation.

Organisms such as Enterobacteriaceae, coliforms, faecal coliforms and *E. coli* are used as indicators of faecal contamination (Matches and Abeyta, 1983) in the environment where the fish are harvested. ICMSF recommended a limit of 10^3 CFU /g for Enterobacteriaceae and a limit of 4×10^2 MPN /g for both coliforms and faecal coliforms (ICMSF, 1986). Results obtained from the present study revealed that the counts of these organisms on fish received in both establishments were within the limits recommended by ICMSF (Table 3, 4). The *E. coli* biotype 1 count was also within the suggested guideline by Shewan (1971) of less than 10^2 /g. The above limits

are based on the fact that these organisms are not naturally present in unpolluted aquatic environment, and fish becomes contaminated due to unhygienic handling and poor processing practices. However, investigations have shown that *E. coli* and faecal coliforms can be present in unpolluted warm tropical waters and that *E. coli* can survive indefinitely in this environment (Hazen, 1988; Fujioka *et al.*, 1988; Toranzos *et al.*, 1988).

Washing of the received whole fish prior to processing caused a decrease in AMCC in both establishments (Table 1). There was also a decrease in H_2S-producing bacteria and members of Enterobacteriaceae (Table 2, 3, 4). In both establishments, washing was performed by gently brushing the surfaces of fish under free flowing water and this removed the gross dirt, blood, slime and the gut contents squeezed out of fish. According to Shewan (1971), more than 90 % of the contaminating bacteria adhering to the slime on the skin and gills of fish can be removed by thorough washing with clean water. In addition, the water used for washing the fish contained about 3 to 5 ppm residual chlorine and this has been reported to cause a decrease of Gram-negative organisms (Gillespie and Macrae, 1975).

In the current study, the AMCC, hydrogen sulphide producing bacteria and Enterobacteriaceae on fish in establishment A decreased remarkably after filleting and after skinning and trimming (Table 1, 2, 3). In establishment B, beheading and gutting operations reduced AMCC, H_2S-producing bacteria and Enterobacteriaceae significantly (Table 1, 2, 3). A further reduction in numbers of these organisms was also noted after trimming. The reduction in microbial load in both establishments may be explained by the fact that the primary sources of bacteria in whole fish are the skin,

gills and the intestines. Consequently there is a considerable reduction in bacterial counts after beheading and gutting, filleting, skinning and trimming. Shewan (1977) reported a similar reduction in bacterial load during the processing of cod.

Although there was a decrease in microbial load during the processing operations in both establishments, certain practices were observed that could lead to an increase in bacterial counts. In establishment A, freshly cut fillets were always allowed to touch the surfaces of the filleting table. According to FAO/WHO (1983), filleting surfaces are likely to be contaminated with microorganisms derived from the skin and the intestinal content of fish. The fillets, therefore, should be lifted by the cutting knife away from the carcass without touching the surface of the filleting table. To avoid cross-contamination between the superficial contamination on the skin and the fillets, arrangement of unskinned fillets in holding crates should be in a skin-to-skin manner. In establishment B, it was observed that during beheading and gutting operations, the knife was sometimes allowed to cut through the intestines releasing the gut contents. Such a practice should be avoided as it exposes the fish muscle to microbial and enzymatic action. In both establishments however, the trimming operations were carried out under running chlorinated water thereby reducing the resulting bacterial contamination.

Establishment hygiene as well as personal hygiene and sanitation are important in fish industry in prevention of contamination of product with microorganisms (Huss, 1994b). In the present study, it was found that fish-contact surfaces and personnel hands were likely sources of contamination of fish with faecal organisms. The occurrence of these organisms on contact surfaces and hands of personnel in the food

industry has been extensively reported. A survey in the fish industry showed that the bacterial count on the processing equipment varied from 10^3 to 10^8 bacteria /cm^2, of which 5-10 % were faecal organisms (Banwart, 1987). In poultry processing plants, working surfaces have been found to be contaminated with various types of coliforms (Schuler and Badenhop, 1972). Bryan (1979) reported the presence of *E. coli* on hands of 13 of 110 (11.8 %) butchers in a meat processing plant. The presence of these bacteria on contact surfaces and hands of personnel indicates general contamination and possible presence of enteric pathogens.

The coliforms, faecal coliforms and *E. coli* biotype 1 detected in the swabs were mainly derived from the surfaces (skin and gills) of the fish being processed. This was well illustrated by the fact that fish contact surfaces at the fish receiving stage had high incidence of these coliforms. Very few landing beaches have potable water and therefore, the fish received at the establishments is not adequately washed prior to receiving. Fish-contact surfaces should therefore be frequently and thoroughly scrubbed and treated with disinfectant. Wherever practicable the surfaces should be continuously flushed with running potable water containing 4 ppm of residual chlorine as recommended by Cheng *et al.* (1985). Personnel hands should also be washed with soap or another cleansing agent before commencing work, on every occasion after visiting toilet, before resuming work and whenever necessary. Although an adequate number of facilities for cleaning and disinfecting hands, equipment and utensils were available in both establishments, it was observed that the facilities were seldom properly utilised.

It was also noted that some hand washing facilities that were provided in the fish processing areas in both establishments had taps that were hand operable. In an attempt to find whether this could be a likely source of faecal contamination, 20 swab samples were collected and analysed for these organisms. All the samples tested positive and this agreed with the findings of Mendes and Lynch (1976), who found faecal organisms on hand operable taps of washrooms and toilets.

The hand operable taps, therefore, could conceivably serve as a source of contamination for the washed hands. It is recommended that hand washing facilities should be of a type not requiring operation by hand and should have adequate supply of water and liquid or powdered soap. In addition, hand washing facilities provided in fish processing areas should be located in full view of the processing floor for ease of supervision.

Chilling and freezing resulted in a reduction in AMCC and counts of hydrogen sulphide producing bacteria, Enterobacteriaceae, coliforms, feacal coliforms and *E. coli* biotype 1 (Table, 1, 2, 3, 4). During superchilling and freezing, the low water activity of the liquid phase in fish caused by concentration of solutes due to the formation of ice as well as the low temperature are at least inhibitory for bacterial activity and have a lethal effect on some organisms (Simmonds and Lamprecht, 1985; Sikorski, 1990).

The AMCC of the processed chilled product from establishment A was lower than that of the frozen product from establishment B (Table 1). The counts of H_2S-producing bacteria in the chilled product were also lower than in the frozen product

(Table 2). A t-test carried out on both microbial parameters revealed that differences among the two products were statistically significant ($p < 0.05$). The frozen product had its skin on and this could probably explain the higher values for both counts. As already mentioned, the skin is one of the primary sources of bacteria in whole fish.

In establishment A, higher count of *S. aureus* was detected (3.5×10^2 CFU /g) from the raw fish than in establishment B (3.2×10^2 CFU /g) (Table 5). However, the difference between the counts was not significant (t-test, $P > 0.05$). Liston (1980) reported that raw fish generally carry *S. aureus* to the extent of 10 to 30 % of total bacterial load. Fish caught from unpolluted waters are generally free of *S. aureus* at the time of harvest (Sikorski *et al.*, 1990). However, fish may become contaminated with this organism during subsequent handling and processing. The principle source of the organism is the fish handler who is a carrier of *S. aureus* or by cross-contamination from another source previously contaminated by humans (Huss, 1994b).

The carrier rate of *S. aureus* among healthy adults has been reported to vary from 25 % to 50 % (Eley, 1994). The organism is normally carried on the hands, ears, nasal cavity and throat. In the present study, *S. aureus* was present in 28.8 % and 29.6 % of the swabs collected from workers' hands in establishment A and B respectively (Table 9). Although personnel in both establishments were provided with gloves, it was observed that they were not maintained in a sound, clean and sanitary condition. Nile perch processed at the establishments was therefore susceptible to contamination with *S. aureus* because most of the processing operations were done manually. However, *S. aureus* counts decreased significantly during the processing operations and reached a

level of less than 10 CFU /g after packing, probably due to the washing of fish in almost every step.

Results from this survey showed that *Salmonella* spp. were present in 5.6 % of incoming whole fish in establishment A (Table 6). *Salmonella* spp. are common in animal faeces and wastes from slaughtering houses and poultry processing plants (Bryan, 1980). However, these organisms sometimes reach fresh water from which fish are caught and eventually reach estuaries. *Salmonella* also can reach these waters through community sewage discharge. It has been demonstrated that the organism can multiply and survive in the estuarine environment for weeks (Rhodes and Kator, 1988; Jimenez *et al.*, 1989). Fish can therefore become contaminated with *Salmonella* while in such polluted waters (Reilly and Twiddy, 1992).

Fish can also become contaminated due to poor sanitary conditions at the landing beaches. Most fish landing villages have been established without proper planning and lack basic sanitary facilities such as latrines (Gitonga, 1998). Very few landing beaches have potable water and landed fish are washed using lake water, which may serve as a source of contamination. While transporting fish from the fishing boats to the fish banda, some fishermen tend to drag the fish on the ground. During this movement, the fish get bruised and may also become contaminated with bacteria of faecal origin. Contamination could also occur through poor personal hygiene of fish handlers, who having had salmonellosis often become carriers of the organism for a period of time after the symptoms have gone. Diseases caused by *Salmonella* are common in Lake Victoria region and about 0.2 to 5 % of those who recover from such diseases become carriers who excrete *Salmonella* in their faeces (Ogwai, 1995).

In the present study, *Salmonella* was isolated from 16.7 % of fish samples collected after filleting in establishment A (Table 6). The source of *Salmonella* that contaminated these fish samples could not be ascertained. However, *Salmonella* was found to be present in 3.4 % of swabs from fish-contact surfaces and 3.8 % of swabs from personnel hands (Table 7, 9). Although *Salmonella* was not detected in any of the fish samples collected from establishment B, fish-contact surfaces were identified as potential sources of contamination since 1.1 % of swab samples tested positive for *Salmonella* (Table 8).

During the course of this study, 30 gut samples were tested for *Salmonella* in order to identify if the organism was established in the digestive systems of Nile perch. One sample tested positive and this was in agreement with data from a study on prawns that showed that *Salmonella* could be present in the gut (Reilly, 1987). As stated earlier, the processing practices in both establishments allowed the cutting of belly cavity resulting in release of gut contents. It is therefore likely that the initial source of *Salmonella* isolated from fish, fish contact-surfaces and personnel hands could have come from the gut contents of fish.

Salmonella ssp. are examined for in fish and fishery products and should not be present in any sample of 25 g (ICMSF, 1986; EEC, 1993). This standard is based on the fact that *Salmonella* spp. are not naturally present in the marine environment, and therefore are unlikely to be detected in freshly harvested fish. The occurrence of *Salmonella* in Nile perch received at the processing plants should be a serious cause for concern for the processors. There is a high risk that if the contaminated fish is accepted into the processing plants, the organism may be detected in the finished

product. There is also an additional risk that cross-contamination between raw fish and processed product may occur in processing establishments. In the present study however, *Salmonella* was not isolated from the finished product in the two establishments. Most of the processing operations in both establishments were carried out under free flowing chlorinated water (residual chlorine level 3 to 5 ppm). This reduces the number of Gram-negative organisms (Gillespie and Macrae, 1975).

In establishment B, cross-contamination was prevented by physical separation of the various operations of fish preparation and processing. Thus, well defined areas were provided for whole fish receiving, fish washing, fish beheading and gutting, fish trimming, and fish packing. The establishment had also provided a separate refuse room for storage of wastes in watertight offal bins. However, the design and layout in establishment A did not provide clear and complete separation of different production areas and the product flow could not preclude cross-contamination. The other effective preventive measures in place in both establishments were avoiding transfer of utensils or containers from one area to the other, and restricting movements of personnel. The establishment also maintained an effective and continuous programme for the control of insects, rodents and other vermin.

V. cholerae was isolated in 1.4 % of the fish samples collected from both establishments (Table 6). The organism was also isolated from 0.7 % of fish-contact surface swabs collected from establishment A (Table 7). All the positive samples had been sampled at the whole fish receiving stage. It should be noted that all the positive samples were isolated when there was a cholera outbreak in the Lake Victoria region. In cholera endemic areas, *V. cholerae* can reach aquatic environments through sewage

discharge (Bryan, 1980; Karunasagar and Karunasagar, 1994). These organisms persist in water only for a short while, but during that time they can either contaminate fish or be ingested by them.

Fish can also become contaminated due to poor sanitary conditions at the landing beaches or if handled by persons infected with *V. cholerae* and who do not practice proper personal hygiene. When such contamination occurs, *V. cholerae* can survive for 1-2 weeks at 0^0C (Ganowiak, 1990) under the chilled transport conditions employed by the fish processing plants. However, in the current study it was found that washing of whole fish prior to processing eliminated this organism. As stated earlier, washing of the whole fish is carried out under running chlorinated water and this reduces the number of Gram-negative organisms (Gillespie and Macrae, 1975). In addition, most of the bacteria adhering to the slime on the skin and gills of fish can be washed away with water (Shewan, 1971).

During the course of this survey, 15 gut samples were tested for the presence of *V. cholerae* to examine if the organism is established in digestive system of Nile perch. Although all samples were negative, other workers have reported the presence of *V. cholerae* in the alimentary tract of fish (Garcia and Zaleski, 1970; Liston, 1980; Hobbs, 1987).

C. perfringens was recovered only from the incoming whole fish and its incidence in both establishments was 0.5 % (Table 6). However, initial washing of whole fish eliminated this organism since it was not isolated at subsequent stages of processing. Ola and Akande (1998) found that *C. perfringens* was present in 2.1 % of samples of

fresh tropical water fish. *C. perfringens* can reach fish in their habitat (particularly near sewage outfalls), from the surface of holds in vessels, from the surfaces of equipment and utensils used for processing or from workers (Bryan, 1980). However, in the present study, *C. perfringens* was not isolated from any swab of fish-contact surface and personnel hands. This organism has a high minimum growth temperature (15^0C) and can be held in check by refrigeration (below 4^0 C) (Ganowiak, 1990) which was observed in the establishments.

The bacterial load of processed fish is dependent on the method of processing as well as environmental factors of the processing plant, especially quality of water and ice used during the operations (Huss, 1994b). According to World Health Organisation (WHO) and European Union (EU) standards for drinking water, the guide level (GL) for Aerobic Plate Count is 10 CFU /ml at 37^0C and 100 CFU /ml at 22^0C (EEC, 1980; WHO, 1984). In addition, coliforms and pathogenic bacteria should be absent in 100 ml of water. The results obtained from the present study (Table 10) revealed that the bacteriological quality of water and ice used during the processing of fish in both establishments was within these standards. It was therefore concluded that the water and ice was of acceptable quality and did not pose any microbiological hazard. The water used in both establishments was derived from municipal water supply and borehole and was treated via use of ultra-violet light and chlorination.

Results of bacteriological examination of water and ice also showed that APC at 22^0C was slightly higher than at 37^0C. This was probably because incubation at 37^0C allows growth of mesophiles while incubation at 22^0C allows growth of both mesophiles and most psychrotrophs (White and Hall, 1985).

Control of fish temperature during handling and processing is the single most important factor in the safety and quality of the processed product (Huss, 1994b). Temperature control is important in preventing growth of pathogenic and spoilage bacteria. In the present study, the temperature of the raw fish received in both establishments was found to be satisfactory as it was below 2^0C (Table 11). This was due to adequate icing of fish at the landing beaches and during transportation.

During processing, it is inevitable that the fish temperature will rise between the time fish are received and the time the finished product can again be placed under chilled conditions. Maintaining a temperature of below 10^0C is considered adequate, if the processing time is not prolonged (Sumner *et al.*, 1982). In establishment A, the temperature of fish along the processing line was in the range of -3.8^0C to 7.2^0C and thus the temperature control was adequate. However, it was noted that the filleting line was being oversupplied resulting in accumulation of raw fish in front of the filleters. In such an event, usually the top layer or the fish most easily accessible to the filleters was the first one to go through the processing line, while the fish at the bottom remained for a longer period of time resulting in temperature rise. This problem could have been avoided if the containers in which the fillets were kept were of a size that would not require long time to fill.

Temperature control in establishment B was not satisfactory at the packing step since the temperature of fish on several occasions was above 10^0C (Table 11). The high temperature was probably due to the final washing of fish in water at 18^0C-20^0C. During the period of study, the establishment installed a chill water facility and this improved the temperature control. The temperature rise could also have been due to

delay in flow of product, which resulted in pile up at packing. Delays of more than a few minutes should be avoided, and if this is not possible, the fish should be chilled, preferably with finely divided ice.

5.1 CONCLUSION AND RECOMMENDATIONS

The results presented here indicated that there was a significant reduction ($P < 0.001$) in microbial load during processing of Nile perch in both establishments and that the microbiological quality of the processed products was satisfactory. However, it was observed that certain microbial hazards existed during the processing operations in both establishments, particularly under circumstances of poor handling of fish. The received raw fish, fish-contact surfaces and hands of personnel handling fish were found to be contaminated with pathogenic bacteria such as *Salmonella* spp. and *V. cholerae*. The presence of such bacteria in Nile perch processing establishments is a serious cause for concern as it justifies the EU ban on the Kenyan fish products. There is a high risk that if contaminated raw fish is accepted into the processing establishments, the organisms may be detected in the processed product. There is also an additional risk that raw fish, fish contact surfaces or hands of personnel may cross-contaminate the processed product in the processing establishments.

From the current study, it is recommended that:

- The Nile perch processing establishments should put in place control measures aimed at eliminating, preventing or minimising the microbial hazards identified in the current study. The critical control points at or by which these microbial hazards can be controlled are (a) fish washing; (b) fish filleting or

beheading and gutting; (c) fillet skinning; (d) control of fish temperature; (e) and sanitation and establishment hygiene procedures.

- Establishment workers must be introduced to the basic principles of food-borne disease prevention and sanitation. This includes an understanding that (a) raw fish received at the establishments can be a source of pathogens; (b) hands should be washed after touching raw fish as well as after using the toilet; and (c) processed product should not be put in contact with raw fish or equipment previously used for raw fish.
- The Nile perch processing establishments should build the capacity to control and identify the microbial hazards.
- Although only certain microbial hazards were observed as demonstrated by laboratory results and temperature measurements during the evaluations in the establishments visited, other hazards no doubt occur from time to time. Additional ones, as well as good practices, must occur in other fish processing establishments. Hence, additional data needs to be collected before the situations can be considered typical of Nile perch processing establishments in Kenya.
- The present study only dealt with the final segment of the Nile perch industry, the processing establishment. Therefore, Data needs to be gathered on the microbial hazards associated with the handling of Nile perch at the fishing grounds and also the landing sites. This should involve monitoring fish handling practices and the sanitary conditions at the landing beaches. The state of Lake Victoria waters also needs to be evaluated for sewage discharges, and monitored by bacteriological surveys to determine the degree of contamination, using suitable indicators of faecal contamination.

- The government/ regulatory agencies should have the capacity to identify the microbial hazards and advice the Nile perch processing establishments accordingly.
- There is need to organise a workshop (s) where the findings of the current study can be shared with the stakeholders.

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APPENDICES

Appendix 1a: Anova for log $_{10}$ of Aerobic mesophilic colony count (AMCC /g) at 30^{0}C of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	20.57	4.11	29.36	P < 0.001
Experimental error	30	4.26	0.14		
Total	35	24.83			

Appendix 1b: Anova for log $_{10}$ of Aerobic mesophilic colony count (AMCC /g) at 30^{0}C of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	40.13	8.03	53.5	P < 0.001
Experimental error	102	15.37	0.15		
Total	107	55.5			

Appendix 2a: Anova for log $_{10}$ of hydrogen sulphide producers count /g at 30^{0}C of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	33.66	6.73	42.06	P < 0.001
Experimental error	30	4.7	0.16		
Total	35	38.36			

Appendix 2b: Anova for log $_{10}$ of hydrogen sulphide producers count /g at 30^{0}C of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	56.5	11.3	49.1	P < 0.001
Experimental error	102	23.9	0.23		
Total	107	80.4			

Appendix 3a: Anova for log $_{10}$ of Enterobacteriaceae count /g of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	11.33	2.27	37.8	P < 0.001
Experimental error	30	1.94	0.06		
Total	35	13.27			

Appendix 3b: Anova for log $_{10}$ of Enterobacteriaceae count /g of Nile perch *(Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	23.91	4.78	79.7	P < 0.001
Experimental error	102	6	0.06		
Total	107	29.91			

Appendix 4a: Anova for log $_{10}$ of coliforms count /g of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	12.66	2.53	28.1	P < 0.001
Experimental error	30	2.74	0.09		
Total	35	15.4			

Appendix 4b: Anova for log $_{10}$ of coliforms count /g of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	31.05	6.21	103.5	P < 0.001
Experimental error	102	6.07	0.06		
Total	107	37.12			

Appendix 5a: Anova for log $_{10}$ of faecal coliforms count /g of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	6.55	1.31	65.5	P < 0.001
Experimental error	30	0.73	0.02		
Total	35	7.28			

Appendix 5b: Anova for log $_{10}$ of faecal coliforms count /g of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	15.19	3.04	101.3	P < 0.001
Experimental error	102	2.83	0.03		
Total	107	18.02			

Appendix 6a: Anova for log $_{10}$ of *E. coli* biotype 1 count /g of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	5.29	1.14	57	P < 0.001
Experimental error	30	0.54	0.02		
Total	35	6.23			

Appendix 6b: Anova for log $_{10}$ of *E. coli* biotype 1 count /g of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	15.28	3.06	102	P < 0.001
Experimental error	102	2.74	0.03		
Total	107	18.02			

Appendix 7a: Anova for log $_{10}$ of *S. aureus* count /g of Nile perch (*Lates niloticus*) along the processing line in establishment A.

Source of variation	df	SS	MS	F	
Sampling point	5	12.2	2.44	81.3	P < 0.001
Experimental error	30	0.93	0.03		
Total	35	13.13			

Appendix 7b: Anova for log $_{10}$ of *S. aureus* count /g of Nile perch (*Lates niloticus*) along the processing line in establishment B.

Source of variation	df	SS	MS	F	
Sampling point	5	43.17	8.63	287.7	P < 0.001
Experimental error	102	3.44	0.03		
Total	107	46.61			