Microbiological Status of Chicken Carcases from a Non-Automated Poultry Processing Plant

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DECLARATION

I declare that this is my own, unaided work. It is being submitted to the University of Pretoria for the degree M Sc (Microbiology). It has not been submitted before for any degree or examination in any other University.

R. Olivier

MARETHA OLIVIER

4 th day of August 1998.

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PREFACE

Some aspects of the work conducted for this dissertation have be presented as papers or posters elsewhere:

CHAPTER 2

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ABSTRACT

This study evaluated the effect of processing on the microbiological status of chicken carcases in a small South African Grade B poultry abattoir. The bacterial numbers after defeathering (6.2 - 6.6 log CFU g^{-1}) and after evisceration (6.3 - 6.4 log CFU g^{-1}) were the highest. Marginally lower total numbers (5.5 - 6.1 log CFU g^{-1}) were obtained after chilling. There was a significant difference ($P<0.05$) in bacterial numbers amongst the different sampling sites in the abattoir during processing. A significant decrease in bacterial numbers ($P<0.05$) was obtained after chilling at 10:30 and 12:30 compared to corresponding numbers of samples taken after defeathering and after evisceration. No significant differences ($P>0.05$) were obtained irrespectively of the days of sampling and times of sampling. *Escherichia coli* was repeatedly isolated from the chicken carcases. *Staphylococcus aureus* was less frequently isolated and presumptive *Salmonella* was isolated only in low numbers.

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

Poultry is a important part of the animal food market and production is increasing to satisfy public demand world-wide (Bryan 1980; Anand *et al.*, 1989). Poultry and its products are also a major dietary item for the South African population (Bok *et al.*, 1986). According to the Directorate of Veterinary Public Health, 4.4 billion rands worth of poultry meat products were purchased by South Africans in 1994 (AFMA, 1996; SAPA, 1996). Therefore, it becomes necessary to maintain absolute hygiene and strict control at different stages of processing to produce a safe and wholesome chicken product.

Healthy chickens ready for processing harbour a tremendous amount and variety of bacteria. These bacteria are present on the surfaces of feet, feathers, skin and also in the intestines. During processing, a high proportion of these organisms will be removed, but further contamination can occur at any stage of the processing operation. The procedure for converting a live, healthy bird into a safe and wholesome poultry product provides many opportunities for micro-organisms to colonise on the surface of the carcase. During the various processing operations, opportunities exist for the contamination of the carcases from the environment, the process in the plant itself, contamination via knives, equipment, the hands of workers and also by cross-contamination from carcase to carcase. Some processing operations encourage an increase of contamination or even multiplication of contaminating organisms. As a result, the microbial population changes from mainly Gram-positive rods and micrococci on the outside of the live chicken to Gram-negative micro-organisms on the finished product (Bryan, 1980; Thomas *et al.*, 1980; Eustace, 1981; Roberts, 1982; Grau, 1986; Bailey *et al.*, 1987; Connor *et al.*, 1987; Banwart, 1989; Mead, 1989). Poultry processing has a number of unique features which make control of microbial contamination more difficult than the processing of any other conventional meat animal. Among them is the rapid rate of processing in some processing plants, a condition which favours the spread of micro-organisms. The carcase must be kept whole throughout the process and the viscera have to be removed rapidly through a small opening in the abdomen without breakage, to minimise contamination of the carcase with intestinal organisms. After defeathering, the skin provides a complex surface with many holes which are capable of trapping bacteria (Mead, 1982; Grau, 1986; Mead, 1989).

The micro-organisms are widely distributed over the carcases under normal circumstances and are spread over the skin during scalding and defeathering and on the inner and outer surfaces during evisceration and further processing (Bailey *et al.*, 1987). Efforts should be made to prevent the build-up of contamination peaks during processing. Rinsing of the carcases, especially during defeathering and evisceration is therefore of great importance (McMeekin *et al.*, 1979a; Brown *et al.*, 1982; Mead, 1982; Anand *et al.*, 1989; Mead, 1989). Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin. The nature and rate of attachment of the micro-organisms depends upon several factors including the bacteria involved and their concentration and also the conditions under which attachment occurs, namely, pH, temperature and contact-time. It was also found that *Pseudomonas* strains attach to meat surfaces more rapidly than any other bacteria (Firstenberg-Eden, 1981). The structure of the skin also has a crucial influence on attachment of bacteria. The organisms adhere by way of flagella and fimbrae and cannot easily be removed by rinsing, especially after a delay. There is still some disagreement on the role and importance of flagella in the attachment process of bacteria to meat. Research also shows that mesophilic bacteria are more heat-resistant when attached to skin than are the same bacteria not attached. (Barnes *et al.*, 1973; Green, 1974; Notermans *et al.*, 1974; Notermans *et al.*, 1975; Harrigan, 1976; Firstenberg-Eden, 1981; Thomas *et al.*, 1981; Faber *et al.*, 1984; Lillard, 1985). The skin serves as a barrier to micro-organisms that might otherwise contaminate the underlying muscle and therefore the deep muscles are normally free of bacteria (Bryan, 1980; Mead, 1982). The few bacteria found in the deep muscle are of types that can only multiply slowly or not at all at low temperatures. The important microbiological changes take place on the surfaces of the carcases. It appears that some parts of the carcase are more favourable than others for bacterial growth, depending on the type of muscle and pH. Studies conducted over the last few years show that the sites most heavily contaminated are the neck skin and less frequently on the back and the area around the vent. Fewer organisms are found around the breast, legs and under the wings. *Acinetobacter* and *Alteromonas* grow better in leg muscle where pH is 6.4 to 6.7 than in breast muscle where pH is 5.7 to 5.9. *Pseudomonas* spp. can grow well at both pH ranges (Patterson, 1972; Barnes *et al.*, 1973; Green, 1974; McMeekin *et al.*, 1979a; Bryan, 1980; Thomas *et al.*, 1981; Mead, 1982; Gill, 1983; Grau, 1986; Anand *et al.*, 1989). The presumable reason for the neck skin being the most heavily contaminated is that the washings

from the rest of the carcase run down the neck while the carcase hangs on the conveyor (Patterson, 1972; Connor *et al.*, 1987).

The literature shows that an aerobic plate count incubated at 35 - 37°C where mesophiles can grow, but most psychrotrophs not, can measure the overall microbiological status of the carcases during slaughter and processing, but does not include the spoilage bacteria that grows and multiplies during chill storage conditions.. When sampling poultry carcases, samples should be taken of surfaces of the skin or the skin itself to evaluate the extent of contamination of the carcases in the processing plant (Bryan, 1980; Brown *et al.*, 1982; Ralph *et al.*, 1984; Zottola *et al.*, 1990). The best representative sample can be obtained by taking a weighed sample of skin and blending it with a diluent for a specified time in a stomacher. This method is the best for removing the micro-organisms existing in the feather follicles, those attached to the skin and those on the surface. Swabbing and rinsing techniques will only recover those organisms loosely attached to the surface. These techniques are useful for estimating organisms that may result from cross-contamination picked up from a source in the processing line (Patterson, 1972; Barnes *et al.*, 1973; McMeekin *et al.*, 1979b; Bryan, 1980; Kilsby, 1982; Brown *et al.*, 1982).

LITERATURE REVIEW

MICROBIOLOGY OF POULTRY

Contaminants may be micro-organisms that cause spoilage of the product or organisms of public health significance. Pathogens associated with poultry are *Salmonella, Staphylococcus aureus, Clostridium perfringens* and *Escherichia coli*. *Listeria monocytogenes* and *Campylobacter jejuni* have also been isolated from poultry. Spoilage bacteria most frequently associated with poultry are *Pseudomonas* spp., *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Aeromonas* spp., *Corynebacterium*, *Flavobacterium*, *Micrococcaceae* and *Enterobacteriaceae*. Poultry is a common vehicle of foodborne illness (See Table 1.1) (Bryan, 1980; Todd, 1980; Smeltzer, 1981; Brown *et al.*, 1982; Mead, 1982; Roberts, 1982; Ralph *et al.*, 1984; Evans, 1986; Gill, 1986; Grau, 1986; Silliker *et al.*, 1986; Cunningham 1987; Banwart, 1989; Mead, 1989;

Zottola *et al.*, 1990; Jones *et al.*, 1991).

PATHOGENS

Salmonella

Salmonella are the main cause of food poisoning from poultry meat (Dougherty, 1976; Todd, 1980). Little is known about the incidence of *Salmonella* in South Africa although figures have been reported by Bok *et al.*, 1986 and Geornaras *et al.*, 1994. There are many sources from which poultry may obtain *Salmonella*, the main sources being from cross-contamination during breeding, hatching and intensive rearing operations. Salmonellas are not part of the normal intestinal microflora of poultry, but are acquired from the farm environment via insects, rodents and birds. Feed is also an important source of salmonellas through contamination of various components of the feed mix. The organisms occur more often in the caecum than in any other region of the gut from where they may be excreted for varying periods, without the host showing any sign of disease (Morris *et al.*, 1970; Mead, 1982; Grau, 1986; Silliker *et al.*, 1986; Mead, 1989; Zottola *et al.*, 1990; Jones *et al.*, 1991). Salmonellas from one flock can contaminate another, usually during conditions of intensive rearing and also when there is inadequate cleaning and disinfecting of the multi-cage transportation lorries used to convey the birds to the abattoir. Studies have also shown that live poultry transported from the farm often introduce *Salmonella* into the processing plant. Such contamination may result in considerable scattering of salmonellae during processing especially in the plucking machines and the scalding tank and may lead to contamination of the final product (McBride *et al.*, 1980; Mead, 1982; Mead, 1989; James *et al.*, 1992).

Clostridium perfringens

Clostridium perfringens is considered to be more widespread in the environment than any other pathogenic bacteria. This organism is commonly present in the intestinal tract of many warm-blooded animals and has been isolated from faecal matter, soil and dust. Raw poultry meat is normally stored at temperatures too low (< 15°C) to permit *Clostridium perfringens* to grow. Therefore, there seems little risk of multiplication in the processing plant. *Clostridium perfringens* is mainly present on processed poultry as spores (Bryan, 1980; Todd, 1980; Mead, 1982; Bailey *et al.*, 1987; Mead, 1989). Only type A strains are normally involved in human

food poisoning and these may be haemolytic, with heat-sensitive spores or non-haemolytic, with spores that are highly heat resistant. These heat-resistant strains can survive normal cooking procedures and if the cooked meat is held under favourable conditions, the organism can multiply to hazardous levels (Todd, 1980; Mead, 1989; Zottola *et al.*, 1990).

Staphylococcus aureus

Food poisoning from poultry meat caused by *Staphylococcus aureus* is much less common than that due to salmonellas or *Clostridium perfringens* (Todd, 1980; Mead, 1982). *Staphylococcus* is important in relation to poultry meat, because it can produce enterotoxins which may cause food poisoning in humans (Notermans *et al.*, 1982). Live poultry carry *Staphylococcus aureus* on skin surfaces and in nasal cavities, but low numbers are also present in the intestinal tract (Todd, 1980; Evans, 1986; Grau, 1986; Mead, 1989). Isolates of *Staphylococcus aureus* from poultry can be subdivided into human, non-human and intermediate types (Gibbs *et al.*, 1978; Mead 1989). It appears that *Staphylococcus aureus* may also be obtained from human sources after hatching and during processing of the carcases (Gibbs *et al.*, 1978; Mead, 1982). Notermans *et al.*, 1982 indicated that after processing, contamination of carcases with this organism increased to $>10^3$ g^{-1} of skin. Defeathering machinery in particular may support the build-up of *Staphylococcus aureus*. Evisceration and chilling are also processing stages which have been incriminated in contaminating carcases with *Staphylococcus aureus* (Gibbs *et al.*, 1978; Todd, 1980; Mead, 1982; Notermans *et al.*, 1982; Mead, 1989).

Campylobacter jejuni

Campylobacter is widely spread in nature and is isolated from wild and domestic animals as well as from the environment. Poultry is a major reservoir of *Campylobacter jejuni*. Many commercial poultry flocks appear to be symptomless carriers of *C. jejuni*, with up to $10^7.g^{-1}$ of gut content being demonstrated in the ileum and caeca of infected poultry and similar levels in the faeces (Genigeorgis *et al.*, 1986; Mead, 1989; Zottola *et al.*, 1990). Some poultry flocks that are negative before slaughter will therefore become contaminated during processing. *Campylobacter* is microaerophilic with a relative high minimum growth temperature (30°) and there seems little likelihood of them multiplying in the processing plant or on the raw, processed product. The main problem in processing is that of cross-contamination (Zottola *et al.*, 1990; Smeltzer, 1981). *Campylobacter* spp. are more sensitive than many other organisms to the

adverse effects of environmental conditions (drying, freezing and cold storage). For this reason, attention has been given to factors influencing the survival of campylobacters in processing. Although freezing is harmfull to *Campylobacter*, it does not eliminate this organism from poultry. Nevertheless, the contamination rate tends to be higher in fresh than in frozen carcases. *Campylobacter* spp. are also more sensitive to chlorine than *E. coli*, but are not eliminated from poultry carcases by immersion chilling in chlorinated water. On the contrary, cooling-water seems to be an important reservoir of this organism: 100-3000 $CFU.ml^{-1}$ were demonstrated and survival over long periods at low temperatures is possible. *Campylobacter* was also isolated from air samples as well as equipment (Cunningham, 1987; Mead, 1989; Zottola *et al.*, 1990).

Listeria monocytogenes

Listeria monocytogenes is widely distributed in nature and the environment. These organisms are isolated from soil, vegetation and faeces of humans and animals, with poultry often being contaminated. Studies also indicated that 57% (20 of 35 samples) and 33% (17 of 51 samples) of market poultry, respectively, contained *L. monocytogenes*. *L. monocytogenes* can multiply at refrigeration temperatures. Data also suggests that *L. monocytogenes* is more heat resistant in meat than *Salmonella*. The necessity of proper hygiene procedures in handling, processing and packaging of poultry is therefore emphasised (Zottola *et al.*, 1990).

SPOILAGE ORGANISMS

The spoilage of raw poultry meat is invariably due to the growth and metabolic activities of specific types of bacteria, the psychrotrophs (Ralph *et al.*, 1984 Kraft, 1986; Mead 1989). Psychrotrophs most frequently associated with poultry are *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Aeromonas* spp., *Flavobacterium* spp., *Corynebacterium, Micrococcaceae, Enterobacteriaceae*, *Serratia liquefaciens*, the pigmented and non-pigmented *Pseudomonas* spp. and also yeast and moulds (Bryan, 1980; Kraft, 1986; Mead, 1989). The bacteria which usually predominate on spoiled carcases held below 10°C are the *Pseudomonas* (*P.*) spp., especially *P. fluorescens*, *P. putida* and *P. fragi* and also *Acinetobacter* and *Moraxella* (Bryan, 1980; Lahellec *et al.*, 1981; Ralph, 1984; Mead, 1989). Some spoilage bacteria originate from the rearing environment and these organisms are carried in large

numbers on the feet and feathers of poultry. These bacteria are not found in the intestines of poultry (Mead, 1982; Grau, 1986; Bailey *et al.*, 1987). Prior to slaughter, the incoming chickens are contaminated with a large number of spoilage bacteria, but most are destroyed when passing through the scald tank, such as *Acinetobacter*, *Moraxella*, *Pseudomonas*, *Corynebacterium* and *Flavobacterium* (Lahellec *et al.*, 1979; Mead, 1989). *Pseudomonas*, however, form a small proportion of psychrotrophic flora on the outside of the chicken (Mead, 1982). Recontamination occurs during various processing stages, because the organisms multiply on all wet surfaces, including the carcases (Bryan, 1980; Mead, 1989). Another possible source of spoilage bacteria is also the processing plant water-supply. The *Pseudomonas* are more resistant to chlorine than *Escherichia coli* and therefore may survive normal water treatment in the processing plant. *Pseudomonas* can be eliminated by super-chlorination of water at the processing plant The quality of water in the processing plant is therefore of great importance. Essential steps to prevent excessive levels of contamination include prompt washing and chilling of eviscerated poultry and effective cleaning and disinfection procedures for equipment and working surfaces at the end of the processing day, prior to the next day's production (Lahellec *et al.*, 1979; Mead, 1989).

The growth of spoilage bacteria and thus the shelf-life of raw poultry meat, stored under chill conditions, will depend on the numbers and types of spoilage organisms present immediately after processing, the storage time and temperature, the type of tissue (skin or muscle), the pH, the redox potential, the type of packaging and the presence or absence of carbon dioxide (Bryan, 1980; Ralph *et al.*, 1984; Mead, 1989).

Time and Temperature

Psychrotrophs can grow at temperatures of -3°C, but most do not multiply above 34°C (Mead, 1989). Psychrotrophic *Pseudomonas* become the predominant flora on the aerobic surfaces of poultry stored at low temperatures and they can multiply the entire time carcases are held at commonly used refrigerator temperatures (Bryan, 1980). There is a simple relationship between storage temperature and shelf-life under aerobic conditions and for any given chill temperature this is related to the doubling time of the spoilage organisms (Barnes, 1976). The differential effect of storage temperature on the microbial growth rates influences the composition of the ultimate spoilage organisms. *Pseudomonas* predominated at spoilage when

poultry carcases were held at 1°C. Above 10°C, however the predominant organisms comprised mainly *Acinetobacter* spp. and *Enterobacter* (McMeekin, 1975; Mead, 1982). Fewer organisms are capable of growth at 4°C and those which do so often undergo a lengthy lag phase (Mead, 1982).

Meat pH and type of muscle

Although most carcase contaminants are found on the skin and over the inner surface of the visceral cavity, the growth of spoilage bacteria during chill storage conditions occurs primarily on cut muscle tissue and in the feather holes (Mead, 1982; Grau, 1986; Mead, 1989). Some parts of the chicken carcase appear to be more favourable than others for bacterial growth, depending on the muscle type and pH (Barnes, 1976). Pigmented and non-pigmented strains of *Pseudomonas* spp. grew equally well in the breast (pH 5,7 - 5,9) and in leg muscle (pH 6,4 - 6,7)(Mead, 1982). *Acinetobacter - Moraxella* spp. grew better in leg muscle, but not in breast muscle, whilst *Shewanella* grew faster in leg than in breast (Mead, 1982; Grau, 1986). Therefore one might expect to find different bacteria growing on the various cut muscle surfaces of spoiling carcases. The possibility also exists that spoilage may be more rapid in the high pH areas (Barnes, 1976).

Packaging and carbon dioxide

Apart from the tendency to retain moisture, the most important property of packaging film in relation to shelf-life is the permeability to oxygen and carbon dioxide (Mead, 1982). It was also shown that chicken carcases stored at 1°C in impermeable vacuum packs (vinylidene chloride-vinyl chloride copolymer) kept for *ca.* 5 days longer than those packed in gas-permeable polyethylene (Barnes, 1976; Mead, 1982). *Pseudomonas* spp. are the principal causes of spoilage on carcases packed in oxygen-permeable films, while *Shewanella putrefaciens* were the principal cause of spoilage of poultry carcases packed in oxygen impermeable films (Barnes, 1976; Bryan, 1980; Gill, 1983).

INFLUENCE OF PROCESSING ON POULTRY

The main operations in processing poultry are as follows: birds are removed from crates, hung by the feet on shackles on a conveyor, stunned by a low voltage electric shock in a water bath

and killed by exsanguination following slitting of the neck and severing the carotid arteries. They are then scalded, defeathered and washed. Heads, feet and the viscera are removed. The carcases are then washed and chilled in cold water or in humidified air. After chilling, the carcases are further processed or packaged and stored chilled or frozen (Fig. 1.1) (McMeekin *et al.*, 1979; Bailey *et al.*, 1987; Bryan, 1980, Mead, 1982; Grau, 1986). During each stage of the process, opportunity exists for the contamination of the carcases with micro-organisms from the environment of the poultry processing plant or by cross-contamination from other birds (McMeekin *et al.*, 1979). Numbers of bacteria on carcase surfaces vary considerably at different stages of processing and increases and decreases in numbers have been demonstrated (Thomas *et al.*, 1980). Defeathering and evisceration are the two stages where bacterial contamination mostly takes place (Mead, 1982; Grau, 1986).

PRE-SLAUGHTER HANDLING AND TRANSPORTATION

For transportation to the processing plant, birds are usually caged in batches. However, stress caused by transport, crowding and exposure to weather conditions may lead to an increased frequency of defecation and discharge of ceacal contents (Grau, 1986; Mead, 1982; Parry, 1989). In the little space available, birds tend to stand in an accumulation of their own droppings. Cages with solid floors used during transportation enable birds to sit in accumulated droppings. On the other hand, cages with perforated floors allow birds at higher levels to contaminate birds at lower levels (Mead, 1982; Grau, 1986; Mead, 1989). There is evidence that stress occurring during transportation can increase the proportion of birds which are intestinal carriers of *Salmonella* (Mead, 1982). It is therefore usual to starve birds before slaughter in order to minimise faecal contamination of carcases during transportation and processing (Anand *et al.*, 1989; Mead, 1989). During unloading, it is inevitable that some birds will struggle and flap their wings as they are hung on the shackles, and this results in a considerable scattering of dust and micro-organisms. The only effective control in preventing the spread of airborne contaminants is the complete separation of this area from the rest of the processing plant (Mead, 1982; Mead, 1989).

SCALDING

Carcases are scalded to loosen the feathers by immersion in a hot water tank, at either 50 - 52°C

(soft scalding) or at 56°C to 60°C (hard scalding) (Bailey *et al.*, 1987; Mead, 1989). During scalding micro-organisms on the skin and feathers and in the faeces of the birds are washed from the birds and continually released into the water of the scald tank. Aerobic plate counts of scald water however, are usually less than $5x10^4$ cfu ml^{-1} of scald water (Mulder *et al.*, 1974; Bryan, 1980). The survival of Enterobacteriaceae and mesophiles is higher at low scald temperatures of 50°C to 54°C than at higher temperatures (Grau, 1986; Anand *et al.*, 1989). At a scald temperature of 61°C, reductions of more than 1000-fold can be obtained, whereas at scald temperatures of 53°C to 55,5°C the counts are reduced by 10 to 100-fold (McBride *et al.*, 1980; Notermans *et al.*, 1980; Grau, 1986). The accumulation and survival of micro-organisms in the scald tank during processing is influenced by the temperature of scalding and the rate at which fresh water is added (Mead, 1982; Bryan, 1986; Bailey *et al.*, 1987). The great reduction in counts during scalding and the absence of *Pseudomonas* indicate that scald water contamination plays a relative minor role in spoilage of chicken carcases (Bailey *et al.*, 1987). Scald temperatures have little effect on the spores of *Clostridium perfringens* in the water (Mead, 1982; Bailey *et al.*, 1987). Evidence also indicates that the shelf-life of carcases is reduced by scalding at temperatures above 58°C. This can be attributed to the fact that scalding at about 58°C - 60°C (hard scalding) and above, followed by mechanical plucking results in removal of the outer epidermal layer (cuticle), whereas scalding at 52°C - 53°C (soft scalding) does not. The cuticle free skin of the carcases serves as a more suitable substrate for spoilage organisms and in particular *Pseudomonas* (Bryan, 1980; Bailey *et al.*, 1987).

DEFEATHERING

During defeathering there is a considerable scattering of micro-organisms from carcase to carcase and also from the defeathering equipment itself. The warm, moist conditions under which these operations take place also favour microbial growth. There are two aspects to the contaminating effect of defeathering. One arises from the extensive aerial scattering of micro-organisms in the vicinity of the machines, and is due to their mechanical action (Mead, 1989). It is therefore necessary to ensure complete separation of the plucking and scalding area from the clean areas of processing (Zottola *et al.*, 1990; Mead, 1989). The other aspect of defeathering hygiene is the nature of the machines themselves, and their siting next to the scald tank, which helps to maintain a warm moist environment suitable for microbial growth. The rubber "fingers" used to remove the feathers harbour micro-organisms and are not easily

cleaned and disinfected (Mead, 1982; Grau, 1986). Micro-organisms can persist in cracks and other imperfections even after vigorous cleaning (Gibbs *et al.*, 1978; Grau, 1986). Up to 10^6 *Staphylococcus aureus* cm^{-2} can be found on the rubber "fingers" of defeathering machines and treatment with 100ppm chlorine for 30min may reduce the counts by only *ca.* tenfold (Gibbs *et al.*, 1978). The counts of both aerobic mesophiles and psychrotrophs on poultry skin can increase during defeathering and also the numbers of Enterobacteriaceae (Lahellec *et al.*, 1979; Thomas *et al.*, 1980). *Salmonella* are also more frequently isolated from carcases after defeathering, than following any other processing operation (McBride *et al.*, 1980). Following a hot or hard scalding, defeathering damages and removes the epidermal layer and exposes a new surface layer. This cuticle-free skin serves as a very suitable substrate for spoilage organisms and the organisms become trapped in the skin follicles and folds (Thomas *et al.*, 1980; Grau, 1986; Connor *et al.*, 1987; Mead, 1989).

EVISCERATION

During evisceration the opportunity exists for contamination with Enterobacteriaceae from the intestinal contents. Careless manual opening of the body cavities and manual evisceration leads to contamination of carcases, especially when the intestines are cut or the vent is inadequately loosened. Cross-contamination can also occur due to workers' hands, evisceration implements and other slaughter equipment (Mead, 1982; Grau, 1986; Mead, 1989). No difference was found between plants using manual evisceration and those with automatic equipment, although automatic evisceration can cause considerable damage to carcases due to rupturing of the intestines when carcases in a particular batch varies in size (Mead, 1989). Aerobic mesophiles on the carcases usually do not increase significantly during evisceration, but the numbers of Enterobacteriaceae and the frequency of contamination with *Salmonella* often increase (Notermans *et al.*, 1980; Grau, 1986). Significant contamination with *Staphylococcus aureus* can occur even though *Staphylococcus aureus* is not detected in the intestinal tract. This contamination comes from sources other than the bird and the contaminating strains also appear to be endemic to the processing plant (Notermans *et al.*, 1982). Washing of carcases after evisceration and before chilling removes organic matter and some of the micro-organisms acquired during evisceration. The visceral cavities also become contaminated during evisceration, especially when the intestines are cut and it is less easily reached by washing with conventional washing equipment (Notermans *et al.*, 1980; Mead, 1982; Connor *et al.*, 1987;

Jones *et al.*, 1991). However, strategically sited spray-washers with high-pressure and the use of water containing at least 40ppm available chlorine are effective in reducing the number of bacteria and 70ppm chlorine almost totally eliminated build-up of bacteria (Notermans *et al.*, 1980; Bailey *et al.*, 1987; Mead, 1989).

CHILLING

In many processing plants, the rate of processing is such that there is little loss of heat from the carcases before if reaches the chilling stage. The deep muscle temperature of the freshly eviscerated carcases is ±30°C and to prevent and limit the growth of spoilage bacteria and pathogens it is necessary that the carcases must be chilled rapidly and efficiently after evisceration to a keep temperature of below 10°C (McMeekin *et al.*a, 1979; Eustace, 1981; Mead, 1989). Two methods of chilling are in common use, one involving dry chilling in cold air and the other immersion of carcases in ice-chilled water (Mead, 1982; Mead, 1989). Continuous immersion chilling is the most widely used method and comprises one or more units, each consisting of a large tank capable of holding many hundreds of carcases, through which water flows continuously. The water can flow with or against the direction taken by the carcases (Bryan, 1980; Mead, 1982). In through-flow systems carcases move in the same direction as the water flow, whereas in counter-flow chillers the birds are moved mechanically in the opposite direction to the flow of in-coming water (Mead, 1982). Hygienic operation of immersion chillers requires measures to prevent a build-up of microbial contaminants in the cooling medium and this depends on the water usage and temperature control. Adequate use of fresh water aids the cooling process and prevents the chiller temperature from reaching a point when bacterial growth becomes a problem (Mead, 1989). The water temperature at the carcase entry and exit points must not exceed 16°C and 4°C respectively (Mead, 1982). Counter-flow immersion chilling (in which carcases at the end of the chilling process come into contact with the cleanest water) effectively decreases counts on carcases and minimises cross-contamination (Bryan, 1980).

Air-chilling, whether as a batch process in a chill room or by continuous air-blast, requires the use of low scald temperatures of *ca.* 50°C. This is to avoid skin damage and colour change of the carcases (Bryan, 1980; Mead, 1989). Air-chilled carcases are always likely to have higher bacterial counts than those chilled in properly controlled immersion systems. Several studies

have confirmed this supposition, although the differences are relatively small and usually less than 10-fold (Mead, 1989). Air-chilling is less likely to cause cross-contamination than water immersion, but micro-organisms may circulate in the currents of cold air and usually there is some degree of contact between individual birds in the chiller (Bryan, 1980; Mead, 1989).

POST-CHILLING HANDLING

Bacterial counts can increase after chilling, because of the transfer of micro-organisms during weighing and packaging. Even at this stage contamination with salmonellas can occur and therefore, the final product should be frozen or transferred to a chill store without delay (Bryan, 1980; Mead, 1989).

THE HAZARD ANALYSIS CRITICAL CONTROL POINT CONCEPT

Foodborne disease caused by micro-organisms associated with poultry processing is of critical importance to the manufacturers as well as the consumer. Principles for controlling most of these disease are well known to public health authorities and emphasis for prevention and control has been put traditionally on inspection. Inspection can never be done frequently or thoroughly enough to provide the degree of food safety that is desired by processors and the public. A tool which the food industry is adopting to aid in the production of safe foods is the Hazard Analysis Critical Control Point (HACCP) system. Steps in the HACCP system are illustrated in Fig.1.2. The HACCP system is a method to assess hazards, estimate risks and establish specific control measures that emphasize prevention and control. A HACCP plan first identifies and assesses all the potential health risks that a particular food may present to the consumer and it focuses on critical factors directly affecting the microbiology of foods. The controls and monitoring necessary to minimise significant risks are then identified and implemented in order to prevent contamination, growth or survival of disease-causing or spoilage bacteria. Microbiological testing plays an essential role in a HACCP plan. It is only through microbiological testing that the safety of the product and the effectiveness of a HACCP plan can be verified. Microbiology testing is used to verify the effectiveness of cleaning and sanitising procedures, the critical control points and the finished product. Therefore microbiology testing has an indispensible role in the HACCP system (Brown *et al.*, 1982; Micrbiology and Food Safety Committee of the National Food Processors Association, 1993;

WHO, 1993; Silliker, 1995).

FOOD STANDARDS FOR POULTRY MEAT

Poultry meat is often involved in food-poisoning incidents, because of contamination of raw poultry meat and also mishandling, especially in homes and food service establishments. Legislation organisations have expressed their concern and have proposed criteria for poultry meat. The International Commission on Microbiological Specifications for Foods (ICMSF,1986) has defined the different terms that should be used for microbiological criteria and these terms should be used as follows:

- "Microbiological criterion" involve the presence of micro-organisms or their toxins or the number of organisms per unit of mass, volume or area, established by use of specific procedures and applied in acceptance sampling of food.
- "Microbiological standard" is a criterion in a law or stipulation controlling foods produced, processed or stored in the area of jurisdiction of a regulatory organisation.
- "Microbiological guideline" is a suggested or recommended microbial limit used by a manufacturer or regulatory organisation in monitoring food, ingredients, processes or systems.
- "Microbiological purchasing specification" is a microbial limit which stipulate the acceptance of a specific food or food ingredient by a food producer or other private or public purchasing organisation.

The microbiological criteria by which foods may be classified as microbiological acceptable or unacceptable include specifications, standards and guidelines. The components of each criterium are the sampling plan, the method of analysis and the microbiological contaminant level or limit that should not be exceeded (i.e. the bases of acceptance). Reaching agreement on a complete specification, standard or guideline is a complicated process. Many questions arise in the process: What foods should be selected and on what basis? What contaminants (i.e. pathogens, indicator groups, etc.) should be specified? What limits should be placed on the presence of each contaminant? How large a sample should be examined and by what method?

The above criteria are normally expressed as a definite value and may be a number of micro-organisms or the presence of a given organism in a specific quantity of sample. The criterion distinguishes between a good quality and a bad quality product. In the last few years, food microbiologists have, however, realised that there cannot be any absolute value that can distinguish between good and bad and that there are microbiological counts which are acceptable, others that are marginally acceptable and then counts or the presence of pathogenic organisms that are a cause of concern and not allowable (Simonsen, 1989). This led to a proposal for the now generally accepted three class sampling plan. A total aerobic count that would include psychrotrophic micro-organisms would be relevant in the monitoring of the hygiene in the processing plant. It would give an evaluation of the hygienic conditions in the processing plant and also of the equipment, machinery and utensils used. These methods would also give the quality control organisation a quantitative figure of the effect of sanitation on the micro-organisms present on the samples examined.

Three different sampling methods have been nominated to use as total aerobic plate counts, namely swabbing, contact plates and the adhesive tape method. When evaluating the three methods with respect to accuracy, applicability in the processing plant, cost, convenience, labour requirements and speed in obtaining results, the swab method and the contact-plate method were equally good and somewhat better than the adhesive tape method.

Standardised methods for microbiological control of poultry meat and products have four purposes namely,

- To serve as basis for judging the relevance of particular screening methods and evaluating the accuracy and exactness of these methods.
- The methods could be used for collecting data when a microbiological criterion is needed and to provide the basis for setting numerical limits. For this reason they must be applicable in situations where a standard or an end-product specification or even a guideline is needed.
- It should be applicable in any dispute.
- It should be capable of detecting small but significant differences in microbiological counts.

For these reasons standardised methods should have a high reproducibility and repeatability. The type of medium used, the incubation time and temperature to choose for standardised methods for the total aerobic count and also how to sample the meat or product is of great importance. Maceration of the neck skin and rinsing of the whole carcase were methods that

were investigated as standardised methods. The conclusion drawn from the tests done on the above sampling methods was that the methods could be useful for a guideline, but not for an end-product specification or a standard. No sampling plan with numerical values has been proposed for poultry products with respect to the total aerobic count. For poultry meat there is a Recommended International Code of Hygiene Practice for Poultry Processing (CAC, 1976), but there are no Codex standards for poultry meat (Brown *et al.*, 1982; Simonsen, 1989).

In South Africa, however, the South African Poultry Association (SAPA) has a Code of Practice for Broiler Processing Plant and according to that Code there is a microbial assessment. According to that Code the microbiological status of a representative range of products and equipment should be performed daily using Rodac contact plates, swab techniques or electro conductance procedures. The following standards should be achieved and are offered as guidelines (Table 1.2)(SAPA, 1993).

MOTIVATION

All the processing plant operations can affect the microbiological quality of the fully processed carcase. Good manufacturing practices (minimum handling of carcases, good sanitation of processing equipment and tools and also water free from contamination) will lead to minimal cross-contamination and the resulting carcases will have an adequate shelf-life to satisfy both the processor and the consumer.

Poultry processing plants in South Africa are graded according to the maximum daily (in a 24 hour cycle) throughput of birds. Grade A poultry abattoirs slaughter > 10 000 birds per day; large Grade B abattoirs, < 10 000; small Grade B abattoirs, < 4 000; Grade C abattoirs, 500 to 800; Grade D abattoirs, < 300 and Grade E abattoirs, < 50 birds per day (Government Gazette 1989).

Although extensive research has been done on the micro-organisms associated with the processing of poultry, no research has been done on this specific Grade B poultry processing plant in South Africa, with a labour-intensive operation and minimal mechanisation. This investigation will provide an opportunity to determine the microbiological status of chicken

carcases at selected control points in a small Grade B poultry abattoir.

Table 1.2 Microbiological standards in a poultry abattoir (SAPA, 1993).

TEST	**TOTAL VIABLE**	**COLIFORMS**	***S. aureus***	***SALMONELLA***
Pre-work surface	< 100/sq cm	< 10 per sq cm	< 10 sq cm	Absent
Skin	< 100,00/g	< 1000 per g	< 100 per g	Absent
Mains water	< 100/100 ml	0/100 ml		
Shelf life + 4°C	> 7 days			

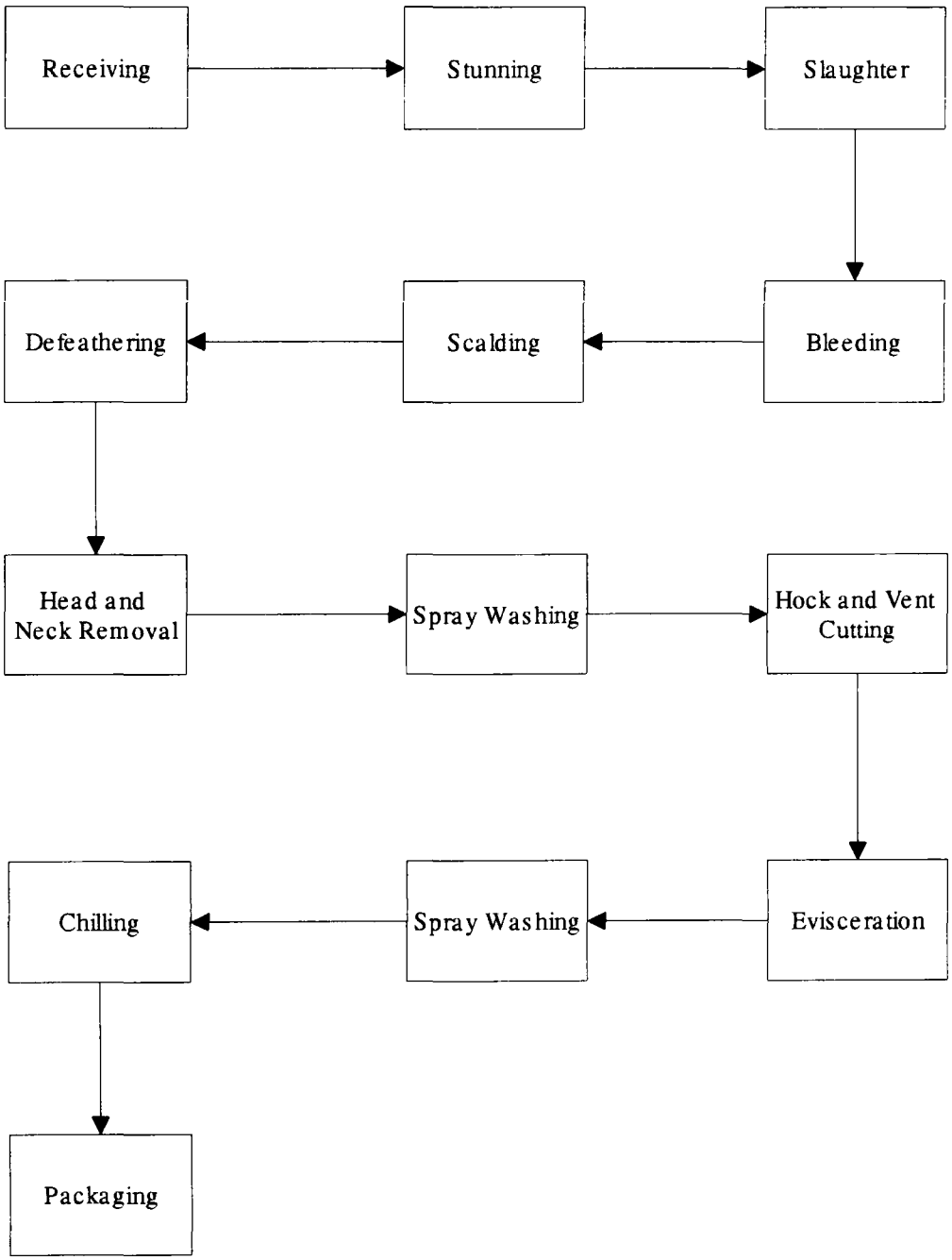

Fig. 1.1. Flowsheet of stages in a typical poultry processing plant.

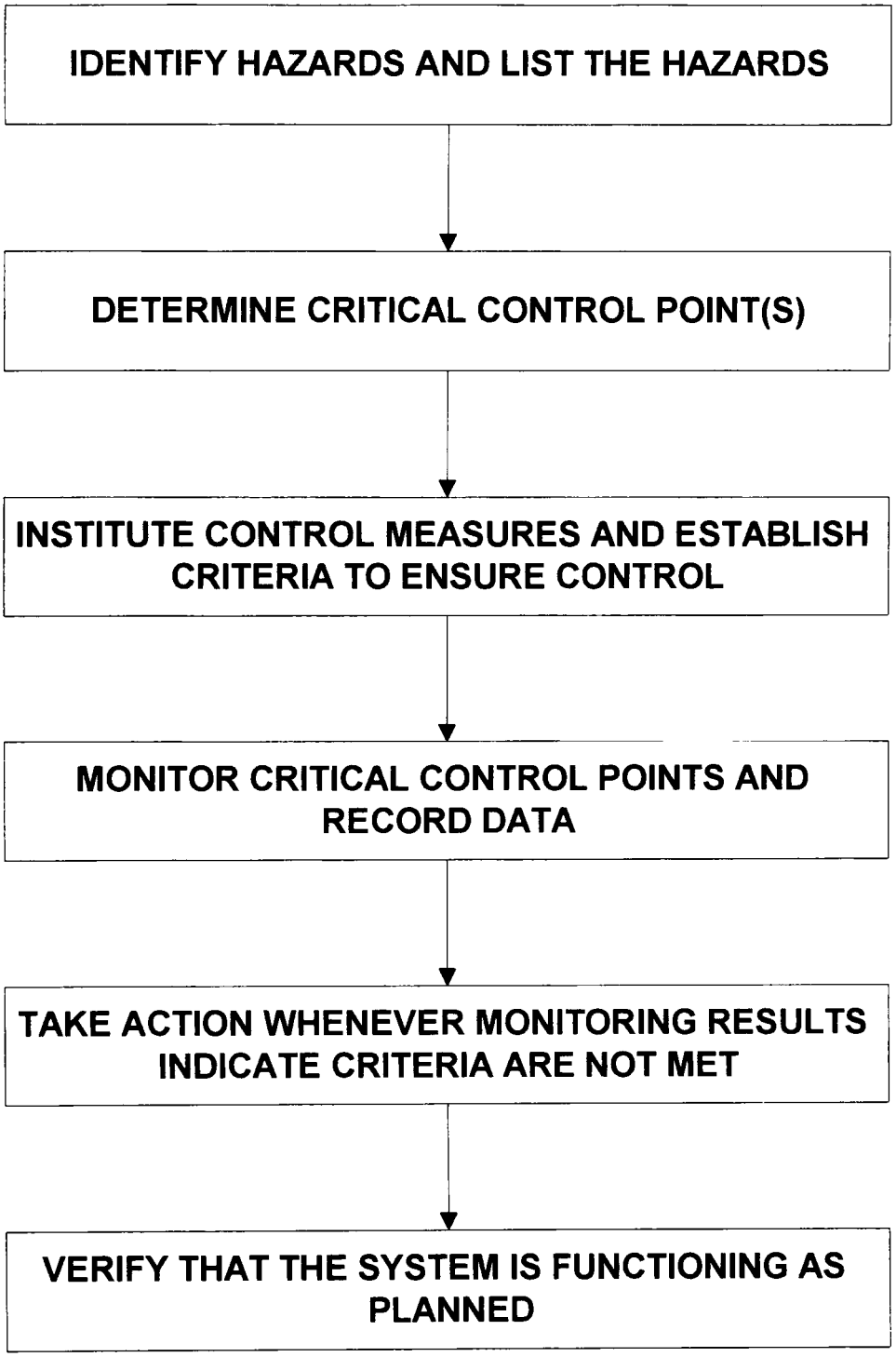

Fig 1.2 The HACCP system

CHAPTER TWO

MICROBIOLOGICAL STATUS OF SELECTED CHICKEN CARCASES FROM A NON-AUTOMATED POULTRY PROCESSING PLANT. (A PILOT STUDY)

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Abstract

The microbiological status of chicken carcases sampled at three different processing points in a South African Grade B poultry abattoir slaughtering *ca.* 750 birds per hour, was determined. Six skin samples and two meat samples were aseptically collected from different sites on each carcase. Total bacterial counts were performed at 25°C, 37°C and 43°C and all colonies from plates showing between 30 and 300 cfu were characterised. Bacterial counts of the skin samples at 37°C were consistently the highest, followed by those at 25°C and then 43°C, but for the two meat samples the highest bacterial counts were found at 37°C and the lowest at 25°C. Neck skin counts were marginally higher than bacterial counts of the other skin samples. The Gram negative genera *Escherichia* and *Acinetobacter* were isolated most frequently at all three incubation temperatures and from all sampling sites, while the dominant Gram positive genera were *Staphylococcus* and *Enterococcus*. *Escherichia* isolates predominanted on the skin sampling site cranio-dorsal to the pygostyle, whilst *Staphylococcus* isolates predominanted on the skin sampling site caudal to the breastbone. Microbiological contamination is a major problem in the abattoir studied and further studies should therefore aim to determine points of maximum contamination in the processing line.

Introduction

The procedure for converting a live bird into a safe and wholesome poultry product provides many opportunities for micro-organisms to colonise the surface of the carcase. A live, healthy bird carries high numbers of bacteria on its feathers, skin and in the intestine (Bryan, 1980; Mead, 1982). These micro-organisms are spread over the skin during scalding and defeathering and on the inner and outer carcase surfaces during evisceration and further processing (Harrigan *et al.*, 1976; McMeekin *et al.*, 1979; Mead, 1982). Defeathering and evisceration are the two stages where most bacterial contamination of the carcases takes place (Mead, 1982; Grau, 1986). During the various processing operations at a typical processing plant opportunities also exist for contamination of carcases from the environment, equipment, the hands of workers and by cross-contamination from other carcases (Bryan, 1980; Thomas *et al.*, 1980; Eustace, 1981; Roberts, 1982; Connor *et al.*, 1987; Banwart, 1989). The carcase sites reported to be most heavily contaminated with bacteria are the neck skin and less frequently the skin on the

back and the area around the cloaca. Fewer bacteria are found around the breast, legs and under the wings (Patterson, 1972; Barnes *et al.*, 1973; Green, 1974; Bryan, 1980; Anand *et al.*, 1989). Spoilage bacteria most frequently associated with poultry processing are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Corynebacterium*, *Flavobacterium*, *Micrococcus* and *Enterococcus* (Bryan, 1980; Banwart, 1989; Mead, 1989). Bacterial pathogens associated with poultry are *Salmonella*, *Staphylococcus aureus*, *Clostridium perfringens*, *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* (Bryan, 1980; Brown *et al.*, 1982; Mead, 1982; Roberts, 1982; Ralph *et al.*, 1984; Grau, 1986; Banwart, 1989; Mead, 1989; Zottola *et al.*, 1990). Although extensive research has been done on the micro-organisms associated with the processing of poultry, no data have been reported from South African Grade B poultry processing plants[1]. Table 2.1 outlines the separation of basic functions in a South African Grade B poultry processing plant. The design is around a three process area system with additional requirements as follows: if up to 10 000 birds are slaughtered per day, a separate process area must be provided for blood, feathers, heads and feet (all handled as dirty product). If up to 4 000 birds are slaughtered per day, blood, feathers, heads and feet are collected and containerised during production in process area I with all the associated hygiene problems as is the case in the abattoir in which the study was conducted. This investigation was undertaken to determine the baseline microbiological status of chicken carcases in a South African Grade B poultry abattoir.

Materials and Methods

Description of processing plant. Chickens were processed at a rate of *ca.* 3 000 birds per day. The birds were soft scalded at 50-52.8°C and defeathered by a single machine. After defeathering, heads and feet were removed manually without separation of clean and dirty functions and dirty hand soiling of the carcases on rehang. Manual pinfeathering followed with minimal handwash and low pressure carcase washing. Carcase abdomens were opened with a knife and the viscera removed by hand or with a spoon. Carcases were then low pressure spray washed and air chilled for *ca.* 50 min. Finally, carcases were packaged whole or portioned and

[1] While doing this research work no data was published on work done in a South African poultry abattoir. By 1998 articles was, however published by Goernaras *et al.*,1996 and Goenaras *et al.*, 1997

despatched chilled.

Sample collection. Six skin samples and two meat samples (each with a mass of *ca.* 5g) were excised aseptically on eight different sites of each of three chicken carcases. One carcase was sampled after defeathering, one after evisceration and the third after chilling. The sampling sites were neck skin, skin from both sides ventral to the wings, skin caudal to the breastbone, skin cranio-dorsal to the pygostyle, skin from the back between the wings, skin *ca.* 2cm cranial to the centre of the breast. A composite meat sample from both sides ventral to the wings and meat caudal to the breastbone were also taken to evaluate the extent of microbial growth to that of the skin samples. This meat samples were taken by aseptically cutting away the surface meat and collecting meat samples from the carcases.

Sample preparation. Samples from individual carcases were separately placed into sterile quarter strength Ringers solution in a 1+9 mass/volume ratio based on their exact mass. Each sample was semi-homogenised for 2 minutes (Seward Medical 400 Stomacher Lab Blender) and tenfold serial dilutions in quarter strength Ringers solution were plated out in triplicate onto Nutrient Agar No.2 (Oxoid) using the spread-plate technique (ICMSF, 1978; Busta *et al.*, 1984; von Holy *et al.*, 1992). Individual plates of each triplicate set were incubated at 25°C, 37°C and 43°C for 48 hours.

Microbiological counts and isolate characterisation. Plates were incubated aerobically and those containing between 30 and 300 colony forming units (cfu) (or the highest number if below 30) were counted and the number of isolates calculated by multiplying with the appropriate dilution factor(10^{-2}). Total aerobic bacterial counts of corresponding sampling sites on the three carcases were meaned and converted to logarithms. All colonies from these plates were isolated and purified on the same medium and characterised to genus level (Brenner, 1984; Holmes *et al.*, 1984; Juni, 1984; Kersters *et al.*, 1984; Palleroni, 1984; Popoff, 1984; Hardie, 1986; Kandler *et al.*, 1986; Keddie *et al.*, 1986; Kloos, 1986; Kocur, 1986; Komagata *et al.*, 1986).

Results

Effect of sampling site and incubation temperature on bacterial counts.

At all three incubation temperatures, the neck skins showed the highest bacterial numbers by a small margin (Figure 2.1). This concurred with findings of previous studies (Bryan, 1980). The presumable reason for the neck skin being the most heavily contaminated is that the washings from the rest of the carcase gravitate to the neck region while the carcase hangs inverted on the conveyor (Patterson, 1972; Connor *et al.*, 1987). Other sites on the chicken carcases that also showed high levels of contamination were the skin cranio-dorsal to the pygostyle, the skin from the back between the wings and the skin *ca.* 2cm cranial to the centre of the breast. The skin samples had significantly higher bacterial numbers than the meat samples, which concurred with findings of previous research work (Barnes *et al.*, 1973; McMeekin *et al.*, 1979; Mead, 1982; Grau, 1986; Banwart, 1989). The meat samples showed the lowest bacterial counts at all three incubation temperatures. The highest bacterial counts on all the skin samples were obtained at 37°C (5.3 - 5.7 log CFU g^{-1}). Marginally lower counts were recorded at 25°C (5.2 - 5.6 log CFU g^{-1}) and at 43°C (4.6 - 5.3 log CFU g^{-1}) the lowest bacterial counts were obtained. In the case of the meat samples the highest bacterial counts were also found at 37°C (4.2 - 4.3 log CFU g^{-1}), but the lowest bacterial numbers were obtained at 25°C (3.7 - 4.1 log CFU g^{-1}).

Effect of sampling site and incubation temperature on the identity of bacterial populations.

The percentage distribution with respect to identity of isolates from the six skin sampling sites on the chicken carcases is shown in Table 2.2. Of the 3 517 bacteria isolated from neck skins, the Gram positive genera *Staphylococcus* and *Enterococcus* collectively amounted to 29.4% of the total with percentages of 18.3 and 11.1%, respectively. The Gram negative genera *Escherichia* (24.2%), *Acinetobacter* (23.9%), *Pseudomonas* (9.4%) and *Citrobacter* (5.5%) amounted to 63.0% of the total. The remaining 7.6% of the isolates were made up by genera individually comprising less than 5% of all isolates. A total of 1 962 bacteria were isolated from skin ventral to the wings on both sides and *Staphylococcus* isolates (36.0%) made up the highest proportion. Gram negative genera comprised 41.7% of the total which was made up of *Escherichia* (19.6%), *Acinetobacter* (11.2%), *Pseudomonas* (5.5%) and *Proteus* (5.4%). The

remaining 4.5% isolates were made up of genera individually totalling less than 5% of all isolates. For skin samples caudal to the breastbone, 1 297 isolates were identified. Gram positive isolates dominated on counts of this sampling site with a percentage of 70.1% of the total. *Staphylococcus* showed the highest percentage (54.5%), followed by *Micrococcus* and *Enterococcus* at 10.0 and 5.6%, respectively. Isolates of the Gram negative genera *Escherichia* (8.4%), *Acinetobacter* (7.7%) and *Pseudomonas* (7.5%) were also encountered. The remaining 6.3% of the isolates were made up by genera individually totalling less than 5% of all isolates. A total of 2 265 bacteria were isolated from skin counts cranio-dorsal to the pygostyle. The Gram positive genera *Staphylococcus* (36.5%), *Enterococcus* (11.2%) and *Micrococcus* (6.6%) collectively amounted to 54.3% of the total. Proportions of the Gram negative genera *Escherichia* and *Acinetobacter* amounted to 27.2 and 13.2%, respectively. The remaining 5.3% of the isolates individually totalled less than 5% of the isolates. From counts on skin from the back between the wings, (1 397 isolates), the Gram positive genus *Staphylococcus* was recovered at the highest frequency (31.9%). The Gram negative genera *Escherichia*, *Pseudomonas*, *Acinetobacter*, *Proteus* and *Citrobacter* comprised noticeable smaller percentages of 17.2, 11.5, 10.7, 10.7 and 6.4%, respectively. The remaining Gram negative isolates individually totalled less than 1% of all isolates. For the 1 399 bacteria isolated from the skin cranial to the centre of the breast, *Staphylococcus*, *Enterococcus* and *Micrococcus* collectively comprised 58.1% of the total, with percentages of 37.7, 10.4 and 10.0%, respectively. The Gram negative genera *Escherichia* and *Acinetobacter* amounted to 34.0% of the total isolates with percentages of 24.0 and 10.0%, respectively. The remaining 7.9% isolates were made up by genera individually totalling less than 5% of all isolates.

Effect of incubation temperature on the identity of bacterial populations.

The percentage distribution of isolates according to identity at the different incubation temperatures is shown in Table 2.3. The genus *Staphylococcus* represented the highest percentage of isolates at an incubation temperature of 25°C. A decline in percentage representation of isolates of this genus occurred at 37°C and at 43°C. *Escherichia* isolates showed the highest predominance at 43°C with a percentage of 56.9%. Lower percentage representation occurred at 37°C and no *Escherichia* was isolated at 25°C. The genus *Enterococcus* was isolated at incubation temperatures of 37°C and 43°C, but not at 25°C. The spoilage bacterium *Pseudomonas* was isolated at 25°C and at 37°C, but not at 43°C.

Acinetobacter and *Proteus* isolates were only recovered at 25°C and *Acinetobacter* isolates showed a strong predominance (40.9%) at this incubation temperature.

Table 2.4 shows the identity of bacterial populations with respect to the different sampling sites at 37°C. It was apparent that at 37°C the highest numbers of bacteria as well as the highest diversity in populations was encountered. High proportions of *Staphylococcus* isolates occurred at all skin sampling sites, while *Micrococcus* and *Enterococcus* were also isolated from all six skin sampling sites. From skin sampled caudal to the breastbone and cranio-dorsal to the pygostyle no *Pseudomonas* were isolated. *Arthrobacter* was only isolated from the skin caudal to the breastbone, while *Proteus* was only isolated from skin from the back between the wings. *Escherichia* was isolated from all the skin sampling sites except from skin from the back between the wings.

Discussion

Total bacterial counts obtained from different skin samples did not show significant differences. Studies done by previous workers showed that the neck skin is the position on the carcases which is usually most highly contaminated (Patterson, 1972; Bryan, 1980). In this study, no noticeable differences in bacterial counts were found between neck skin and other skin sampling sites. The entire carcase was therefore contaminated, with no significant concentration of bacteria at any particular area. Noticeable differences in bacterial counts were, however, found between the skin samples and meat samples. The reason for this is that bacteria occur mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces under the skin and not in the actual carcase meat of healthy birds (Barnes *et al.*, 1973; Bryan, 1980). The skin therefore serves as a barrier to micro-organisms that might contaminate the underlying carcase meat (Barnes *et al.*, 1973; Harrigan, 1976; McMeekin *et al.*, 1979; Bryan, 1980).

The potential poultry pathogens *Escherichia* and *Staphylococcus* occurred at all the skin sampling sites. The genus *Escherichia* showed the highest percentage prevalence on skin sampled cranio-dorsal to the pygostyle, whereas the genus *Staphylococcus* showed a high prevalence on skin sampled caudal to the breastbone. The most probable reason for the high proportion of *Escherichia* at this sampling site was that this area can become contaminated with *Enterobacteriaceae* from the intestinal contents. Careless manual opening of the abdomen and

manual evisceration causes soiling of the area cranio-dorsal to the pygostyle, where the spooned viscera hang over the pygostyle on the dorsal aspect of the back. This is compounded when the intestines are lacerated or the cloaca is inadequately loosened (Bryan, 1980; Notermans *et al.*, 1980; Grau, 1986; Connor *et al.*, 1987). The reason for the high prevalence of *Staphylococcus* could have been the poor personal hygiene and technique of the workers opening the abdomen. With the predominantly practised technique of hand evisceration observed in the abattoir under study and infrequent hand washing, a high prevalence of bacteria related to human contact was expected at this sampling site. *Staphylococcus aureus* is also carried on the live chicken and could also be a possible source of *Staphylococcus* contamination (Bryan, 1980; Grau, 1986; Mead, 1989).

The documented poultry spoilage genera *Acinetobacter* and *Pseudomonas* were isolated from all skin sampling sites with the exception of skin cranio-dorsal to pygostyle and skin *ca.* 2cm cranial to the centre of the breast, where no *Pseudomonas* isolates were found. These bacteria are common environmental contaminants. Consequently the feathers, feet and bodies of the birds are likely to be contaminated with these bacteria. The process water can also be contaminated with *Acinetobacter* and *Pseudomonas* bacteria (Bryan, 1980; Grau, 1986; Mead, 1989). In this abattoir carcases were processed in an immersion tank, the water of which was not changed at regular intervals. This practise can lead to contamination of the carcases with *Pseudomonas* and *Acinetobacter*. Poultry spoilage bacteria like *Acinetobacter* and *Pseudomonas* can also multiply on the wet carcase surfaces and on soiled surfaces of equipment. As is customary in poultry processing, the carcase surfaces are kept as wet as possible during production and in addition dirty contact surfaces and equipment can lead to multiplication of these spoilage bacteria (Bryan, 1980; Mead, 1989). In this abattoir contact with surfaces is excessive through design faults and the hygiene of these contact surfaces is poor.

The most diverse bacterial populations were isolated on skin from the back between the wings. Gram negative bacteria mostly *Escherichia* dominated at the neck skin sampling sites and on skin from the back between the wings. Contamination of the neck skin commonly arises when the washings from the rest of the carcase gravitate to the neck area while the carcase hangs on the conveyor (Patterson, 1972; Connor et al.,1987). Skin from the back between the wings is contaminated due to the spooned viscera which hang against this area for individual offal item

harvesting. At all the other skin sampling sites Gram positive bacteria showed a high predominance, especially at the sampling site skin caudal to the breastbone. This could be attributed to poor handling techniques, inadequate cleaning of equipment and other poor personal hygiene practices. The genus *Staphylococcus* was isolated at all three incubation temperatures, but the highest percentage occurrence was obtained at 25°C. The most probable reason for this is that *Staphylococcus* are mesophilic and optimum growth is between 17 and 30°C (Bryan, 1980; Grau, 1986). Conversely, *Escherichia* isolates showed strong predominance at 43°C, but no *Escherichia* were isolated at 25°C. *Escherichi*a mainly occur in the intestinal tract of the carcases, where the body temperature is *ca.* 42°C which could explain the high frequency of isolation at 43°C (Grau, 1986). *Pseudomonas* was isolated at 25°C and 37°C, but was not isolated at 43°C and *Acinetobacter* was only isolated at 25°C. This can be explained by the psychrotrophic nature of these isolates which reportedly only grow between -3°C and 34°C (Bryan, 1980; Grau, 1986).

Conclusion

It can be concluded that microbiological contamination was a major problem in the abattoir studied here. Further studies should therefore aim to determine points of maximum contamination in the processing line. Although no noticeable differences were found in bacterial counts between the six skin sampling sites, it was decided that composite skin samples are taken for such studies to minimise the influence of uneven distribution of bacteria on counts. The taking of meat samples from healthy birds seems to be unnecessary as very few bacteria was isolated from the meat samples. The most suitable incubation temperature indicated by this study was 37°C, since the highest diversity in bacterial populations and also the highest counts were found at this temperature. Aerobic plate counts incubated at 35 - 37°C also reportedly measure in-process contamination of chicken carcases since mesophiles can, but most psychrotrophs cannot grow at this temperature and are thus a useful indicator of microbiological status as a result of processing (Bryan, 1980; Brown *et al.*, 1982; Ralph *et al.*, 1984; Zottola *et al.*, 1990). The bacterial counts obtained from the chicken carcases were high and the spoilage bacteria *Pseudomonas* and *Acinetobacter* were consistently isolated from carcases. It is therefore necessary to keep the initial bacterial numbers low to improve the shelf-life of the product, because psychrotrophic bacteria can continued multiply during chilled storage at a range of -2°C to 2°C (super chill range)(Bryan, 1980; Mead, 1989). However the cold chain is

broken in this abattoir as the despatch area is not temperature controlled, therefore the temperature at the keel will be in excess of the prescribed 10°C maximum, ideal for more rapid replication of *Pseudomonas* and *Acinetobacter*. The potentially pathogenic genera *Escherichia* and *Staphylococcus* were also isolated at all the skin sampling sites in relative high frequency and could pose a health risk to the consumer.

Table 2.1 The separation of basic functions in a South African Grade B poultry processing plant.

Process area I	Stunning Bleeding	Dirty function
	Scalding Defeathering Singeing Head and feet removal Carcase wash Product refrigeration (rough offal)	Dirty function
Process area II	Evisceration Viscera handling Carcase wash	Clean function
Process area III	Portioning and deboning Carcase and portion wrapping and packing	Clean function
	Product refrigeration Product despatch	Clean function

Table 2.2 Characterisation of 118 bacteria isolated from aerobic plate counts at six sampling sites on chicken carcases and incubated at three temperatures.

	Sampling Sites								
Bacterial Genus (%)	1 (35)[a]	2 (19)	3 (13)	4 (23)	5 (14)	6 (14)	**Range**		
Escherichia	24.2	19.6	8.4	27.2	17.2	24.0	8.4	-	27.2
Acinetobacter	23.9	11.2	7.7	13.2	10.7	10.0	7.7	-	23.9
Staphylococcus	18.3	36.0	54.5	36.5	31.9	37.7	18.3	-	54.5
Enterococcus	11.1	10.1	5.6	11.2	5.7	10.4	5.6	-	11.1
Pseudomonas	9.4	5.5	7.5	-	11.5	-	0	-	11.5
Citrobacter	5.5	-	-	-	6.4	-	0	-	6.4
Micrococcus	-	7.7	10.0	6.6	5.7	10.0	0	-	10.0
Proteus	-	5.4	-	-	10.7	-	0	-	10.7
Miscellaneous[b]	7.6	4.5	6.3	5.3	0.2	7.9		-	
Gram-negative genera	63.0	41.7	23.6	40.4	56.5	34.0	23.6	-	63.0
Gram-positive genera	29.4	53.8	70.1	54.3	43.3	58.1	29.4	-	70.1
Total	100%	100%	100%	100%	100%	100%		-	

1: Neck skin, 2: Skin from both sides ventral to wings, 3: Skin caudal to breastbone,
4: Skin cranio-dorsal to pygostyle, 5: Skin from back between wings, 6: Skin *ca.*2cm cranial to centre of breast.
[a]Number of isolates at each site is shown in brackets.
[b]Genera individually totalling less than 5% of isolates.

Table 2.3 Characterisation of 120 bacteria isolated from chicken carcase samples, from three processing stages and incubated at three temperatures.

	Incubation temperature		
Bacterial Genus (%)	25°C (43)a	37°C (53)	43°C (24)
Staphylococcus	42.3	30.0	19.9
Escherichia	-	24.0	56.9
Enterococcus	-	15.1	14.0
Micrococcus	-	11.5	-
Pseudomonas	5.7	8.3	-
Acinetobacter	40.9	-	-
Proteus	5.2	-	-
Miscellaneous	5.9	11.1	9.2
Gram-negative genera	51.8	32.3	56.9
Gram-positive genera	42.3	56.6	33.9
Total	100%	100%	100%

[a]Number of isolates is shown in brackets.

Table 2.4 Characterisation of 52 bacteria isolated from aerobic plate counts at six sampling sites on chicken carcases and incubated at 37°C

	Sampling Sites								
Bacterial Genus (%)	1 (15)[a]	2 (8)	3 (6)	4 (9)	5 (6)	6 (7)	**Range**		
Escherichia	24.6	30.1	9.3	31.9	-	30.7	9.3	-	31.9
Staphylococcus	24.1	26.1	46.7	28.3	29.2	35.3	24.1	-	46.7
Enterococcus	18.4	11.9	9.9	19.3	13.6	13.9	9.9	-	19.3
Pseudomonas	14.5	10.3	-	-	6.6	5.0	0	-	14.5
Citrobacter	8.8	-	-	-	9.3	-	0	-	9.3
Micrococcus	7.9	9.5	21.3	12.9	11.9	12.5	7.9	-	21.3
Arthrobacter	-	-	6.7	-	-	-	0	-	6.7
Proteus	-	-	-	-	25.5	-	0	-	25.5
Miscellaneous[b]	1.7	12.1	6.1	7.6	3.9	2.6	1.7	-	12.1
Gram-negative genera	47.9	40.4	9.3	31.9	41.4	35.7	9.3	-	47.9
Gram-positive genera	50.4	47.5	84.6	60.5	54.7	61.7	47.5	-	84.6
Total	100%	100%	100%	100%	100%	100%		-	

1: Neck skin, 2: Skin from both sides ventral to wings, 3: Skin caudal to breastbone,
4: Skin cranio-dorsal to pygostyle, 5: Skin from back between wings, 6: Skin *ca.*2cm cranial to centre of breast.
[a]Number of isolates at each site is shown in brackets.
[b]Genera individually totalling less than 5% of isolates.

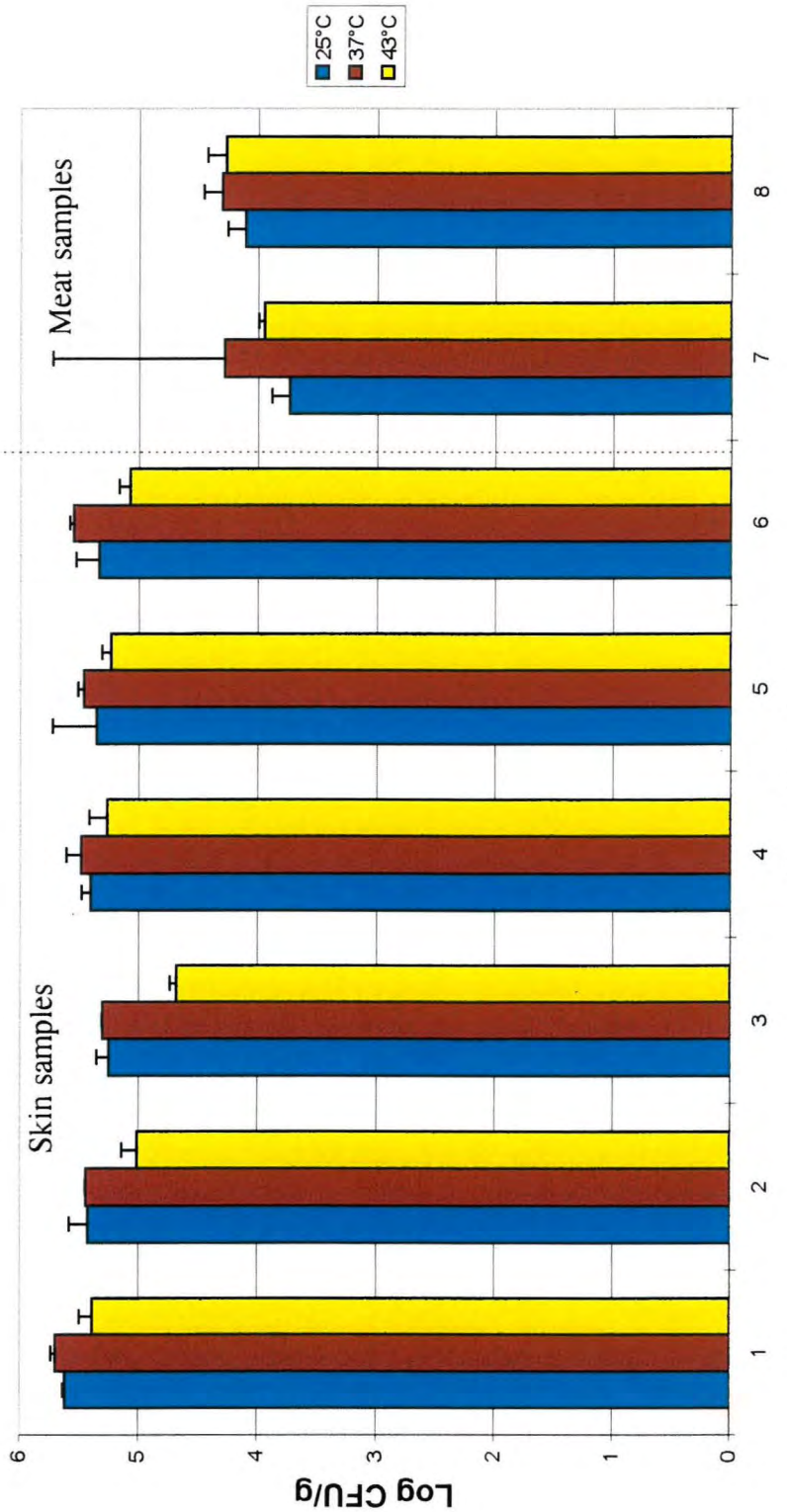

Fig. 2.1. Mean bacterial counts at eight sampling sites on chicken carcases from three sampling points (after defeathering, after evisceration and after chilling) at three incubation temperatures.
1: Neck skin, 2: Skin from both sides ventral to wings, 3: Skin caudal to breastbone, 4: Skin cranio-dorsal to pygostyle, 5: Skin from back between wings, 6: Skin ca. 2cm cranial to centre of breast, 7: Meat from both sides ventral to wings, 8: Meat caudal to breastbone.

CHAPTER THREE

AEROBIC BACTERIAL COUNTS AND CHARATERISATION OF BACTERIA ISOLATED FROM CHICKEN CARCASES AT SELECTED SAMPLING POINTS IN A PROCESSING PLANT

Abstract

A South African poultry abattoir with minimal mechanisation was chosen to evaluate the effect of processing on the microbiological status of chicken carcases. Skin samples were taken aseptically at six different locations from each of three chicken carcases sampled at three different times at three sampling points during processing. Total bacterial counts were determined on Nutrient agar incubated at 37°C for 48 hours. Isolation of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* was made using selective media. Total bacterial numbers after defeathering (6.2 - 6.6 log CFU g^{-1}) and after evisceration (6.3 - 6.4 log CFU g^{-1}) were the highest. Marginally lower total bacterial numbers (5.5 - 6.1 log CFU g^{-1}) were obtained after chilling. *E. coli* and *S. aureus* were isolated frequently at all the sampling points. The frequency of isolation of *Salmonella* was low. *Escherichia* dominated at all three sampling points. At the sampling points, after defeathering and after chilling, *Staphylococcus* showed high incidence of prevalence. Statistically significant differences ($P < 0.05$) were found amongst sampling points on the different days of sampling.

Introduction

Poultry is an important dietary food component in South Africa. Production, as in the rest of the world, is increasing to satisfy a growing demand (Todd, 1980; Parry, 1989; Simonsen, 1989). During the processing stages, a high proportion of the micro-organisms on the live chicken will be removed by means of scalding and spray washing, but further contamination can occur at any stage in the processing line (McMeekin *et al.*, 1979a; Mead, 1982; Bailey *et al.*, 1987; Connor *et al.*, 1987). Reduced shelf-life and danger to public health is of concern to processors and consumers of poultry meat alike. To produce a quality poultry product, hygiene and actions or activities to minimise or reduce hazards at the different stages of processing is necessary (FAO/WHO, 1993). Strict control can be accomplished by implementing the Hazard Analysis Critical Control Point (HACCP) system. The system identifies critical operations on the processing line and provides ways for monitoring and controlling them on an ongoing basis. Microbiological testing to determine bacterial populations before and after the introduction of a HACCP system is essential to assess which points could be considered critical control points (CCP's) in the first place and to determine the value of this system in poultry production

(Brown *et al.*, 1982; Bryan *et al.*, 1991; WHO, 1993; Silliker, 1995). During processing opportunities exist for contamination of the carcases from the environment via aerosols and process-water, equipment, worker's hands and also by cross-contamination from other carcases (McMeekin *et al.*, 1979a; Bryan, 1980; Bailey *et al.*, 1987; Mead, 1989; Zottola *et al.*, 1990). Some processing operations may cause an increase of contamination or even a multiplication of organisms (McMeekin *et al.*, 1979a; Bryan, 1980; Mead, 1982; Bailey *et al.*, 1987; Connor *et al.*, 1987). Consequently, the micro-organism population changes from mainly Gram positive rods and micrococci on the outside of the live chicken to Gram negative micro-organism on the finished product (Thomas *et al.*, 1980; Banwart, 1989; Mead, 1989).

Spoilage bacteria grow mainly on the skin surfaces, in the feather follicles and on cut muscle surfaces. The intact skin serves as a barrier to micro-organisms that might otherwise contaminate the underlying muscle and therefore the muscle surfaces and deep muscle are normally free of bacteria (Barnes *et al.*, 1973; McMeekin *et al.*, 1979a; Bryan, 1980; Mead, 1982). The few bacteria isolated from the deep muscle are those that can only multiply slowly or not at all at low temperatures (Bryan, 1980). Spoilage bacteria most frequently associated with poultry are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alteromonas putrefaciens*, *Corynebacterium*, *Flavobacterium*, Micrococcaceae and Enterobacteriaceae. Pathogens associated with poultry are *Salmonella, Staphylococcus aureus, Clostridium perfringens* and *Escherichia coli*. *Listeria monocytogenes* and *Campylobacter jejuni* have also been isolated from poultry (Bryan, 1980; Brown *et al.*, 1982; Mead, 1982; Grau, 1986; Cunningham, 1987; Banwart, 1989; Mead, 1989; Zottola *et al.*, 1990). An increase in aerobic mesophiles (*Micrococcus, Streptococcus* and lactobacilli), *Staphylococcus aureus* and Enterobacteriaceae occurs during defeathering, due to a considerable scattering of micro-organisms within the defeathering machines and inadequate maintenance and cleaning of the defeathering machines (Bryan, 1980; Thomas *et al.*, 1980; Mead, 1982; Grau, 1986). The Enterobacteriaceae counts increase during evisceration, but there is no significant increase in the *Micrococcus* counts during this process. An increase in *Staphylococcus aureus* can occur from sources other than the birds. *Salmonella* counts can also increase, after exposure and opening of the intestines (Notermans *et al.*, 1980; Notermans *et al.*, 1982; Grau, 1986; Connor *et al.*, 1987; Anand *et al.*, 1989; Mead, 1989).

Washing reduces the count of mesophiles, Enterobacteriaceae and coliforms. The incidence of *Salmonella* on the carcases also decreases after washing. Carcases can however be contaminated by *Pseudomonas* spp. during washing due to contamination of process water with *Pseudomonas* (Lahellec *et al.*, 1979; Bryan, 1980; Thomas *et al.*, 1980; Lahellec *et al.*, 1981; William *et al.*, 1992).

During chilling an increase in psychrotrophic bacteria can occur, but a reduction in mesophilic bacteria and coliforms occurs. The levels of *Salmonella* were also reduced during immersion chilling (Morris *et al.*, 1970; Mead, 1989). Spoilage at chill temperatures is of the biggest concern and *Pseudomonas* usually predominates on poultry at chill temperatures (McMeekin *et al.*, 1979a; Bryan, 1980; Eustace, 1981; Gill, 1983; Grau, 1986; Banwart, 1989; Mead, 1989; Zottola *et al.*, 1990).

The objectives of this study were to identify sampling points and time of sampling during processing in a South African Grade B poultry abattoir in order to:

- determine the numbers of total aerobic bacteria
- determine the pathogens
- determine the dominant organisms

on chicken carcases to evaluate the influence of processing on the microbiological status of chicken carcases.

Materials and methods

Processing plant studied

A description of the processing plant and details of the process can be found in Chapter 2.

Sampling procedure

The time of sampling was selected to monitor the slaughter process throughout the processing plant. The sampling points in the abattoir was after defeathering, after evisceration and after chilling (Fig. 3.1) (Mead, 1982; Grau, 1986; Mead, 1989). The sites of sampling on the chicken carcases were selected to represent the sites of highest contamination on the carcases

(Patterson, 1972; Barnes *et al.*, 1973; Green, 1974; Bryan, 1980; Anand *et al.*, 1989). Skin samples were taken aseptically from each of three chicken carcases selected at three different times at three sampling points (Fig. 3.2). This sampling was repeated four times on different days of the week from May until June. Six skin samples (each with a mass of *ca.* 1-2 g) were taken from the following sites on three randomly selected chicken carcases: neck skin, skin from both sides ventral to the wings, skin caudal to the breastbone, skin cranio-dorsal to the pygostyle, skin from back between the wings and skin *ca.* 2 cm cranial to the centre of the breast. The six skin samples from the different sampling points on the selected chicken carcases were pooled to form a composite sample with a mass of ca. 34 - 36g (n = 1, Fig.3.2).

Microbiological analysis

This composite sample was placed into quarter strength Ringers solution (Oxoid) in a 1+9 mass/volume ratio based on exact mass. Each composite sample was semi-homogenised for 2 min (Seward Medical 400 Stomacher Lab Blender). Tenfold serial dilution's in Ringers solution (Oxoid) were plated out in duplicate on Nutrient Agar (Oxoid) using the spread-plate technique (ICMSF, 1978; Busta *et al.*, 1984; von Holy *et al.*, 1992) and incubated aerobically at 37°C for 48 h. Plates showing between 30 and 300 colony forming units (cfu) (or the highest number if below 30) were counted. Bacterial counts obtained from the plates of each duplicate set were meaned, converted to logarithms and analysed statistically using the SAS Programme, Version 6.1 (SAS Institute Inc., Carp, North Carolina, USA).

***Escherichia coli*, presumptive *Salmonella* and *Staphylococcus aureus* isolation.**

a) *Escherichia coli*. The prevalence of *E. coli* was determined by spread plating duplicate 1 ml samples of the semi-homogenised composite skin samples in the quarter strength Ringers solution (Oxoid) onto Biolab Violet Red Bile Mug Agar (Combined)(C144) and incubated at 37°C for 48h. The plates were examined under the longwave UV light (366nm). All colonies showing a blue fluorescence in the surrounding medium were counted and were considered to be *E. coli*.

b) Presumptive *Salmonella*. To determine the presence of presumptive *Salmonella*, 1 ml samples of the semi-homogenised composite skin samples were inoculated into 10 ml of

Buffered Peptone Water pH 7.0 (Oxoid) (CM509) and incubated at 37°C for 24h. The pre-enrichment broth (0.1 ml) was inoculated into 10 ml of Oxoid Selenite Broth Base (CM395) for enrichment and incubated at 37°C for 24h. Oxoid XLD Medium (CM469) was used for the isolation of presumptive *Salmonella*. The identity of presumptive *Salmonella* colonies was confirmed by the urease, lysine decarboxylase and hydrogen sulfide on triple sugar iron agar, tests.

c) ***Staphylococcus aureus.*** The prevalence of *Staphylococcus aureus* was determined by spread plating duplicate 1 ml samples of the semi-homogenised composite skin samples in the quarter strength Ringers solution (Oxoid) onto Oxoid Baird-Parker Medium Base (CM275) containing Oxoid Egg Yolk Tellurite Emulsion (SR54) supplement and incubated at 37°C for 48h. Presumptive *S. aureus* colonies were tested for the ability to produce coagulase with Oxoid Staphtect tests (DR650).

Isolation and characterisation of the micro-organisms.

The plates were incubated aerobically and the predominance was determined on the highest dilution showing growth. All the colonies (350) on the Nutrient Agar plates of the highest dilution showing growth were purified on the same medium and characterised. All the Gram positive organisms (sampling point 1, 59 isolates; sampling point 2, 30 isolates and sampling point 3, 20 isolates) were identified with API 20 STREP, STAPH and CORYNE (Bio Mérieux S.A., Marcy L'Etaile, France) identification strips. All the Gram negative colonies (sampling point 1, 94 isolates; sampling point 2, 109 isolates and sampling point 3, 38 isolates) were identified with MICROBACT 24E (Disposable Products Pty. Ltd., Technology Park, South Australia) identification strips.

Results and discussion

Prevalence of pathogens on chicken carcases.

The prevalence of *E. coli* was high throughout the process and was also isolated in high numbers namely >300/g. *Staphylococcus aureus* was isolated in low numbers (<10/g). Although *Salmonella* species were isolated from the chicken carcases (*S. muenchen*, *Salmonella* I Rof and *S. kallo*), they were part of the well recognised non-pathogenic species (Table 3.1)

(Eley, 1992).

Total bacterial numbers

At the first sampling point (after defeathering) bacterial numbers increased between 08:30 and 12:30 (6.2 - 6.6 log CFU g^{-1}) (Fig.3.3). The bacterial numbers after evisceration were marginally higher (6.3 - 6.4 log CFU g^{-1}) than the other two sampling points at 8:30 and 10:30, but not at sampling time 12:30. After chilling a decrease in bacterial numbers was observed from 08:30 to 12:30 (6.1 - 5.5 log CFU g^{-1}). The lowest bacterial numbers were also obtained at this sampling site.

There was a significant difference ($P < 0.05$) in bacterial numbers amongst the different sampling sites during processing (Fig.3.4). No significant differences ($P > 0.05$) were obtained irrespective of the days of sampling and times of sampling (Fig. 3.5 and Fig. 3.6).

The taking of composite skin samples was possibly an experimental design error, because it limited the statistical analysis that could be done on the results obtained from samples on the carcases. Due to the composite skin samples it was not possible to statistically compare the bacterial numbers at the different sampling times and days.

Aerobic plate counts of sampling sites after defeathering and after evisceration did not differ significantly ($P > 0.05$) between 8:30, 10:30 and 12:30 (6.2, 6.3, 6.6, 6.3, 6.4, 6.1 log CFU g^{-1}, respectively) (Fig. 3.3). Also, after chilling at 8:30, the bacterial numbers did not differ significantly ($P > 0.05$) (6.1 log CFU g^{-1}) from the other two sampling sites. A significant decrease in bacterial numbers ($P < 0.05$) was, however, obtained after chilling at 12:30 (5.5 log CFU g^{-1}) compared to corresponding numbers of samples taken after defeathering at 12:30 (Fig. 3.3).

The bacterial numbers increased over time during the processing day at sampling point, after evisceration (Fig. 3.3). This could be as a result of contamination from the environment, equipment, workers' hands and also by cross-contamination from other carcases. Because of the position of the defeathering machine next to the scald tank where the environment is warm and moist and which is conducive to microbial growth the carcases can easily become

contaminated with micro-organisms from the outside of the carcases (Mead, 1982; Grau, 1986). Soil, dust and bacteria on the skin, feathers, in the faeces and the respiratory tract enter the water as the birds are moved continuously through the scald tank. Continuous replacement of fresh water and flow patterns together with the temperature of the water in the scald tank is therefore of great importance (Mulder *et al.*, 1978; Bailey *et al.*, 1987). At the abattoir, in which the poultry processing was studied, no replacement of the water in the scald tank with clean water occurred during the processing day. Introduction of replacement water during the processing day, to reduce the build-up of contaminants in the scald tank , was also emphasised by Bryan, 1980 and Mead, 1982.

There is also a considerable scattering of micro-organisms during defeathering, which leads to further contamination and cross-contamination possibilities (Mead, 1982; Grau, 1986; Mead, 1989). With the passage of warm, moist carcases through the defeathering machine, bacteria colonise the rubber "fingers" (Mead, 1982; Grau, 1986; Mead, 1989). These are difficult to clean and bacteria persist on them, leading to further contamination (Bryan, 1980; Mead, 1982; Grau, 1986; Mead, 1989). The problem is compounded by the lack of machine maintenance and failure to replace the " fingers" when they harden and crack with excessive wear and tear. Normally defeathering (picking) takes place through a series of in-line mechanical defeathering machines in this size of abattoir with this production through-put, to efficiently remove all the feathers from a carcase (Mead, 1982; Parry, 1989). In the abattoir studied there was only one defeathering (picking) machine. This single machine is not automatically adjustable and therefore not capable of removing all the feathers on the carcases, leaving feathers on the wings, hocks and pygostyle. Pin feathering of the remaining feathers was incompletely done in haste by hand causing cross-contamination (Bailey *et al.*, 1987).

During defeathering, micro-organisms become attached to the newly exposed skin of the carcases and persist in the relaxed and expanded feather follicles and could cause colonisation (Bryan, 1980; Grau, 1986; Bailey *et al.*, 1987). The nature and rate of attachment of the micro-organisms (*S. aureus*, salmonellae and coliforms) depends upon several factors including the type of bacteria (presence/absence of flagella and fimbrae) and their number and also the conditions of pH, temperature and contact-time under which attachment occurs (Bryan, 1980; Mead, 1982). The structure of the skin (channels and crevices in the skin) also has an influence

on the attachment of bacteria, because of the trapping of bacteria in the feather follicles, channels and crevices in the skin. The organisms (Enterobacteriaceae and *S. aureus*) adhere by way of flagella and fimbrae and cannot easily be removed, especially after the delay in time in rinsing the carcases, caused by the removing of feathers by hand in this abattoir (Bryan, 1980; Mead, 1982; Mead, 1989). Repeated spray cleaning of the carcases to prevent adherence of bacteria, especially during defeathering and evisceration is therefore important (Notermans *et al.*, 1975; Bryan 1980; Thomas *et al.*, 1980; Firstenberg-Eden, 1981; Mead, 1982; Faber *et al.*, 1984; Lillard, 1985; Grau, 1986; Anand *et al.*, 1989; Mead, 1989). In the abattoir studied no rinsing of the carcases was done after defeathering, which would lead to build-up of contamination (Fig. 4.3).

Prior to evisceration the carcases were submerged in a container of stagnant water. It is logical to assume that this would lead to further contamination, with a build-up of bacteria occurring in such water (Mead, 1982). Immersion in stagnant water is not conducive to removal of bacterial contamination and therefore it is legislated in South Africa that free running water be used (Government Gazette, 1969). Head and feet removal from the carcases in this abattoir was done by hand with knives, just after defeathering and before the carcases were sampled. The head and then one foot were removed with a knife. The carcases were then rehung on the conveyor after which the other foot was removed. Excessive manual handling of carcases took place at this stage of the process. Infrequent hand washing, poor personal hygiene of the workers and insufficient cleaning of equipment such as knives led to further build-up of bacteria. All the above practices will contribute to increased bacterial numbers over the time of sampling at this specific sampling site in the abattoir studied. This was in agreement with similar observations by Bailey *et al.*, 1987.

At the second sampling point (after evisceration) the highest bacterial numbers were obtained at 8:30 and 10:30 (Fig. 3.3). Evisceration is one of the processing operations where most bacterial contamination of the carcases can take place and offers many opportunities for carcase to carcase contamination and increased bacterial load (Mead, 1982; Grau, 1986; Bailey *et al.*, 1987; Anand, 1989). Careless manual opening of the abdomen and manual evisceration leads to contamination of carcases, especially when the intestines are lacerated or the vent is inadequately loosened. Cross-contamination can also occur due to workers' hands, evisceration

implements (spoons) and other slaughter equipment such as hand tools (Mead, 1982; Grau, 1986; Bailey *et al.*, 1987; Anand *et al.*, 1989; Mead, 1989). The hand evisceration technique practised in the abattoir under study, infrequent hand washing and poor personal hygiene, resulted in increased bacterial loads. Cross-contamination also occurred due to contact between carcases on the processing line and due to rubbing of the carcases against the taps of the evisceration troughs and against the evisceration trough itself. Bailey *et al.*, 1987 also noted that any location where carcases must be handled or are allowed to rub against each other or evisceration equipment (troughs and taps) has the potential for cross-contamination. Due to infrequent and insufficient cleaning of evisceration implements in this abattoir increased build-up of contaminants occurred as the day progressed. The steriliser design in the abattoir under study was a water container (like a waterbath) with a electric element for heating the water. The water temperature in the container was ±50°C and not 82°C as prescribed in South African legislation for sterilising evisceration implements (Government Gazette, 1969; SAPA, 1993). The evisceration implements were only cleaned with cold water and without any detergent, adding to possible cross-contamination of the carcases in this abattoir.

The lowest bacterial numbers were obtained after chilling (Fig. 3.3). This concurred with previous work done on poultry processing (Bryan, 1980; Anand *et al.*, 1989). Chilling can delay the growth of psychrotrophic bacteria and prevent an increase of micro-organisms of public health significance (Bryan, 1980; Mead, 1982; Anand *et al.*, 1989). In the abattoir under study, the carcases should have been chilled for eg. 50 min. in an on-line air-chiller to reduce the carcase temperature from *ca.* 30°C to *ca.* 10°C. Carcase dwell time was, however, only 35 min. in the air-chiller. The deep breast temperature of carcases (keel) never reached 10°C or less, but varied between 19°C and 22,3°C (See Fig. 3.7) (SAPA, 1993). At the first two sampling points bacterial numbers increased during the course of sampling time, but after chilling bacterial numbers decreased (6.1 - 5.5 log CFU g^{-1}) (Fig. 3.3). Monitoring the temperature in the air-chiller, when the first carcases enter the chiller, at the beginning of a slaughtering day, the temperature was not -6°C, as prescribed in the abattoir for the effective use of the on-line air-chiller to sufficiently reduce the temperature of the carcases. During the processing day the temperature in the air-chiller, however declined from -3°C to the prescribed -6°C (Fig. 3.8). The decrease in bacterial counts from sampling time 8:30 to 12:30 can be attributed to the fact that the bacterial growth on the chicken carcases was inhibited more as the

temperature in the air-chiller declined during the processing day. The decrease can also possibly be that the incubation temperature of 37°C was to high to isolate the bacteria which would survive chilling at -6°C.

Characterisation of microbial populations during processing.

The percentage distribution with respect to identity of bacteria isolated from chicken carcases sampled at three different sampling points is shown in Table 3.2.

At the first sampling point (after defeathering), a total of 153 bacteria were isolated. The highest number of bacteria were isolated at this sampling point. The Gram negative genera *Escherichia* (28.4%), *Acinetobacter* (17.0%) and *Pseudomonas* (9.5%) amounted to 61.5% of the total. The Gram positive genera *Staphylococcus* and *Micrococcus* collectively amounted to 36.3% of the total with percentages of 28.8% and 7.5%, respectively. The remaining 2.2% of the isolates were made up of genera individually comprising less than 5% of all isolates (Fig. 3.9).

A total of 139 bacteria were isolated from the sampling point after evisceration. The Gram negative genera dominated at this sampling point with a percentage of 77.7% of the total. *Escherichia* showed the highest percentage (36.0%), followed by *Acinetobacter* and *Pseudomonas* at 24.1% and 17.6%, respectively. The Gram positive genus *Staphylococcus* was recovered at a percentage of 18.4%. The remaining 3.9% isolates were made up of genera individually totalling less than 5% of all isolates (Fig. 3.10). At the sampling point after chilling, only 58 bacteria were isolated. The Gram negative genera *Escherichia* (34.5%), *Acinetobacter* (15.5%) and *Pseudomonas* (15.5%) collectively amounted to 65.5% of the total. Proportions of the Gram positive genera *Staphylococcus* and *Micrococcus* amounted to 25.9% and 6.0%, respectively. The remaining 2.6% of the isolates individually totalled less than 5% of the isolates (Fig. 3.11).

At the first sampling point (after defeathering), the Gram negative genus *Escherichia* showed the highest percentage prevalence (35%), followed by the Gram positive genus *Staphylococcus*. This was in agreement with reports from Grau (1980), Mead (1982) and Mead (1989). They reported that during defeathering the *Escherichia* and *Staphylococcus* numbers increase. The

high percentage of *Escherichia* could be attributed to the considerable scattering and spreading of micro-organisms in the air around and in the defeathering machine and also as a result of contamination from carcase to carcase. The outside of the chickens (skin, feathers and feet) are normally contaminated with Enterobacteriaceae and these micro-organisms from one carcase, at this stage, can cross-contaminate large numbers of other carcases. During defeathering scattered micro-organisms in the defeathering machine become attached to the newly exposed skin of the carcases. After defeathering the cuticle-free skin provides numerous sites that have a crucial influence on the attachment of the bacteria. The organisms attached to the skin in such a way that they cannot easily be removed by rinsing, especially *Staphylococcus* and *Escherichia* (Notermans *et al.*, 1975; Bryan, 1980; Thomas *et al.*, 1980; Mead, 1982; Anand *et al.*, 1989). Also, the rubber "fingers" of the defeathering machine are very difficult to clean, in particular when they become worn and cracked and could harbour micro-organisms like *Staphylococcus* (Bryan, 1980; Mead, 1982) . There are normally up to three defeathering machines in series to remove all the feathers from a carcase (Mead, 1982; Parry, 1989). In the abattoir studied there was only one defeathering (picking) machine. This single machine is not adjustable to remove all the feathers on the carcases, leaving many feathers on the wings, hocks and pygostyle. Pin feathering of the remaining feathers was done by hand causing cross-contamination mainly with *Staphylococcus*. The literature also stated that defeathering machines and defeathering equipment can cause contamination with *Staphylococcus* (Gibbs *et al.*, 1978; Notermans *et al.*, 1982; Grau, 1986; Mead, 1989).

At the second sampling point (after evisceration) the percentage Gram negative genera (77.7%) dominated over the Gram positive genera (18.4%). At this sampling point the percentage *Escherichia* isolated (36%) was also the highest of all the sampling points. The literature showed that the carcases can become contaminated with Enterobacteriaceae from the intestinal contents at this sampling point (Mead, 1982; Grau, 1986; Connor *et al.*, 1987; Anand *et al.*, 1989). Careless manual opening of the abdomen and manual evisceration causes soiling of the carcases in this abattoir. This is aggravated when the intestines are ruptured or the cloaca is inadequately loosened (Bryan, 1980; Notermans *et al.*, 1980; Grau, 1986; Connor *et al.*, 1987). Spray washing at various stages during evisceration is needed to control the feacal contamination and the build-up of contaminants during evisceration (Mulder *et al.*, 1974; Notermans *et al.*, 1980). The percentage prevalence of *Pseudomonas* was also the highest at

this sampling point. This concurred with previous work done on poultry processing (Mead, 1989). This could be attributed to the fact that *Pseudomonas* are a common environmental organisms. The process water can be contaminated with *Pseudomonas*. Poultry spoilage bacteria like *Pseudomonas* can also multiply on the wet carcase surfaces and on soiled surfaces of equipment (Lahellec *et al.*, 1979; Grau, 1980; Mead, 1982).

At the third sampling point (after chilling) a high percentage of Gram negative bacteria (65.5%) were obtained and in particular *Escherichia*. The most probable reason for the high prevalence of these bacteria at this sampling point was that there was only one place after evisceration where spray washing of the carcases took place. This spray washing was not effective enough to remove or drastically decrease the bacterial load on the carcases, due to the high line speed of the carcases at this stage of processing. Notermans *et al.*, 1980 stated that effective washing at more than one place after evisceration is necessary to reduce the bacterial load on the carcases. The carcases were not washed with water under pressure and this adversely effect the efficiency of washing at this stage (Government Gazette, 1969; Mead, 1989). Carry-over of micro-organisms from one stage (evisceration) to another (chilling) occurred here due to the absence of adequate cleaning after evisceration. Adequate cleaning after evisceration and before cooling to reduce the bacterial load was also emphasised by Mulder *et al.*, 1974. The reason for the high prevalence of *Staphylococcus* at this stage could have been the poor personal hygiene of the workers and also the technique of hand wiping of the carcases at the stage of spray washing, after evisceration and before chilling. Therefore, a high prevalence of human related bacteria such as *Staphylococcus* was expected at this stage (Gibbs *et al.*, 1978). It was also indicated in previous findings that chilling had little effect on the reduction in *Staphylococcus* counts (Notermans *et al.*, 1982; Grau, 1986) Micro-organisms can also circulate in currents of cold air in the chiller and can contaminate the carcases (Bryan, 1980; Mead, 1989).

Conclusion

The microbiological survey of the carcase processing showed that bacterial contamination was a problem in this abattoir since high bacterial numbers were obtained throughout the course of the four sampling days. This survey also showed that the processing stages in this abattoir where most bacterial contamination occurred were defeathering and evisceration and that more strict

control should be carried out at these two stages to ensure hygiene of the carcases for a safe and wholesome poultry product. With the implementation of the Hazard Analysis Critical Control Point system this strict control can be carried out to ensure that the process is improved step by step to achieve acceptable microbiological standards (Table 3.3) (SAPA, 1993). The bacterial numbers obtained in this study were high (Table 3.1), compared to the standards set by SAPA, 1993 (Table 4.3). It is also recommended that efforts should be made to prevent the build-up of bacteria during processing. Thorough and frequent spray washing and rinsing, especially during defeathering and evisceration, will reduce the bacterial load. High pressure spray washers, strategically sited at different stages, will reduce the number of bacterial contaminants. This would apply particularly after opening of the carcase, after exposure of the intestines and after removal of the intestines during the evisceration process. Frequent rinsing of the defeathering machine (after every tea and lunch break during the processing day), with clean water and a disinfectant, will also reduce bacterial build-up on the defeathering "fingers". The installation of a counter-current system when scalding to ensure that the carcases have contact with the cleanest water when exiting the scald tank will decrease bacteria build-up. The continuous overflow of contaminated scald water and the simultaneous introduction of fresh water will also remove contaminants of the scald tank. The installation of more defeathering machines (a minimum of two defeathering machines in series is recommended) will also prevent the build-up of bacteria and specific *Staphylococcus* in the single defeathering machine, because pin feathering and hence excessive manual handling of the carcases will be reduced. Preventing the rupturing of the intestines and subsequent contamination of the carcases during evisceration will further decrease bacterial contamination and more specifically Enterobacteriaceae. Avoiding of hand wiping of the carcases at spray washing should also prevent the high prevalence of bacteria such as *Staphylococcus*. This would also prevent a lot of cross-contamination and carry-over of micro-organisms from one stage to another. It is also recommended that the air-chiller should be turned on timeously to ensure that the temperature in the chiller is -6°C before slaughter starts in the morning. Regular monitoring (at least twice per shift) of the chiller temperature, the temperature of the carcases at the keel as well as the dwell-time of the carcases in the chiller is also recommended, to ensure that the recommended temperature will be maintained (SAPA, 1993).

Table 3.1 Prevalence of *Escherichia coli*, presumptive *Salmonella* and *Staphylococcus aureus* isolated at the different sampling points over the four days of sampling.

Sampling Day	Sampling Point (SP)	Sampling Time	Foodborne Pathogens (no. of positive sample/total no. of samples)(% prevalence)		
			Escherichia coli	Presumptive *Salmonella*	*Staphylococcus aureus*
1	1	8:30	>300/g	2/5 (40%)	ND
		10:30	>300/g	ND	ND
	2	8:30	>300/g	¾ (75%)	ND
		10:30	>300/g	3/5(60%)	ND
	3	8:30	ND	ND	ND
		10:30	>300/g	ND	ND
2	1	8:30	>300/g	2/6 (33%)	1/5 (20%)
		10:30	ND	ND	ND
	2	8:30	>300/g	3/5 (60%)	ND
		10:30	>300/g	2/5 (40%)	ND
	3	8:30	ND	¼ (25%)	ND
		10:30	>300/g	1/5 (20%)	ND
3	1	8:30	>300/g	ND	2/4 (50%)
		10:30	ND	ND	ND
		12:30	>300/g	ND	1/3 (33%)
	2	8:30	>300/g	3/5 (60%)	ND
		10:30	ND	1/3 (33%)	1/5 (20%)
		12:30	>300/g	ND	ND
	3	8:30	ND	ND	ND
		10:30	>300/g	ND	ND
		12:30	>300/g	ND	ND
4	1	8:30	>300/g	ND	2/5 (40%)
		10:30	ND	ND	ND
		12:30	>300/g	2/3 (66%)	ND
	2	8:30	ND	4/5 (80%)	ND
		10:30	ND	2/6 (33%)	ND
		12:30	>300/g	ND	ND
	3	8:30	>300/g	ND	ND
		10:30	>300/g	ND	ND
		12:30	>300/g	ND	ND

ND - Not detected

Table 3.2 Characterisation of 350 bacteria isolated from chicken carcases sampled at three different sampling points.

	Sampling points (SP's)			
Bacterial Genus (%)	**SP 1 (153)[a]**	**SP 2 (139)**	**SP 3 (58)**	**Range**
Escherichia	35	36	34.5	34.5 - 36.0
Staphylococcus	28.8	18.4	25.9	18.4 - 28.8
Acinetobacter	17	24.1	15.5	15.5 - 24.1
Pseudomonas	9.5	17.6	15.5	9.5 - 17.6
Micrococcus	7.5	0	6.0	0 - 7.5
Miscellaneous[b]	2.2	3.9	2.6	2.2 - 3.9
Gram-negative genera	61.5	77.7	65.5	61.5 - 77.7
Gram-positive genera	36.3	18.4	31.9	18.4 - 36.3
Total	100%	100%	100%	-

SP 1: After defeathering, SP 2: After evisceration, SP 3: After chilling.

[a] Number of isolates at each sampling point is shown in brackets.

[b] Genera individually totalling less than 5% of isolates.

Table 3.3 South African Poultry Association standards for microbiological counts of poultry carcase skin.

Test	**TOTAL VIABLE**	**COLIFORMS**	***S. aureus***	**SALMONELLA**
Skin	< 100/g	< 1000/g	< 100/g	Absent

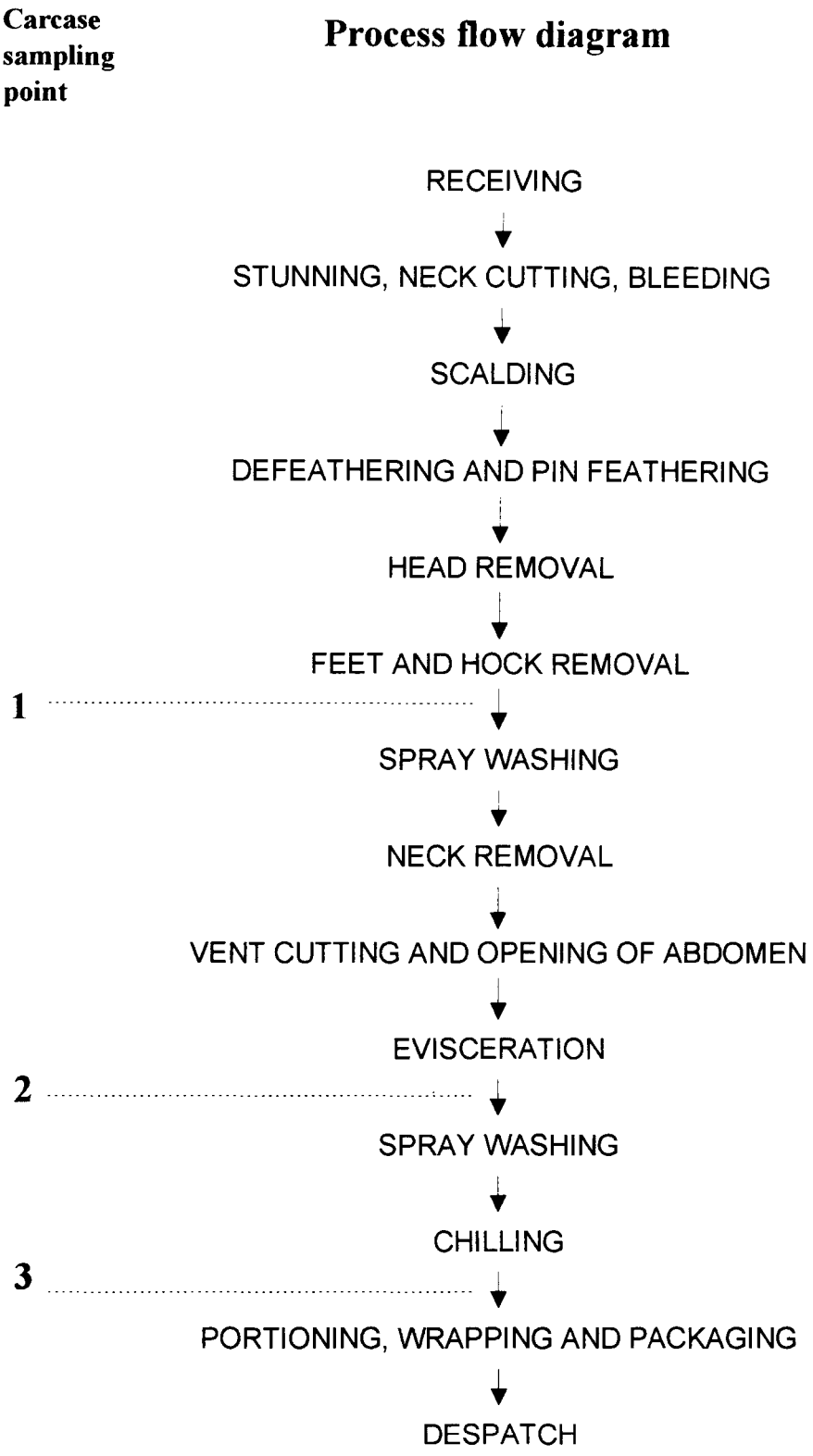

Fig. 3.1 Process flow diagram showing carcase sampling sites in a small South African Grade B poultry abattoir.

* Carcase sampling points in the abattoir.

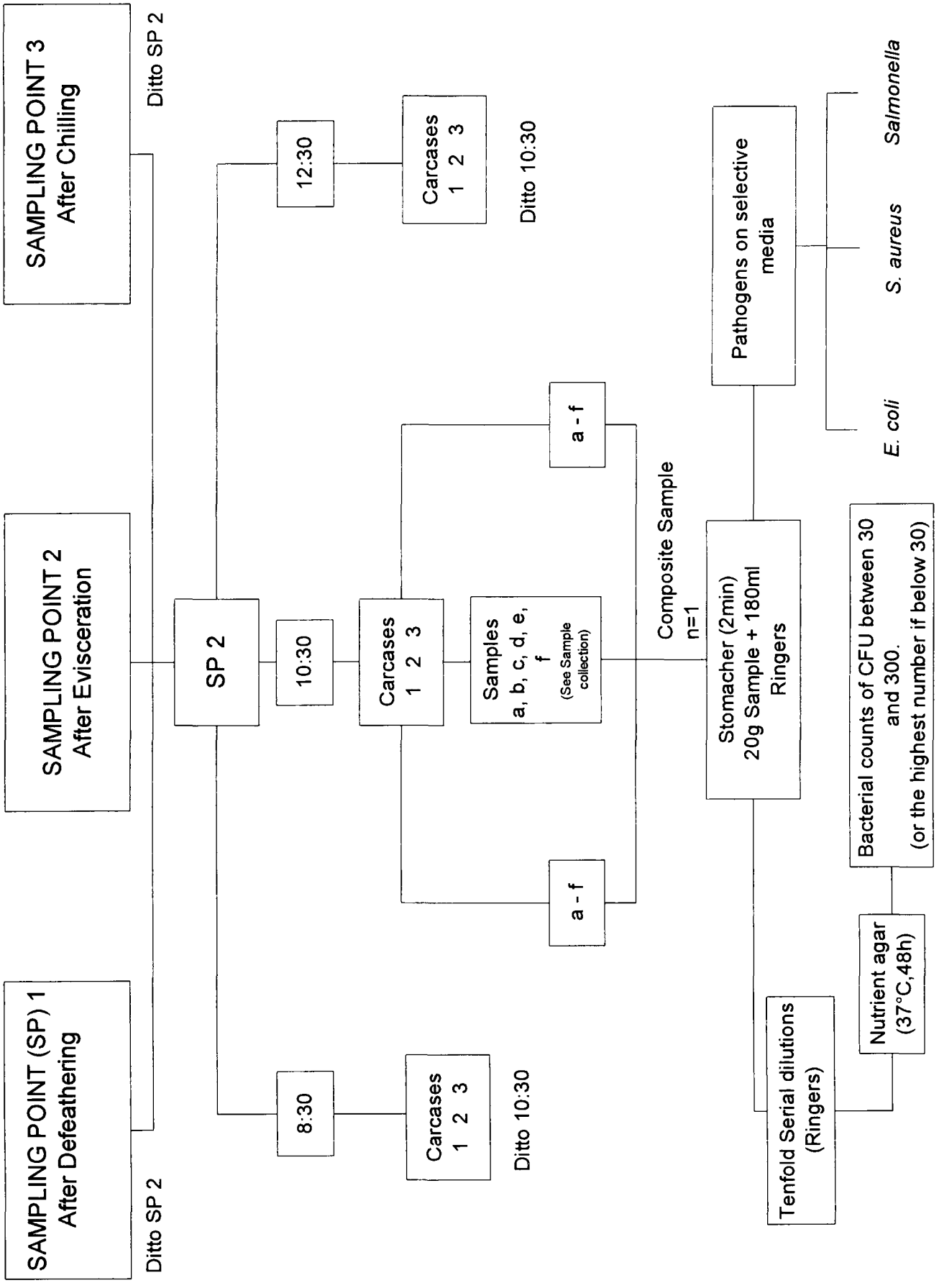

Fig. 3.2 Flow diagram of methods used for the sample collection and preparation in the survey.

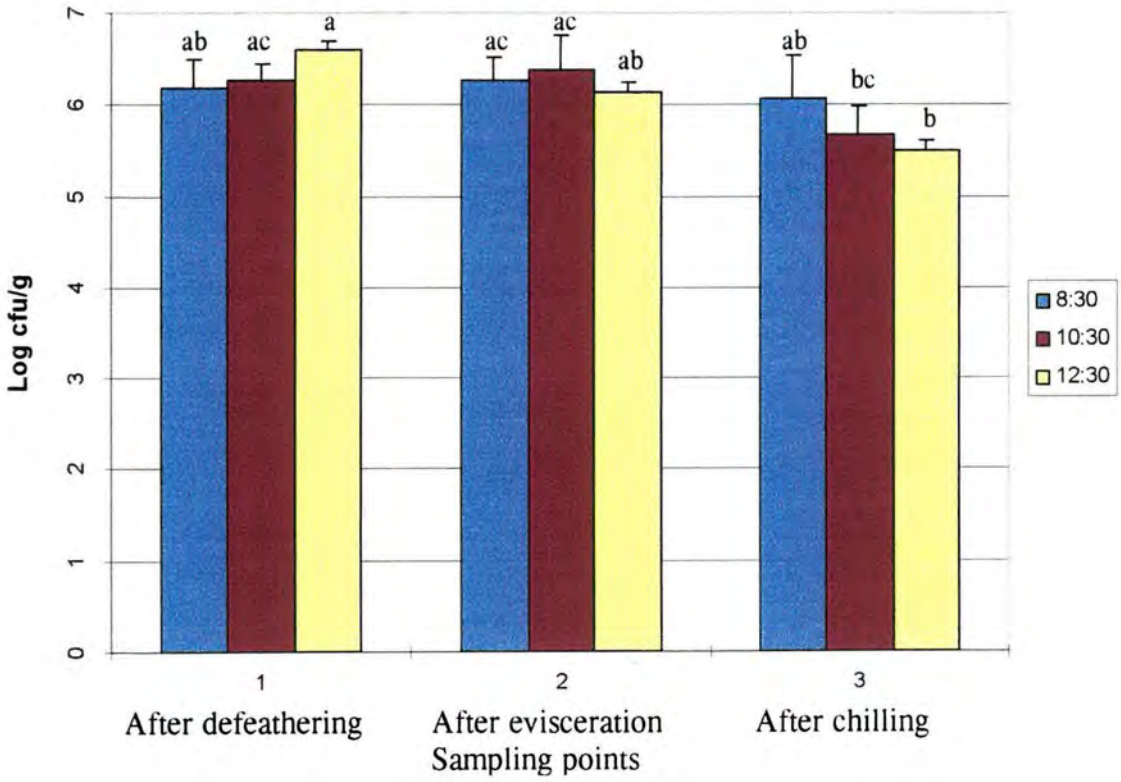

Fig. 3.3 Bacterial counts of the three different sampling points with regard to time of sampling. Means with different superscripts indicate statistically significant differences ($P<0.05$) by ANOVA

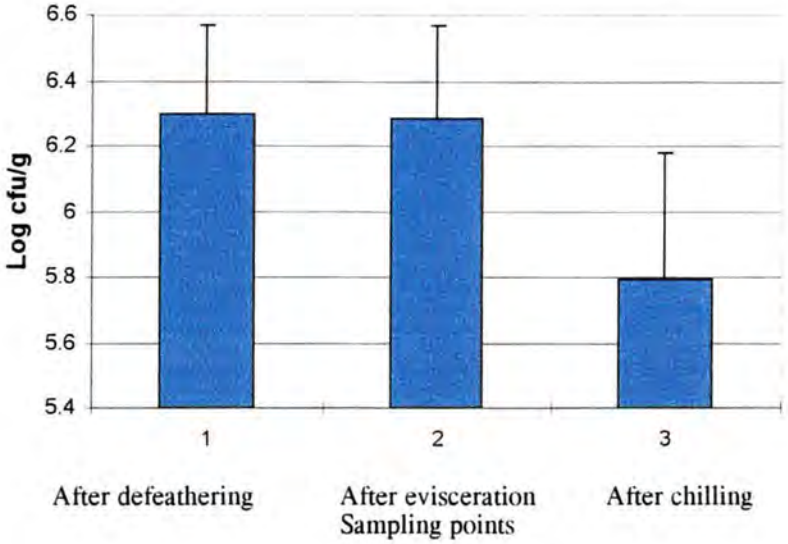

Fig. 3.4 Bacterial counts of chicken carcases sampled at the different sampling points over the four days of sampling.

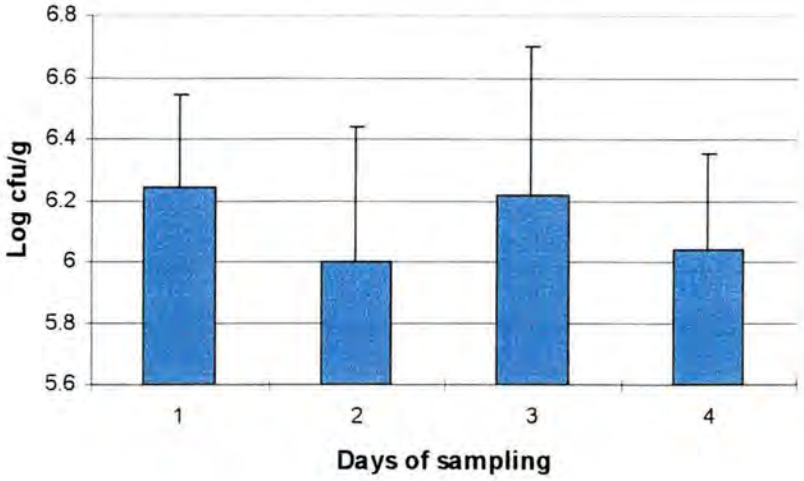

Fig.3.5 Bacterial counts of chicken carcases sampled over the four different days of sampling.

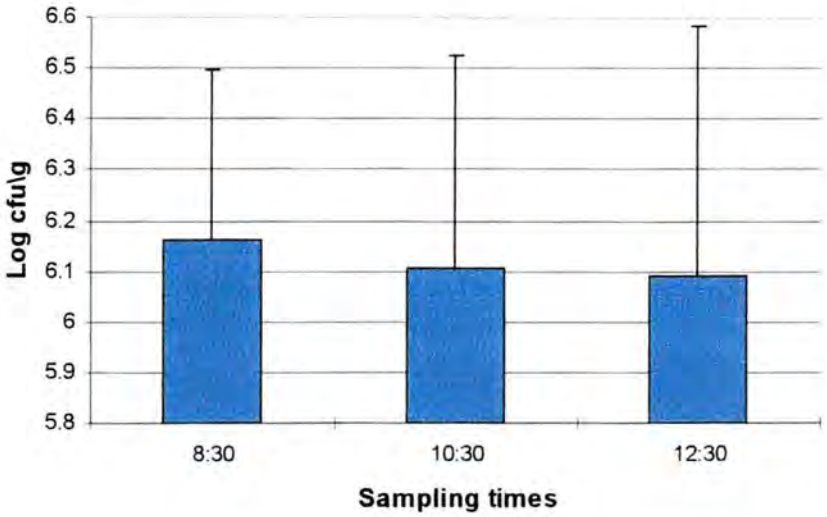

Fig. 3.6 Bacterial counts of the chicken carcases sampled at the three different sampling times over the four days of sampling.

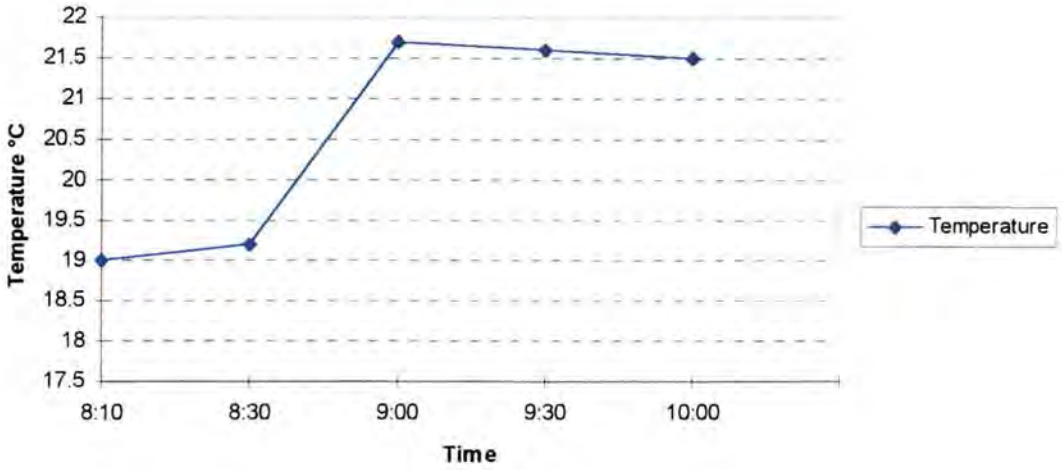

Fig.3.7 Deep breast temperature of randomly selected chicken carcases sampled after chilling during processing in the abattoir.

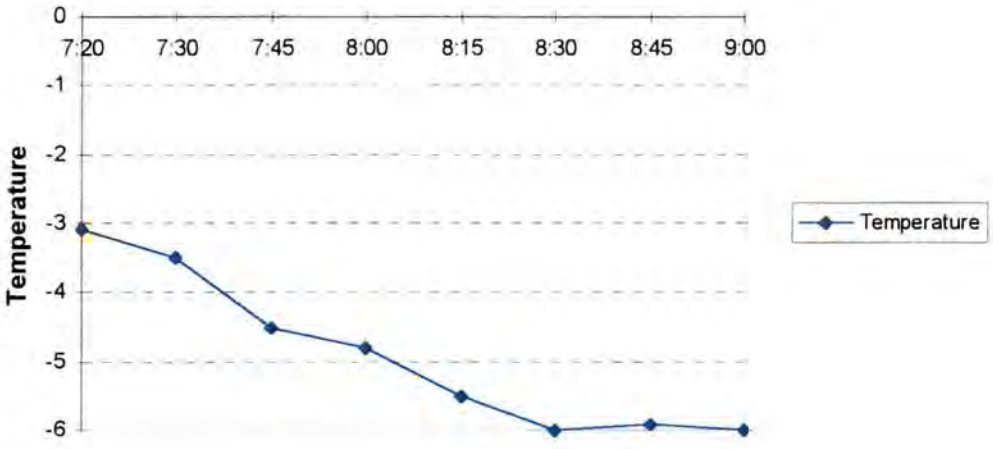

Fig. 3.8 Temperature in the air-chiller versus time monitored during a slaughtering day.

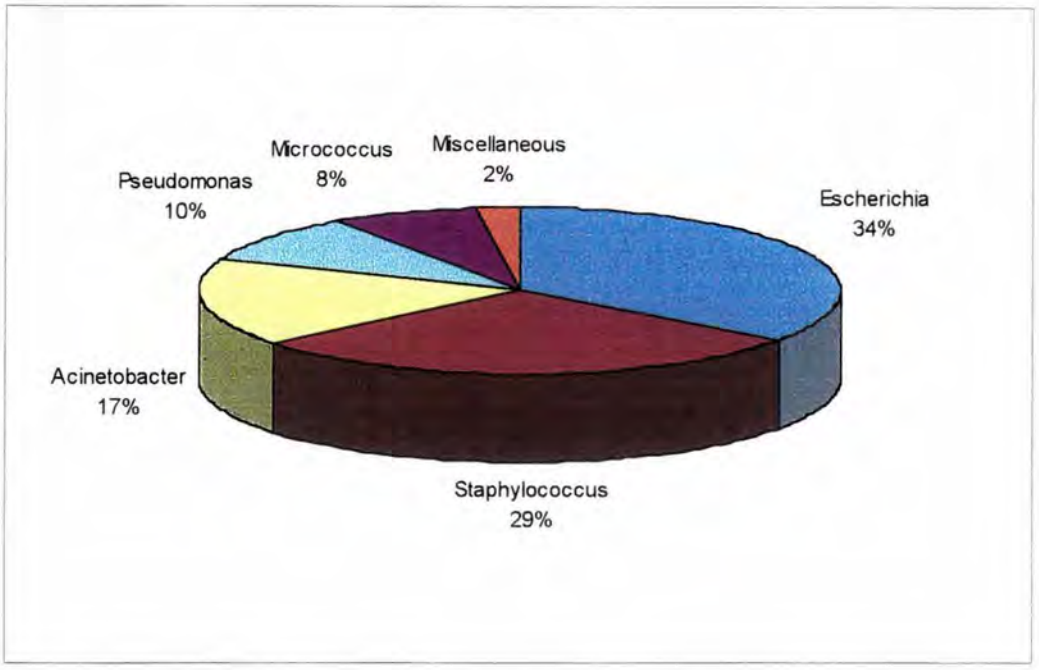

Fig. 3.9 Characterisation of 153 bacteria isolated from chicken carcases sampled after defeathering.

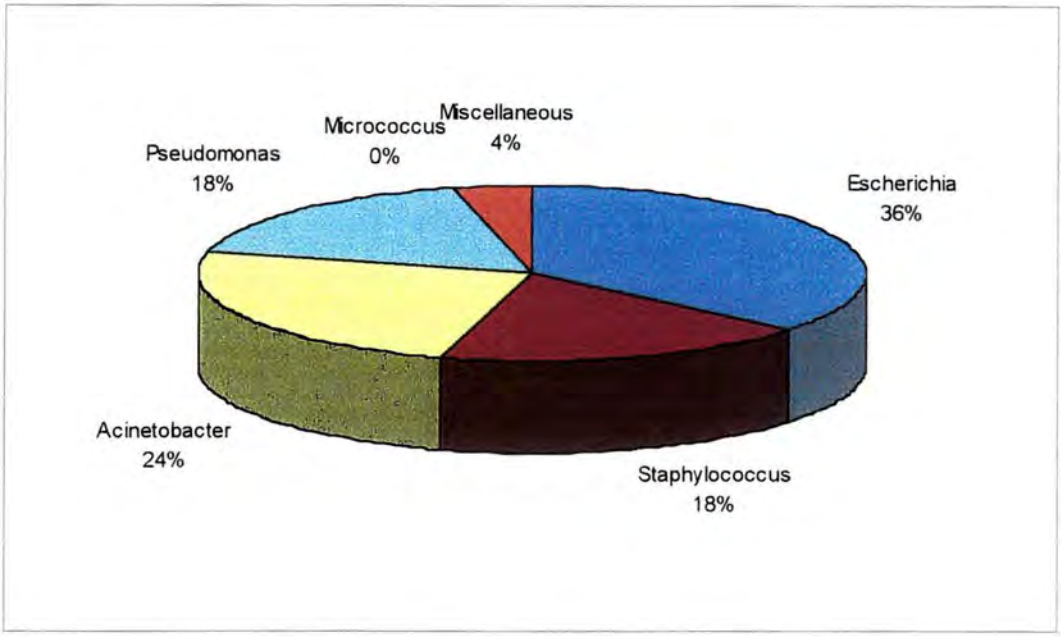

Fig. 3.10 Characterisation of 139 bacteria isolated from chicken carcases sampled after evisceration.

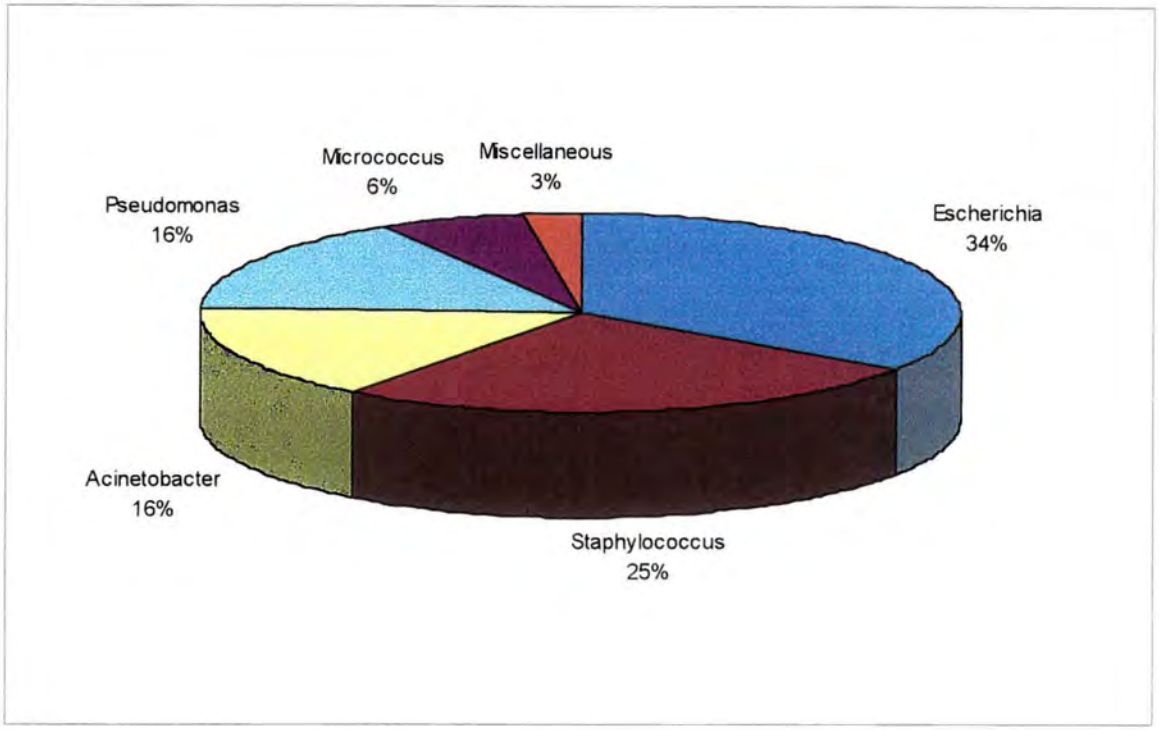

Fig. 3.11 Characterisation of 58 bacteria isolated from chicken carcases sampled after chilling.

CHAPTER FOUR

GENERAL CONCLUSIONS

This study indicated that microbiological contamination was a major problem in this abattoir and also indicated that the process in the abattoir lead to an build-up of contamination through the day. In the abattoir studied the water in the scald tank was never replaced during the processing day. It can be assumed that a build-up of bacteria in the scald tank occurred due to soil, dust, bacteria on the skin, feathers and in the faeces of the birds as they were moved through the scald tank. Further evidence to support this apparent contribution of contamination of the scald tank water was the high counts of Gram negative and specific the *Escherichia* counts that were isolated at this sampling point. The literature (Bryan, 1980; Grau, 1986) indicated that the outside of the chickens (feathers and skin) and also the faeces of the chickens are contaminated with Enterobacteriaceae. In further studies it is recommended that the water of the scald tank must also be monitored for the level of contamination. It is also recommended that the water in the scald tank in this abattoir must be replaced continuously throughout the day and also the temperature of the water should be monitored. The literature shows that control of microbial levels in the scald tank depends upon water usage and temperature. The survival of Enterobacteriaceae, salmonellae and aerobic mesophiles is higher at the low scald temperatures (53%) (Bryan, 1980; Grau, 1986; Mead, 1989). Monitoring the temperature of the scald tank water was also never noted during the study. The installation of a counter-current system when scalding to ensure that the carcases have contact with the cleanest water when exiting the scald tank will decrease the build-up of contamination on carcases. The continuous overflow of contaminated scald water and the simultaneous introduction of fresh water will remove contaminants from the scald tank water and will prevent excessive accumulation of bacteria in the scald tank, plus the destruction of some bacteria by the heat process.

The considerable scattering of feathers and therefore bacteria in the vacinity of the defeathering machine leads to further contamination and also cross-contamination, due to excessive contact between the carcases in the defeathering machine. With the passage of the warm, moist carcases through the defeathering machine, bacteria colonise the rubber "fingers". These rubber "fingers" are difficult to clean and bacteria persist on them, leading to further contamination. The literature also showed (Gibbs *et al.*, 1978; Grau, 1986) that up to 10^6 *Staphylococcus* per cm^2 can be found on the rubber "fingers" of the defeathering machine and can be transferred to the carcases. Although, not investigated in this study, this could be a

possible source of the high *Staphylococcus* (28.8%) counts obtained at this stage in the processing of the carcases. The problem is compounded by the lack of machine maintenance and failure to replace the "fingers" when they harden and crack with excessive wear and tear. Normally defeathering takes place through a series of in-line mechanical defeathering machines to efficiently remove all the feathers from the carcases. In the abattoir studied there was only one defeathering machine. This single machine is not automatically adjustable and therefore not capable of removing all the feathers on the carcases, leaving many feathers on the carcases. Pin feathering of the remaining feathers was done by workers' hands. This could also lead to an additional source of cross-contamination, because of poor personal hygiene of the workers. During the study it was never observed that the workers washed their hands during the processing day which could lead to tremendous cross-contamination of carcases and could also be a possible source of the high *Staphylococcus* obtained at this stage of processing. It is also recommended that in a future study, the workers' hands should be tested for *Staphylococcus* counts to establish the extent of contamination from the workers. The installation of more defeathering machines will also lead to a decrease in bacterial contamination, because pin-feathering and hence excessive manual handling of the carcases will be reduced.

After evisceration the highest bacterial numbers were obtained at sampling time 8:30 and 10:30 (6.3 - 6.4 log CFU g^{-1}) than at any of the other two sampling points at the same time of sampling. The highest number of Gram negative (77.7%) bacteria was also isolated at this sampling point. These results agreed with findings of Mead (1982), Grau (1986) and Bailey *et al.*, (1987) who reported that the numbers of Enterobacteriaceae increased during evisceration. The high number of Gram negative bacteria and in particular *Escherichia* (36.6%) was not unexpected since rupturing of the viscera during this stage of processing was frequently observed in this abattoir, resulting in the spillage of faecal material over the carcases and leading to contamination of the carcases and equipment. The hand evisceration technique practised in this abattoir, and infrequently hand washing can also result in the build-up of contamination at this stage. During this study it was also noted that the evisceration equipment (evisceration spoons) was not sterilised as prescribed in South African legislation for sterilising evisceration equipment. The evisceration equipment was only dipped in cold water without any detergent, adding to more contamination of carcases. Excessive rubbing of the carcases against the taps of the evisceration trough and against the evisceration trough itself, was noted in the

abattoir and this could also lead to further build-up of contaminants. In a future study it is also recommended that the evisceration trough should be tested for the type and percentage of faecal contamination. It can be concluded that the above mentioned practises reinforced the results of the high bacterial counts of Gram negative (77.7%) bacteria obtained at this stage.

The percentage prevalence of *Pseudomonas* (17.6%) was also the highest at this sampling point. Although not tested in this study the source of *Pseudomonas* was speculated to be the processing water, because *Pseudomonas* is a common environmental bacteria. *Pseudomonas* can multiply on the wet soiled surfaces of equipment and the evisceration trough itself and this could also lead to the contamination of the carcases with *Pseudomonas*. Mead 1989, also reported that the *Pseudomonas* counts increased in numbers during evisceration.

At the third sampling point high levels of Gram negative bacteria (65.5%) were also isolated. These high numbers were most likely due to a carry over of bacteria from evisceration to chilling as a result of insufficient spray washing of the carcases after evisceration and before chilling. In the abattoir studied there was only one place after evisceration where spray washing took place and this was not done with high-pressure jets to increase the efficiency of washing. In-plant chlorination of the water used for washing of carcases could also lead to a decrease in bacterial contamination of carcases. The literature shows that chlorination of spray water at a level of 40 to 60ppm can lead to reduction of bacterial loads (Bryan, 1980; Bailey et al., 1987). No in-plant chlorination of water was noted during the duration of the study. In-plant chlorination may also reduce microbial contamination of working surfaces and equipment in the processing plant, thus facilitating plant cleaning and disinfection at the end of the working day. It is also recommended that the dwell time of the carcases in the air-chiller should be monitored to ensure that the deep breast temperature of carcases reached 10°C or less as prescribed (SAPA). The temperature in the air-chiller should also be monitored to ensure that the keel temperature of carcases could be reached and consequently inhibit the growth of spoilage bacteria.

Results from this study revealed the problem areas in the abattoir under study eg. scalding (although not tested), defeathering, evisceration and chilling. It is also advisable that in a further study the effect of packaging on the microbial load of the carcases should be establish to

determine the extent of contamination at that stage of processing. Since the study indicated the possibility of contamination of the product by abattoir equipment (eg. defeathering machine, evisceration implements) and abattoir surfaces (eg. evisceration troughs, taps of evisceration troughs), a re-evaluation of existing daily cleaning and disinfection regimes of these equipment and surfaces should be considered to eliminate or decrease microbial contamination

Out of the above problems encountered in the abattoir studied, it can be concluded that a system is necessary to control and eliminate all these problems to ensure that a safe product could be delivered. With the implementation of the HACCP system in the abattoir all the processing operations in the abattoir can be monitored step by step and improved to achieve the acceptable microbiological standards. To implement the HACCP system seven basic principles must be taken into account:

- Conduct a hazard analysis. Prepare a flow diagram of the steps in the process. Identify and list the hazards and specify the control measures.
- Identify the critical control points (CCP) in the process.
- Establish target levels and tolerance which must be met to ensure each CCP is under control.
- Establish a monitoring system to ensure control of the CCP by scheduled testing or observations.
- Establish the corrective action to be taken when monitoring indicates that a particular CCP is moving out of control..
- Establish effective record-keeping procedures that document the HACCP system.
- Establish verification procedures which include appropriate supplementary tests to confirm that HACCP is working effectively.

In the HACCP flow diagram all the steps in the processing of chicken carcases in this abattoir must be identified. The steps can be divided into the three different process areas or sections in this Grade B poultry abattoir. At each step in the process in the abattoir, all the microbiological hazards must be identified eg. workers' hands, equipment and tool surfaces, the environment (water, ice, air, litter) and the product itself. All the microbiological monitoring sites must also be identified at each step in the process. The microbiological hazards in the abattoir can be rated as critical, significant, important, potentially important and unimportant according to the hazards' influence on the safety of the product. Between each sampling point there are also

factors (eg. head pulling and trachea, hock cutting, washing, pin feathering, open body cavity, neck removal, drawing the viscera, manual sizing, portioning etc.) involved which could be a hazard to the product and these factors should also been taken in consideration and should be monitored and controlled. With the suggestions been made to improve the product and decrease contamination at each sampling point and with the implementation of HACCP, the acceptable microbiological standards should be achievable.

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