ANOLYTE AND CATHOLYTE AS DISINFECTANTS IN A POULTRY PROCESSING PLANT

Prof T E Cloete, Dept of Microbiology and Plant Pathology, University of Pretoria

1. 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).

2. LITERATURE REVIEW

2.1 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).

2.2 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 Georgnaras *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°C) 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).

2.3 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 days 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).

2.4 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).

2.5 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).

2.6 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).

2.7 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).

Table 1.1 Micro-organisms associated with poultry.

Pathogens	**Spoilage Bacteria**
Salmonella	*Acinetobacter*
Clostridium perfringens	*Shewanella*
Staphylococcus aureus	*Pseudomonas* spp.
Yersinia	*Flavobacterium*
Campylobacter	*Moraxella*
Escherichia coli	*Aeromonas*
Listeria monocytogenes	*Enterobacter* spp.
	Corynebacteria
	Micrococcus

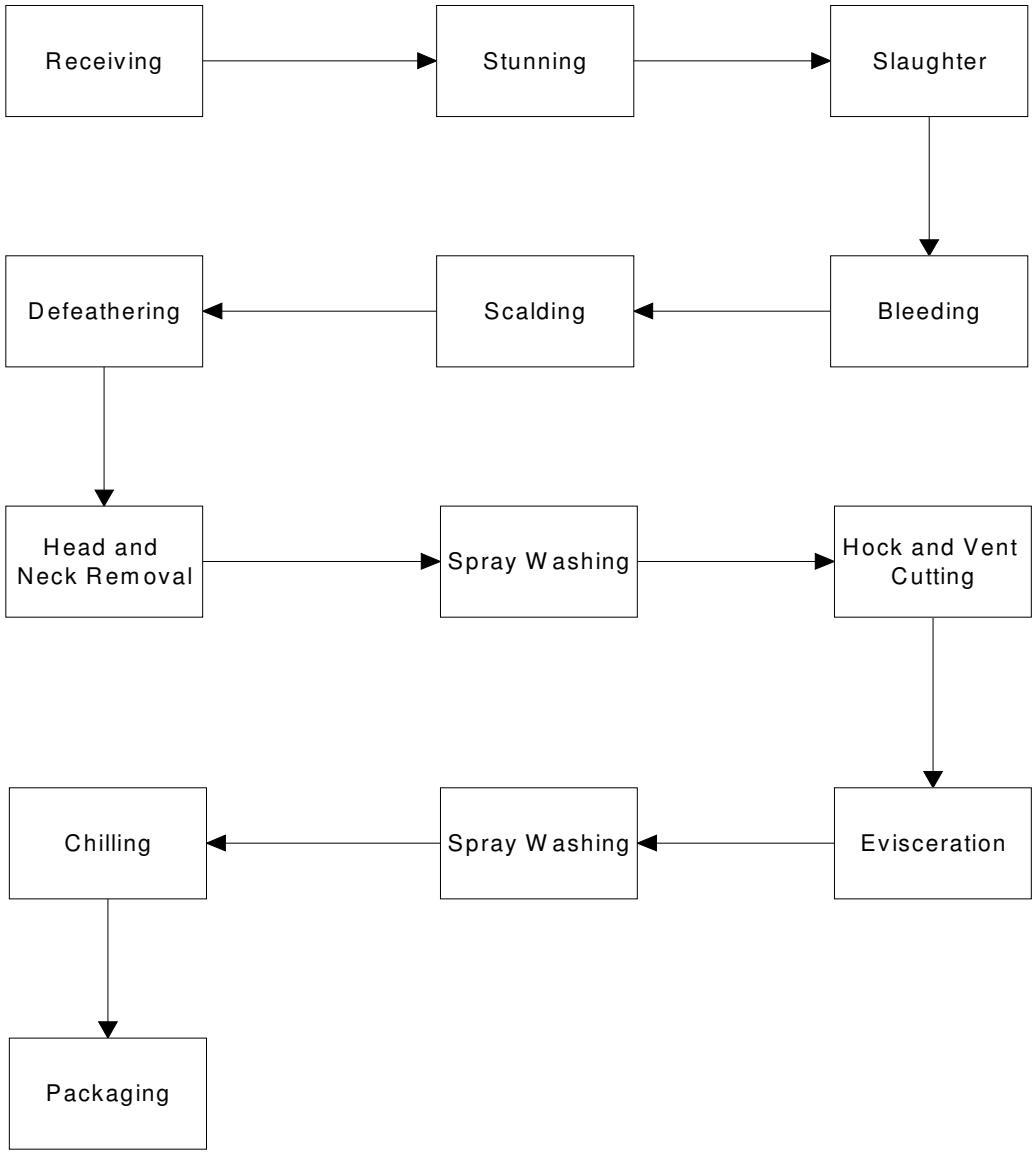

Fig. 1.1. Flowsheet of stages in a typical poultry processing plant.

3. MATERIALS AND METHODS

3.1 Disinfectant solutions

3.1.1 Laboratory evaluation (Anolyte)
Anolyte for minimum inhibitory concentration (MIC) determinations under laboratory conditions was supplied by Radical Waters. The MIC was determined on the same day the Anolyte solution was obtained.

3.1.2 Poultry Plant evaluation (Anolyte)
Fresh Anolyte solution was prepared on the day of evaluation for a particular application.

3.1.3 Catholyte evaluation during scalding
Catholyte was supplied by Radical Waters, for evaluation during scalding.

NOTE:0,01 % Sodium thioglycolate was added to all test solutions to neutralize the anolyte, before conducting microbiological analysis.

3.2 **Applications, sample collection and preparation**

3.2.1 Applications and sample collection

- Pre-evisceration spray application: During this application, chicken carcases were sprayed with 100 ml of a 1:10 Anolyte solution.
- Post-evisceration spray application: During this application, chicken carcases were sprayed with 1000 ml of a 1:10 Anolyte solution.
- Spinchiller application: Chicken carcases were submerged in a 1:10 Anolyte solution at 10°C for 30 min.
- Catholyte scalding application: Feathered chickens were submerged in a 100 % Catholyte solution for 7 min at a temperature of 54°C $\pm$ 2°C.

For each of the above applications, control samples of chickens were randomly taken off the processing line.

3.2.2 Sample preparation

A composite meat and skin sample was prepared, by taking samples from the neck, back between the wings and under the wing 5g of sample was added to 45 ml of sterile Ringers solution to give a 1:10 dilution. From this a dilution series (10^{-1} - 10^{-6}) was prepared for plating.

3.3 **Microbiological analysis**

This composite sample was placed into quarter strength Ringers solution in a 1+9 mass/volume ratio based on exact mass. Each composite sample was homogenised for 20 min (Seward Medical 400 Stomacher Lab Blender). Tenfold serial dilution's in Ringers solution were plated out in duplicate on Nutrient Agar for the total bacteria count and on McConkey agar for the coliform bacteria count, 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.

4. RESULTS

Table 1 Microbiological quality of chicken carcass sprayed with 100 ml of anolyte immediately after defeathering, compared to the standard practise

Anolyte	Total bacteria cfu/g		
1.	200 000	-	200 000
2.	60 000	80 000	70 000
3.	16 200	10 600	13 400
4.	10 800	7 600	9 200
5.	240 000	360 000	300 000
6.	220 000	130 000	17 5000
7.	20 000	15 000	17 500
8.		1 400	1 400
9.		820 000	820 000
10.	220 000	190 000	205 000
		x	$1{,}81 \times 10^5$
		Range	$1{,}4 \times 10^3$ - $8{,}2 \times 10^5$

Control	Total bacteria cfu/g		
1.	590 000	800 000	695 000
2.	580 000	600 000	590 000
3.	90 000	150 000	120 000
4.	160 000	180 000	170 000
5.	230 000	260 000	245 000
6.	30 000	46 000	38 000
7.	38 600	54 600	46 600
8.	990 000	1150 000	1070 000
9.			
10.	700 000	50 000	375 000
		x	$3{,}7 \times 10^5$
		Range	$3{,}8 \times 10^4$ - $1{,}07 \times 10^6$

Spraying the chickens with anolyte immediately after defeathering, resulted in a 1 log difference in the bacterial number on average compared to the standard practise. This was considered a significant difference.

Anolyte	Coliform bacteria cfu/g		
1.	11 100	9 300	10 200
2.	7 800	8 300	8 050
3.	30 500	30 000	32 500
4.	130 000	17 000	73 500
5.	12 500	12 200	12 350
6.	100 000	130 000	115 000
7.	7 100	6 300	6 700
8.	18 000	14 500	16 250
9.	840 000	770 000	805 000
10.	20 000	ND	20 000
		x	1.09×10^5
		Range	$8{,}05 \times 10^3$ - $8{,}05 \times 10^5$

Control	Coliform bacteria cfu/g		
1.	590 000	800 000	695 000
2.	11 900	8 900	10 400
3.	110 000	90 000	100 000
4.	40 600	38 900	39 750
5.	16 200	17 800	17 000
6.	-	-	-
7.	90 000	280 000	185 000
8.	-	-	-
9.	10 200	11 000	10 600
10.	-	180 000	180 000
		x	$1{,}54 \times 10^5$
		s	$1{,}0 \times 10^4$ - $6{,}95 \times 10^5$

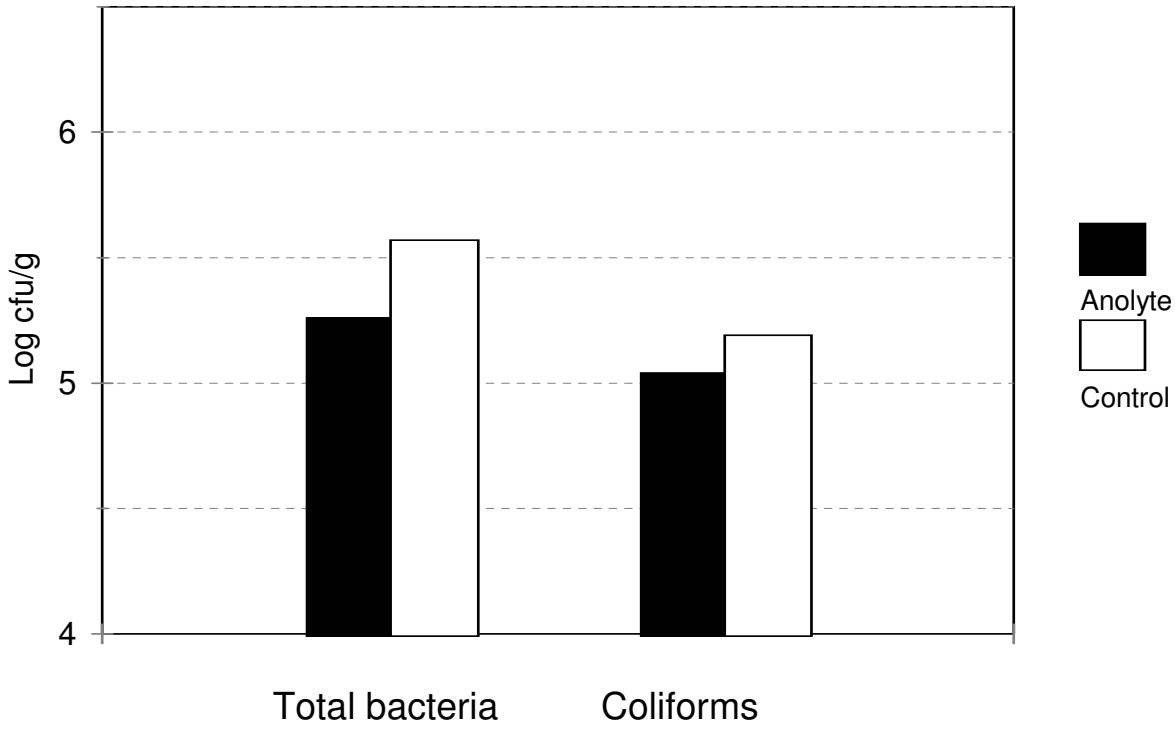

Figure 1 Microbiological quality of chicken carcass sprayed with 100 ml of anolyte immediately after defeathering, compared to the standard practise

No significant difference in the number coliform bacteria was observed between the anolyte spray and the control (Figure 1).

Table 2 Microbiological quality of chicken carcases using anolyte in a simulated spinchiller application compared to the standard practise

Anolyte	Total bacteria cfu/g		
1.	250 000	340 000	295 000
2.	440 000	460 000	450 000
3.	140 000	-	140 000
4.	70 000	-	70 000
5.	16 200	-	16 200
6.	60 000	-	60 000
7.	20 800	18 200	19 500
8.	10 400	14 500	12 450
		x	$1{,}32 \times 10^5$
		Range	$1{,}24 \times 10^4$ - $4{,}5 \times 10^5$

Control	Total bacteria cfu/g		
1.	15 500	-	15 500
2.	490 000	-	490 000
3.	23 800	-	23 800
4.	14 500	-	10 750
5.	8 000	7 000	7 500
6.	13 300	8 300	10 800
7.	9 900	-	9 600
8.	27 500	9 300	18 400
9.	5 100	19 300	12 200
		x	$6{,}66 \times 10^4$
		Range	$9{,}6 \times 10^3$ - $4{,}9 \times 10^5$

During the simulated spinchiller application, the average total number of bacteria in the control was lower than the anolyte treatment. The anolyte treatment was nevertheless within the same range and hence not considered significantly different.

Anolyte	Coliform bacteria cfu/g		
1.	2 000	2 000	2 000
2.	6 500	ND	6 500
3.	5 100	4 000	4 550
4.	ND	8 000	8 000
5.	ND	6 000	6 000
6.	9 600	8 800	9 200
		X	$6{,}04 \times 10^3$
		Range	$2{,}5 \times 10^3$ - $8{,}0 \times 10^3$

Control	Coliform bacteria cfu/g		
1.	ND	5 800	5 800
2.	-	4 200	4 200
3.	19 100	14 900	17 000
4.	11 600	-	11 600
5.	8 000	6 900	7 450
6.	4 500	7 500	6 000
7.	4 700	2 700	3 700
		x	$7{,}96 \times 10^3$
		Range	$3{,}7 \times 10^3$ - $1{,}1 \times 10^4$

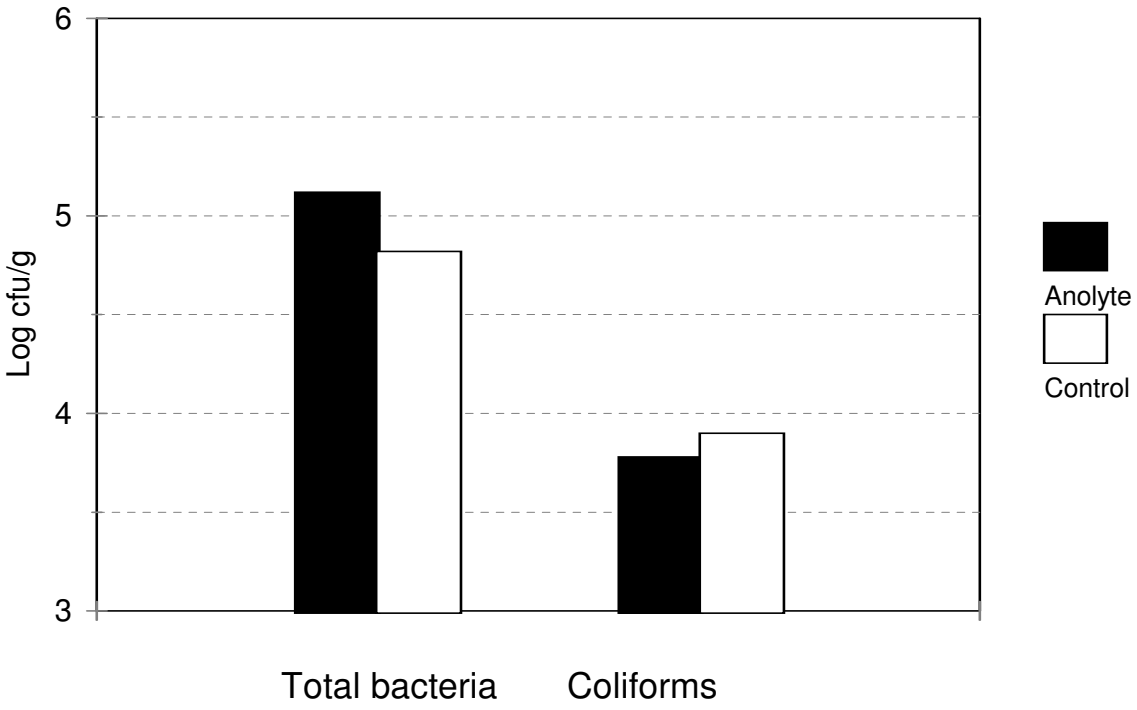

Figure 2 Microbiological quality of chicken carcases using anolyte in a simulated spinchiller application compared to the standard practise

As with the total bacteria count, no significant difference was noted between the anolyte treatment and control (Figure 2).

Table 3 Microbiologial quality of chicken carcases sprayed with 1 000ml of anolyte, compared to the standard practise

Anolyte	Total bacteria cfu/g		
1.	28 400	ND	28 400
2.	300 000	130 000	215 000
3.	670 000	980 000	825 000
4.	920 000	1000 000	960 000
5.	39 000	49 800	44 400
6.	19 900	12 100	16 000
7.	840 000	910 000	875 000
8.	37 500	42 800	40 150
9.	24 600	36 200	30 400
10.	77 000	42 000	59 500
		x	$3{,}09 \times 10^5$
		Range	$2{,}84 \times 10^4$ - $9{,}6 \times 10^5$

Control	Total bacteria cfu/ml		
1.	43 600	ND	43 600
2.	17 000	19 100	18 050
3.	39 000	25 600	32 300
4.	110 000	20 000	65 000
5.	270 000	280 000	275 000
6.	29 600	300 000	164 800
7.	2600 000	270 000	1435 000
8.	980 000	ND	980 000
9.	110 000	270 000	190 000
10.	24 000	27 300	25 650
		x	$3{,}22 \times 10^5$
		Range	$1{,}80 \times 10^4$ - 1.4×10^6

Anolyte	Coliform bacteria cfu/ml		
1.	2 500	2 200	2 350
2.		72 000	72 000
3.	22 000	26 000	24 000
4.	4 000	4 200	4 100
5.	7 100	8 400	7 750
6.	1 600	3 300	2 450
7.	41 000	39 400	40 200
8.	22 100	17 900	20 000
9.	11 600	16 200	13 900
10.	14 300	12 700	13 500
		x	$2{,}00 \times 10^4$
		Range	$4{,}1 \times 10^3$ - $7{,}0 \times 10^4$

Control	Coliform bacteria cfu/ml		
1.	16 600	19 600	18 100
2.	7 000	1 900	4 450
3.	11 200	8 800	10 000
4.	6 200	6 100	6 150
5.	18 600	ND	18 600
6.	8 600	18 800	13 700
7.	25 500	20 300	22 900
8.	240 000	15 000	127 500
9.	11 700	12 000	11 850
10.	12 300	ND	12 300
		x	$2{,}45 \times 10^4$
		Range	$4{,}45 \times 10^3$ - $1{,}95 \times 10^5$

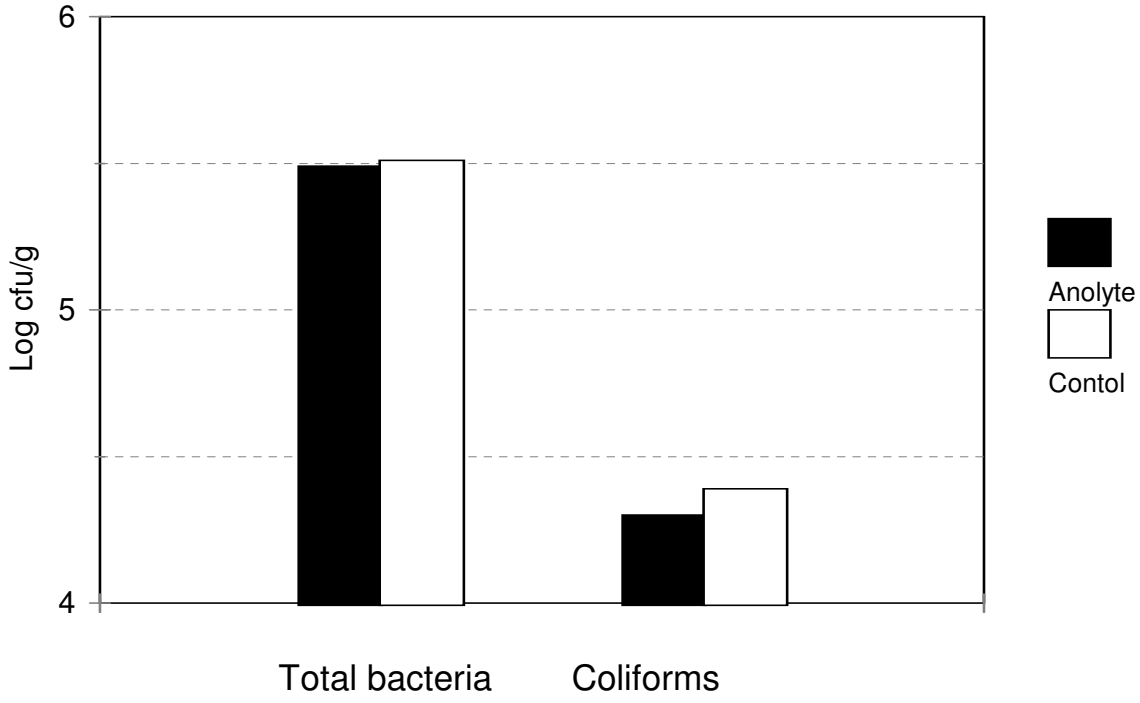

Figure 3 Microbiologial quality of chicken carcases sprayed with 1 000ml of anolyte, compared to the standard practise

Neither the total bacteria number, or the coliform bacteria number differed significantly between the anolyte treatment and the control (Figure 3).

Table 4 Microbiological quality of chicken skin after scalding at 54°C in catholyte solution, compared to the standard practice

Catholyte	Total bacteria cfu/g		
1.	200 000	150 000	175 000
2.	690 000	590 000	640 000
3.	460 000	580 000	520 000
		x	$4{,}45 \times 10^5$
		Range	$1{,}75 \times 10^5$ - $6{,}4 \times 10^5$

Control	Total bacteria cfu/g		
1.	960 000	1280 000	1120 000
2.	350 000	220 000	285 000
3.	590 000	-	590 000
4.	240 000	-	240 000
5.	800 000	630 000	715 000
		x	$5{,}9 \times 10^5$
		Range	$2{,}4 \times 10^5$ - $1{,}2 \times 10^6$

Catholyte	Coliform bacteria cfu/g		
1.	700 000	920 000	810 000
2.	9 700	910 500	460 100
3.	150 000	180 000	165 000
4.	15 000	90 000	52 500
		x	$3{,}7 \times 10^5$
		Range	$5{,}3 \times 10^4$ - $8{,}1 \times 10^5$

Control	Coliform bacteria cfu/g		
1.	260 000	230 000	245 000
2.	510 000	760 000	635 000
3.	6 000	5 900	5 950
4.	20 000	40 000	30 000
5.	3 600	7 000	5 300
		x	$1{,}84 \times 10^5$
		Range	$5{,}3 \times 10^3$ - $6{,}35 \times 10^5$

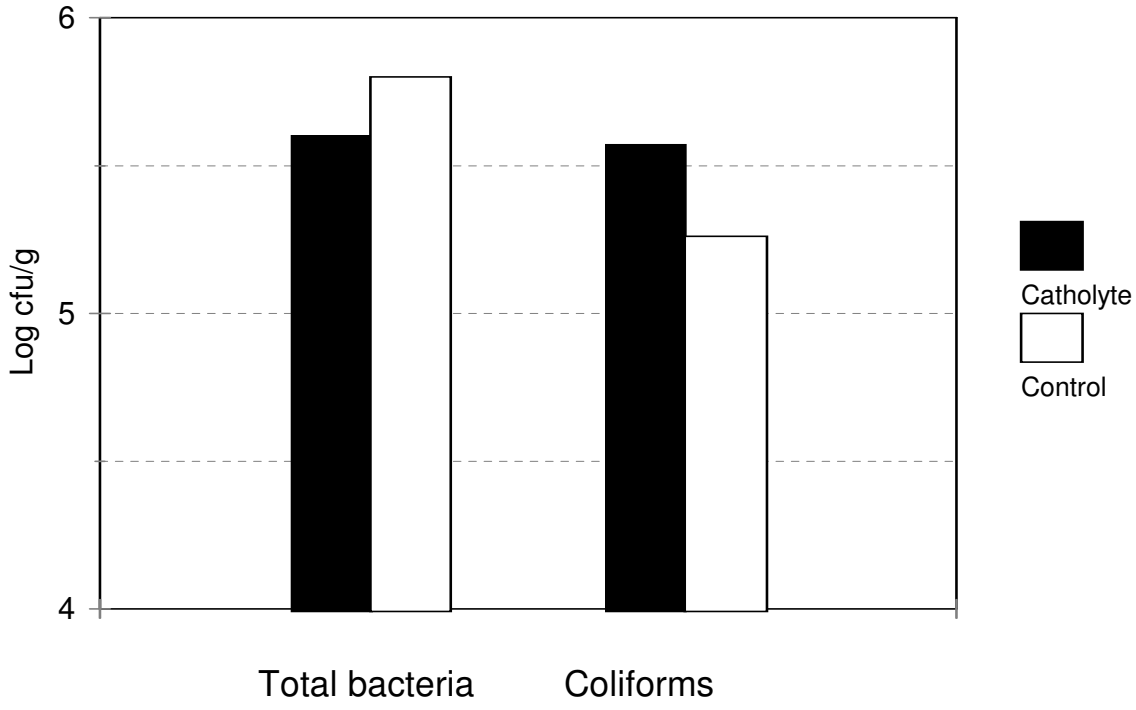

Figure 4 Microbiological quality of chicken skin after scalding at 54°C in catholyte solution, compared to the standard practice

Both the total bacteria and coliform bacteria numbers on the skin of chicken were concluded, that no significant difference existed between the two treatments (Figure 4).

Table 5 Microbiological quality of feathers after scalding at 52°C in catholyte compared to the standard practice

Catholyte	Total bacteria cfu/g		
1.	60 000	80 000	70 000
2.	1140 000	320 000	730 000
3.	1500 000	1500 000	1500 000
4.	15 000	20 000	17 500
		x	$5{,}79 \times 10^5$
		Range	$1{,}75 \times 10^4$ - $1{,}5 \times 10^6$

Control	Total bacteria cfu/g		
1.	500 000	340 000	420 000
2.	330 000	780 000	550 000
3.	270 000	500 000	385 000
		x	$4{,}5 \times 10^5$
		Range	$4{,}2 \times 10^5$ - $3{,}85 \times 10^5$

The average number of bacteria for the catholyte treatment compared to the control was exactly the same. The range was also similar for the two treatments.

Catholyte	Coliform bacteria cfu/g		
1.	<10	<10	<10
2.	<10	<10	<10
3.	<10	<10	<10
4.	<10	<10	<10
5.	<10	<10	<10
		x	<10
		Range	<10

Control	Coliform bacteria cfu/g		
1.	15 000	15 000	15 000
2.	7 200	10 200	8 700
3.	8 900	13 400	11 150
4.	6 100	15 200	10 650
5.	15 000	60 000	37 500
		x	$1{,}66 \times 10^4$
		Range	$8{,}7 \times 10^3$ - $3{,}75 \times 10^4$

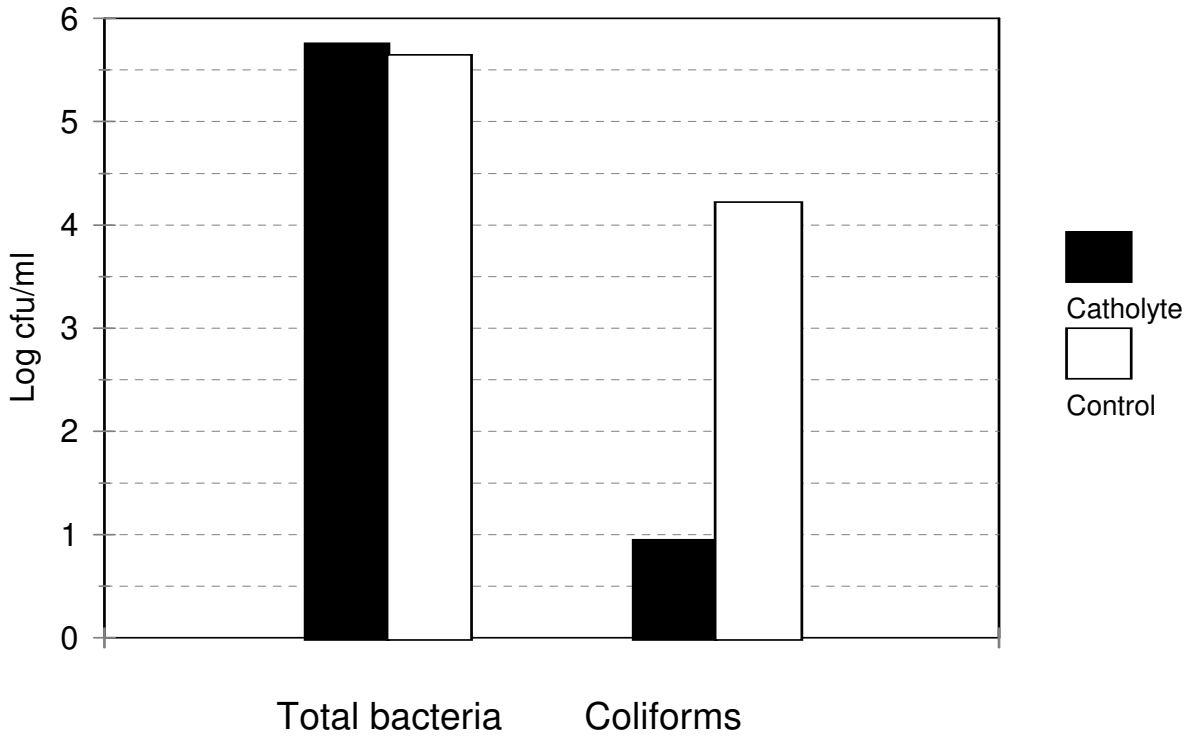

Figure 5 Microbiological quality of feathers after scalding at 52°C in catholyte compared to the standard practice

No coliform bacteria were detected on the feathers, treated with the catholyte, in contrast to the control. This suggested, that the catholyte, either despersed all the coliforms, or killed all the coliforms on the feathers (Figure 5).

5. DISCUSSION

During the laboratory evaluation of the anolyte solution, a 1:10 dilution gave the best result, using *E.coli* as test organism. The 1:20 and lower dilutions, did not result in a significant decrease of *E.coli,* using ca. 10^6 organisms as a challenge. Hence, it was decided to use the 1:10 dilution in our evaluation.

The anolyte spray compared very favourably with the current practise in all applications. The most promising result was obtained immediately after defeathering, where the anolyte resulted in a significant decrease in the bacterial numbers compared to the control. It is highly recommended that this practise be followed, since it will reduce the bacterial numbers right at the outset and very early on during processing.

Both the post-eviration spray application and the spinchiller application compared favorably with the current practice.

Catholyte used during the scalding application, did not result in an improved result, with regards to the bacterial numbers on the skin, nor on the feathers. It should also be noted, that should the catholyte disperse the bacteria during scalding, without also killing the bacteria, it might result in a higher level of contamination, because bacteria attached to the feather will be dispersed, which may not be case with the current practise.

6. CONCLUSIONS

It was concluded, that the anolyte solution (1:10 dilution) could replace the current practise and especially if it is introduced directly after defeathering it could have major benefits.

The use of catholyte during the scalding application is not recommended.

7. RECOMMENDATIONS

1. The cost-effectivity of the anolyte solution (1:10 dilution) should be determined. If it is not comparable to the current practise, a comparable dilution of the anolyte should be evaluated.

2. A study should be conducted to determine the consistency of the anolyte product, with respect to its disinfection capability between different batches.

2. The antimicrobial properties of the catholyte solution should be determined.

8. REFERENCES

1. AFMA. 1996 Braaikuikenbedryf in Suid-Afrika. *AFMA MATRIX* **5**(3): 27

2. Anand S.K., Mahapatra C.M., Pandey N.K. and Verma S.S. 1989 Microbial changes on chicken carcasses during processing. *Indian J. Poult. Sci*. **24**(3): 203 - 209

3. Bailey J.S., Thomson J.E. and Cox N.A. 1987 Contamination of poultry during processing. [Edited by Cunningham F.E. and N.A. Cox] In *The microbiology of poultry meat products*. Academic Press, Orlando. 193 - 211.

4. Banwart George J. 1989 Basic Food Microbiology. 2nd ed. Published by Van Nostrand Reinhold.

5. Barnes Ella M., Impey C.S. and Parry R.T. 1973 The Sampling of Chickens, Turkeys, Ducks and Game Birds. [Edited by Board R.G. and D.W. Lovelock] In *Sampling - Microbiological Monitoring of Environments*, Society of Applied Bacteriology Technical Series No.7, Academic Press, London. 63 -73

6. Bok H.E., Holzapfel W.H., Odendaal E.S. and van der Linde H.J. 1986 Incidence of foodborne pathogens on retail broilers. *International Journal of Food Microbiology* **3**: 273 - 285

7. Brenner, D.J. 1984 Family *Enterobacteriaceae*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath PHA. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. I.* Williams and Wilkins, Baltimore, USA. 409 - 498.

8. Brown M.H. and Baird-Parker A.C. 1982 The microbiological examination of meat. [Edited by Brown M.H.] In *Meat Microbiology*. Applied Science Publishers, London. 423 -520

9. Bryan F.L. 1980 Poultry and meat products. [Edited by Silliker J.H., Elliot R.P., Baird-Parker A.C., Bryan F.L., Christian J.H.B., Clark D.S., Olson J.C. and T.A. Roberts] In *Microbial Ecology of Foods, Vol.2: Food Commodities*. (ICMSF) 410 - 458

10. Busta F.F., Peterson E.H., Adams D.M. and Johnson M.G. 1984 Colony counts methods. [Edited by Speck M.L.] In *Compedium of Methods for the Microbiological Examination of Foods*, 2nd ed. American Public Health Association (APHA) Washington, DC. 62 - 83

11. Codex Alimentarius Commission (CAC) 1976 Recommended International Code of Hygiene Practice for Poultry Processing. CAC/RCP 14-1976, 2nd edn.

12. Connor J.T., McMeekin T.A. and Patterson J.T. 1987 Prevention of microbial contamination in the poultry processing plant. [Edited by Smulders F.J.M.] In *Elimination of Pathogenic Organisms from Meat and Poultry*. Proceedings of the International Symposium: Prevention of Contamination, and Decontamination in the Meat Industry. Zeist, The Netherlands, 2 - 4 June 1986. Elsevier Science Publishers. 163 - 175

13. Cunningham F.E. 1987 Types of microorganisms associated with poultry carcasses. [Edited by Cunningham F.E. and N.A. Cox] In *The microbiology of poultry meat products*. Academic Press, Orlando. 29 - 42.

14. Dougherty T.J. 1976 A study of *Salmonalla* contamination in broiler flocks. *Poultry Science* **55**: 1811 - 1815

15. Eley, 1992 Microbial Food Poisoning. Chapman and Hall, London

16. Eustace I.J. 1981 Control of bacterial contamination of meat during processing. *Food Technology in Australia* **33**(1): 28 - 32

17. Evans J.B. 1986 Staphylococci. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.6: Meat and Health*. Elsevier Science Publishers Ltd. 157 - 183

18. Farber J.M. and Idziak E.S. 1984 Attachment of psychrotrophic meat spoilage bacteria to muscle surfaces. *Journal of Food Protection* **47**(2): 92 - 95

19. Firstenberg-Eden R. 1981 Attachment of bacteria to meat surfaces: A review. *Journal*

of Food Protection **44**(8): 602 - 607

20. Genigeorgis C., Hassuneh M. and Collins P. 1986 *Campylobacter jejuni* infection on poultry farms and its effect on poultry meat contamination during slaughter. *Journal of Food Protection* **49**: 895 - 903

21. Geornaras I., Dykes G.A. and von Holy A. 1994 Microbial populations associated with refrigerated poultry. South African Journal of Science **90**: 579 - 582

22. Goernaras I., de Jesus A.E., van Zyl E. and von Holy A. 1996 Bacterial populations associated with poultry processing in a South African abattoir. *Food Microbiology* **13**: 457 - 465

23. Goernaras I., de Jesus A.E., van Zyl E. and von Holy A. 1997 Bacterial populations of different sample types from carcasses in the dirty area of a South African poultry abattoir. *Journal of Food Protection* **60**: 551 - 554

24. Genstat 5 Committee of the Statistics Department. 1990 Rothamsted Experimental Station Genstat 5 Reference Manual, Release 2.2. Clarendon Press, Oxford.

25. Gibbs P.A., Patterson J.T. and Thompson J.K. 1978 The distribution of *Staphylococcus aureus* in a poultry processing plant. *Journal of Applied Bacteriology* **44**: 401 - 410

26. Gill C.O. 1983 Meat spoilage and evaluation of the potential storage life of fresh meat. *Journal of Food Protection* **46**(5): 444 - 452

27. Gill C.O. 1986 The control of microbial spoilage in fresh meats. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.2: Meat and Poultry Microbiology*. Avi Publishing Company, Inc. 49 - 88

28. Government Gazette, No. 11881, May 1989, No. R. 1028. Animal Slaughter, Meat and Animal Products Hygiene Act, 1967 (Act. No. 87 of 1967) - Grades and Requirements for Abattoirs pp. 5 - 9. Republic of South Africa Government Printers.

29. Government Gazette, No. 2540, October 1969, No. R. 3505. Standing Regulations under the Animal Slaughter, Meat and Animal Products Hygiene Act, 1967 (Act No. 87 of 1967) Part XVIII pp. 34 - 44. Republic of South Africa Government Printers.

30. Grau F.H. 1986 Microbial ecology of meat and poultry. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.2: Meat and Poultry Microbiology*. Avi Publishing Company, Inc. 1 - 36

31. Green S. 1974 Microbiology in the poultry processing industry. *Process Biochemistry* **9**(1): 27 - 28

32. Hardie J.M. 1986 Genus *Streptococcus*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II.* Williams and Wilkins, Baltimore, USA. 1043 - 1071.

33. Harrigan W.F. and McCane M.E. 1976 Laboratory methods in food and diary microbiology. Academic Press Inc. London

34. Holmes B., Owen R.J. and McMeekin T.J. 1984 Genus *Flavobacterium*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. I.* Williams and Wilkins, Baltimore, USA. 353 - 360.

35. International Commision on Microbiology Specifications for foods (ICMSF) 1978 *Micro-organisms in Foods*. 1 Their Significance and Methods of Enumeration, 2nd ed. University of Toronto Press, Canada

36. James W.O., Williams O.W., Prucha J.C., Johnston R. and Chritensen W. 1992 Profile of selected bacterial counts and *Salmonella* prevalence on raw poultry in a poultry slaughter establishment. *JAVMA* 200(1): 57 - 59

37. Jones F.T., Axtell R.C., Rives D.V., Scheideler S.E., Tarver F.R., Walker R.L. and Wineland M.J. 1991 A survey of *Salmonella* contamination in modern broiler production. *Journal of Food Protection.* **54**(7): 502 - 513

38. Juni E. 1984 Genus *Acinetobacter*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. I*. Williams and Wilkins, Baltimore, USA. 303 - 307.

39. Kandler O. and Weiss N. 1986 Genus *Lactobacillus*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II*. Williams and Wilkins, Baltimore, USA. 1209 - 1234

40. Keddie R.M., Collins M.D. and Jones D. 1986 Genus *Arthrobacter*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II*. Williams and Wilkins, Baltimore, USA. 1288 - 1301.

41. Kersters K. and de Ley J. 1984 Genus *Agrobacterium*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. Vol. I.* Williams and Wilkins, Baltimore, USA. 244 - 254.

42. Kilsby D.C. 1982 Sampling schemes and limits. [Edited by Brown M.H.] In *Meat Microbiology*. Applied Science Publishers, London. 387 - 421

43. Kloos E.W. and Schleifer K.H. 1986 Genus *Staphylococcus*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II*. Williams and Wilkins, Baltimore, USA. 1013 - 1035.

44. Kocur M. 1986 Genus *Micrococcus*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II*. Williams and Wilkins, Baltimore, USA. 1004 - 1008.

45. Komagata K. and Suzuki K. 1986 Genus *Aureobacterium*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. II*. Williams and Wilkins, Baltimore, USA. 1323 - 1325.

46. Kraft A.A. 1986 Psycrotrophic organisms. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.2: Meat and Poultry Microbiology*. Avi Publishing Company, Inc. 191 - 208

47. Lahellec C. and Colin P. 1981 *Pseudomonas* as spoilage agents in poultry: Observations concerning strains isolated at different stages of processing and during storage. [Edited by Roberts T.A., Hobbs G., Christian J.H.B. and N. Skovgaard] In *Psychrotrophic Micro-organisms in Spoilage and Pathogenicity*. Academic Press, London. 269 - 274

48. Lahellec C. and Colin P. 1979 Bacterial flora of poultry: Changes due to variations in ecological conditions during processing and storage. *Archiv Für Lebensmittelhygiene* **30** : 95 - 98

49. Lillard H.S. 1985 Bacterial cell characteristics and conditions influencing their adhesion to poultry skin. *Journal of Food Protection* **48**(9): 803 - 807

50. McBride G.B., Skura B.J., Yada R.Y. and Bowmer E.J. 1980 Relationship between incidence of *Salmonella* contamination among pre-scalded, eviscerated and post-chilled chickens in a poultry processing plant. *Journal of Food Protection* **43**(7): 538 - 542

51. McMeekin T.A. 1975 Spoilage association of chicken breast muscle. *Applied*

Microbiology **29**(1): 44 - 47

52a. McMeekin T.A. and Thomas C.J. 1979 Aspects of the microbial ecology of poultry processing and storage: A Review. *Food Technology in Australia* **31**: 35 - 43

52b. McMeekin T.A., Thomas C.J. and McCall D. 1979 Scanning electron microscopy of micro-organisms on chicken skin. *Journal of Applied Bacteriology* **46**:195 - 200

53. Microbiology and Food Safety Committee of the National Food Processors Association, 1993 Implementation of HACCP in a food processing plant. *Journal of Food Protection* **56** (6): 548 - 554

54. Mead G.C. 1989 Hygiene problems and control of process contamination. [Edited by Mead G.C.] In *Processing of Poultry*. Elsevier Science Publishers Ltd. 183 - 220

55. Mead G.C. 1982 Microbiology of poultry and game birds. [Edited by Brown M.H.] In *Meat Microbiology*. Applied Science Publishers Ltd. 67 - 101

56. Morris G.K. and Wells J.G. 1970 *Salmonella* contamination in a poultry-processing plant. *Applied Microbiology* **19** (15): 795 - 799

57. Mulder R.W.A.W. and Veerkamp C.H. 1974 Improvements in poultry slaughterhouse as a result of cleaning before cooling. *Poultry Science* **53**: 1690 - 1974

58. Mulder R.W.A.W., Dorresteijn L.W.J. and Van Der Broek J. 1978 Cross-contamination during the scalding and plucking of broilers. *Br. Poult. Sci*. **19**: 61 - 70

59. Notermans S., Dufrenne J. and Van Leeuwen W.J. 1982 Contamination of broiler chickens by *Staphylococcus aureus* during processing; incidence and origin. *Journal of Applied Bacteriology* **52**: 275 -280

60. Notermans S., Terbijhe R.J. and Van Schothorst M. 1980 Removing faecal contamination of broilers by spray - cleaning during evisceration. *Br. Poult. Sci*. **21**: 115 - 121

61. Notermans S. and Kampelmacher E.H. 1975 Further studies on the attachment of bacteria to skin. *Br. Poult. Sci*. **16**: 487 - 496

62. Notermans S. and Kampelmacher E.H. 1974 Attachment of some bacterial strians to the skin of broiler chickens. *Br. Poult. Sci*. **15**: 573 - 585

63. Olivier, M., Cloete, T.E., Veary, C.M and von Holy, A. 1996 Microbiological status of selected chicken carcases from a non-automated poultry processing plant. *Journal of Basic Microbiology*. **36**(1): 41 - 49

64 Olivier, M., Cloete, T.E., Veary, C.M. and von Holy, A. 1995 Microbiological status of selected chicken carcases from a non-automated poultry processing plant. *Proceedings of the Thirteenth Biennial SAAFoST International Congress*. Durban, South Africa. p96.

65. Palleroni N.J. 1984 Genus *Pseodomonas*. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In *Bergey's Manual of Systematic Bacteriology, Vol. I*. Williams and Wilkins, Baltimore, USA. 141 - 199.

66. Parry R.T. 1989 Pre-slaughter handling and processing. [Edited by Mead G.C.] In *Processing of Poultry.* Elsevier Science Publishers Ltd. 65 - 101

67. Patterson J.T. 1972 Microbial sampling of poultry carcasses. *Journal of Applied Bacteriology* **35**: 569 - 575

68. Popoff M. 1984 Genus Aeromonas. [Edited by Murray R.G.E., Brenner D.J., Bryant M.P., Holt J.G., Krieg N.R., Moulder J.W., Pfennig N., Sneath P.H.A. and Staley J.T.] In Bergey's Manual of Systematic Bacteriology, Vol. I. Williams and Wilkins, Baltimore, USA. 545 - 548.

69. Ralph W.J. and Tompkin R.B. 1984 Meat and poultry products. [Edited by Speck Marvin L.] In *Compedium of Methods for the Microbiological Examination of Foods*, 2nd ed.

American Public Health Association (APHA) Washington, D.C. 611 - 623

70. Roberts D. 1982 Bacteria of public health significance. [Edited by Brown M.H.] In *Meat Microbiology*. Applied Science Publishers Ltd. 319 - 386

71. Silliker J.H. 1995 Microbiological testing and HACCP programs. *Diary, Food and Environmental Sanitation* **15** (10): 606 - 610

72. Silliker J.H. and Galois D.A. 1986 *Salmonella*. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.2: Meat and Poultry Microbiology*. Avi Publishing Company, Inc. 209 - 229

73. Simonsen B. 1989 Microbiological critera for poultry products. [Edited by Mead G.C.] In *Processing of Poultry*. Elsevier Science Publishers Ltd. 221 - 250

74. Smeltzer T.I. 1981 Isolation of *Campylobacter jejuni* from poultry carcases. *Australian Veterinary Journal* 57: 511 - 512

75. South African Poultry Association (SAPA). 1996 Whats happening in the poultry and egg industry*? Food Industries of South Africa* (9): 9 - 15

76. South African Poultry Association (SAPA). 1993 Code of Practice for Broiler Processing Plant (Abattoirs) Annexure G

77. Statgraphics: Statistical graphics system by statistical graphics corporation, Plus Ware Software Products, STSC inc. USA

78. Thomas C.J. and McMeekin T.A. 1981 Attachment of *Salmonella* spp. to chicken muscle surfaces. *Applied and Environmental Microbiology* **42**(1): 130 - 134

79. Thomas C.J. and McMeekin T.A. 1980 Contamination of broiler carcass skin during commercial processing procedures: an Electron microscopy Study. *Applied and Environmental Microbiology* **40**(1): 133 - 144

80. Todd E.C.D. 1980 Poultry-associated foodborne disease - Its occurrence, cost, source and prevention. *Journal of Food Protection* **43**(2): 129 - 139

81. Von Holy A., Holzapfel W.H. and Dykes G.A. 1992 Bacterial populations associated with Vienna sausage packaging. *Food Microbiology* **9**: 45 - 53

82. WHO, 20th Session ,1993 Codex Guidelines for the application of the Hazard Analysis Critical Control Point (HACCP) System. Codex Alimentarius Commission., Annex II, III and IV . 17 - 25

83. Zottola E.A. and Smith L.B. 1990 Pathogenic bacteria in meat and meat products. [Edited by Pearson A.M. and T.R. Dutson] In *Advances in Meat Research, Vol.6: Meat and Health*. Elsevier Science Publishers Ltd. 157 -183