

**DETERMINATION OF OPTIMAL BRINING LEVELS AND EFFECTIVE
CHLORINATED ANTIMICROBIALS IN THREE SELECTED
COMMERCIAL POULTRY ABATTOIRS OF SOUTH AFRICA**

by

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DECLARATION

I, Malesela Dennis Mashishi, declare that “Determination of Optimal Brining Levels and Effective Chlorinated Antimicrobials in three selected commercial poultry abattoirs of South Africa” is my unaided own work.

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DEDICATION

I dedicate this dissertation to The Lord Jesus Christ for surrendering his own life for us to be cleansed our sins through his blood so that we can enter the Kingdom of Heaven (1 John 1:17).

ABSTRACT

Two experiments were conducted to determine the optimal brining levels and effective chlorinated antimicrobials in three selected commercial poultry abattoirs of south Africa. The objective of the study was two fold: Firstly, the primary objective of the study was to determine the optimal inclusion level of brine for application in chicken processing to elongate the shelf life by reducing spoilage bacteria under refrigeration stage while the secondary objective of the study was to determine the most effective chlorinated antimicrobial to be applied in poultry processing plants to reduce spoilage bacteria. For each experiment, a complete randomized design was used. The general linear model procedure was used to determine the effects of brining and chlorine antimicrobials on the Psychrotrophic bacterial load of individually quick frozen (IQF) chicken portions. Simultaneously, a quadratic type equation was used to determine the optimal inclusion level of brine in relation to the responses of Psychrotrophic bacterial loads. The results indicated that control samples (0% brine) had higher ($P < 0.05$) bacterial load than all samples injected with various injection levels. There were significant differences ($P < 0.05$) between samples injected with 15% and 20% for all major abattoirs combined. However, there were no differences ($P > 0.05$) between samples injected with 20% and 25% brine, respectively. In addition, there was a significant difference ($P < 0.05$) in Psychrotrophic bacterial load between the samples treated with 25% and 30% brine inclusion level as well as those treated with 30% and 35% brining levels in all abattoirs. Furthermore, the results of the study also showed that Acidified Sodium Chloride had significantly lower bacterial load than both aqueous chlorine and chlorine dioxide. However, the effect of percentage brining on average Psychrotrophic bacterial count had minimum quadratic values of $24.45 - 0.517 \text{ brining} + 0.805 \text{ brining}^2$ with $r = 0.995$; $r^2 = 0.989$, with optimum percentage brining dose being 43.08%. By extension, the result implies that the lowest reduction in spoilage bacteria is attained at 43.08% of brine inclusion level. These findings have implications on the most effective and convenient antimicrobial to be used in chicken abattoirs as well as reduction of psychrotrophic bacterial load on individually quick frozen (IQF) chicken portions.

TABLE OF CONTENTS

CONTENTS	PAGE
CHAPTER ONE: INTRODUCTION	1
1.1 BACKGROUND	1
1.2 PROBLEM STATEMENT	2
1.3 MOTIVATION OF THE STUDY	2
1.4.1 Aim of the study	3
1.4.2 Objectives of the study	3
1.5 RESEARCH ASSUMPTION	3
1.6 SIGNIFICANCE OF THE STUDY	3
1.7 LIMITATION OF THE STUDY	4
CHAPTER TWO: LITERATURE REVIEW	5
2.1 INTRODUCTION	5
2.2 BRINE	9
2.2.1 Salt	12
2.2.2 Nutritional properties	13
2.3 BACTERIOLOGY	14
2.3.1 Bacteria responsible for spoilage	14
2.3.2 Microbiological quality	16
2.3.2.1 Mechanism of spoilage	16
2.3.2.2 Potential of Hydrogen (pH)	17
2.4 LEGISLATIONS	18
2.4.1 Industry overview	18
2.4.2 South Africa	19

2.5 CHLORINE ANTIMICROBIALS	21
2.5.1 Chlorine Dioxide	23
2.5.2 Acidified Sodium Chloride	24
2.5.3 Aqueous Chlorine	26
2.6 CONCLUSION	27
CHAPTER THREE: MATERIALS AND METHOD	29
3.1 STUDY AREA	29
3.2 EXPERIMENTAL PROCEDURE	30
3.3 DATA COLLECTION	34
3.4 STATISTICAL ANALYSIS	35
3.5 ETHICAL AND LEGAL CONSIDERATION	36
3.6 DISSEMINATION OF THIS STUDY	36
CHAPTER FOUR: RESULTS	37
4.1 BRINE	37
4.2 CHLORINE ANTIMICROBIALS	38
4.3 INFERENTIAL ANALYSIS (REGRESSION)	38
4.4 OVERVIEW EFFECTS OF BRINING AND ANTIMICROBIALS	39
4.5 OPTIMAL INCLUSION LEVELS OF BRINING USING QUADRATIC	41
CHAPTER FIVE: DISCUSSION	42
5.1 BRINE	42
5.2 SALTS	43
5.3 ANTIMICROBIALS	44
CHAPTER SIX: CONCLUSION AND RECOMMENDATION	46
REFERENCES	47

LIST OF TABLES

TABLE	PAGES
Table 2.1: Brine formulations and ingredients declared for RSA IQF	10
Table 2.2: The ingredients of brine in RSA	10
Table 2.3: The brine ingredients in USA	11
Table 2.4: The classification of microorganisms according to pH levels	17
Table 2.5: Bacterial counts (log ₁₀ cfu/mL) recovered on plate count agar	23
Table 2.6: Mesophiles on whole carcass dipped into ASC solution	25
Table 2.7: Psychrotrophs on whole carcass dipped into ASC solution	26
Table 4.8: Effects of different levels of brining on Psychrotrophic bacteria load	37
Table 4.9: Effects of chlorine antimicrobials on Psychrotrophic bacteria load	38
Table 4.10: The optimal brining levels using quadratic model	39
Table 4.11: Brining and antimicrobials effects bacterial load	40
Table 4.12: Reported brining levels in South Africa	41

LIST OF FIGURES

FIGURES	PAGES
Figure 3.1: Fomaco brine injection Machine	30
Figure 3.2: Flow diagram of first Psychrotrophic bacterial count	32
Figure 3.3: Flow diagram of second Psychrotrophic bacterial count	33
Figure 3.4: Flow diagram of the entire experimental design of the study	35
Figure 4.5: Quadratic function showing the optimum brining levels	39

CHAPTER ONE: INTRODUCTION

1.1 Background

The treatment of poultry meat with an aqueous solution containing, amongst other ingredients, salt with the objective of preserving the meat, enhancing its flavour and tenderising it has been a practice of the commercial poultry processing industry for some time. In the United States of America, for instance, a variety of patents seeking better ways of preserving eviscerated poultry carcasses were registered from the 1950s – these inventions related to the treatment, through injection or immersion, of poultry carcasses in brine solutions with the following claimed benefits: increased shelf-life, inhibition of bacteria growth, increased moisture and nutrient retention, amongst others (Buchanan, 1955; Nelson, 1958; Libby, 1970). Brining has evolved with technological innovations and can now be applied to individually quickly frozen (IQF) poultry portions; this improves shelf-life, meat flavour and tenderness. In arguing for higher brine inclusion levels in South African IQF portions, the South African Poultry Association pointed out that lower regulated brine inclusion levels would increase the price of chicken and hamper product quality (South African Poultry Association v Minister of Agriculture (39597/2016) [2016] ZAGPPHC 862). However, in recent years in South Africa, there have been reported cases of the abuse of brining, with some commercial processors over-injecting chicken portions with brine injection levels as high as 60% (Vutula, 2011).

While original brine treatment compositions were based on aqueous solutions of monosodium glutamate (MSG), contemporary formulations of brine are based on sodium and phosphate chloride with ingredients including flavours, antioxidants, hydrocolloids, non-meat proteins, and starch (Moholisa, Roodt, Bothma, de Witt, and Hugo, 2014). As such, high levels of brine inclusion will result in high levels of salt in poultry products, putting consumers at risk of suffering from ailments associated with high sodium intake (Alvarado and McKee, 2007; Ellinger, 1972). Moreover, the poultry industry practices the recirculation of the brine solution used in the injection process. For this reason, it should be taken into account that microbial safety of chicken injected with brine is compromised (Kutu, 2014). It is reported that, in pork, that recirculation of brine results in contamination and bacterial growth (Gill, McGinnis, Houde, Lamoureux, and Leblanc, 2005).

Poultry processing plants make use of chlorine antimicrobials (aqueous chlorine, chlorine dioxide and Acidified Sodium Chlorite) to abate the effects of spoilage bacteria (Cressey *et al.* (2008) as cited by Hernando *et al.*, 2013). Resultantly, this practice has highlighted the urgent need for improved food regulation and consumer protection owing to human ailments that may be attributed to the consumption of products with chemical exposure. Concerns around brining practices by certain poultry producers warranted a study on brine usage in poultry processing plants to determine the optimal brining level, increase the scientific knowledge on the brining of poultry meat and abattoir safety practices. Thus, the objective of this study was to determine the optimal inclusion level of brine and most economical and effective chlorine disinfectant to apply in South African poultry abattoirs.

1.2 Problem statement

Knowledge of the optimal brining levels application in the South African poultry processing industry is limited. Consequently, there is a lack of consensus between the industry and regulators on what constitutes the most optimal level of brine injection so as to both reap the benefits of brine treatment of meat and mitigate risks that may result from over-brining. Often, the over application gives rise to consumers not getting value for money spent on processed chicken. Not only does such meat contain more water than it should, but also has high amounts of salts. As suggested by Morrison *et al.* (2011), high levels of salt in processed chicken places consumers at risk of developing health problems. Furthermore, the use of chlorine disinfectants in the poultry processing industry is a cause for concern regarding meat safety. The effectiveness of such disinfectants on reducing spoilage bacteria also requires investigation. It is envisaged that the determination of an optimal brine application level and the most effective, less harmful and less costly disinfectant to use when reducing spoilage bacteria will help to quell concerns relating to treatment of poultry meat with brine solutions and the use of chlorine-based disinfectants.

1.3 Motivation

In 2010, alleged brine abuse and contravention of regulations on food safety by one of South Africa's largest poultry processing plants drew negative attention to poultry meat processors and the process of preserving poultry meat through the injection of a brine

solution (Ottermann, 2010). The Department of Agriculture, Forestry and Fisheries (DAFF), as result of conflicting discourses arising out of the earlier mentioned case, proposed new brine injection limits. These were, however, unacceptable to the South African Poultry Association (SAPA), which proposed a brine injection limit of of 80:20 (80% chicken; 20% brine). In view of these differences, DAFF commissioned the Agricultural Research Council (ARC) to conduct a study based on poultry brine injection in South Africa (Vutula, 2011). Eventually, in 2016, the Minister of Agriculture, Forestry and Fisheries announced new limits for brine treatment of poultry meat – with a 85:15 ratio of chicken/brine (85% chicken: 15% brine). The Minister was then taken to the Northern Gauteng High Court by SAPA, which opposed the new limits and argued for brining limits within 20% and 25% - however, this case was dismissed (South African Poultry Association v Minister of Agriculture (39597/2016) [2016] ZAGPPHC 862). In view of these developments, a scientific enquiry was necessary to ensure that poultry producers and consumers find common ground on brine injection limits. The results of this study will also ensure that arguments on both sides of the poultry brining debate can be based on credible evidence. The objective of the present study was therefore to determine the level of inclusion of brine for optimal productivity in commercial poultry processing plants in South Africa.

1.4 Aim and Objectives of the study

1.4.1 Aim of the study:

The aim of the study was to make a contribution towards the determination of optimal brining level and effective chlorinated antimicrobials on processed chicken in commercial poultry abattoirs.

1.4.2 Objectives of the study:

The objectives of the study were:

- To determine the optimal inclusion level of brine to be applied in chicken preservation in relation to microbial levels of thawed meat 6 months after refrigeration.
- To determine the type of chlorinated antimicrobial to be used in three selected commercial poultry abattoirs after 6 months of shelf life.

1.5 Research assumption/Hypothesis

The study was based on the following hypothesis:

- There is no optimal inclusion level of brine to be applied in chicken preservation in relation to microbial levels of thawed meat 6 months after refrigeration.
- There is no type of chlorine antimicrobial to be used in poultry abattoir after 6 months of shelf life.

1.6 Significance of the study

The findings of this study are expected to provide optimal levels of brine to apply in 3 selected commercial poultry processing plants in South Africa in order to maximise productivity and reduce health risks to consumers using quadratic functions. Furthermore, it is expected that the findings of this study will contribute positively towards the understanding of the effects of using chlorine disinfectants aimed at reducing spoilage bacteria known as Psychrotrophic bacteria in chicken carcasses.

1.7 Limitation of the study

The current study undertaken had some limitations such as availability of funding to cover more abattoirs, materials required and availability of chemicals in South Africa.

CHAPTER TWO: LITERATURE REVIEW

2.1. Chicken preservation and usage

Chicken is a primary source of protein in many households owing to its high nutritional value and affordability. However, the chicken is highly perishable with short shelf life even when refrigerated. To mitigate this, the agro-processing industry has explored means in finding a suitable treatment for preservation in order to increase the shelf life of processed chicken products (Khaled *et al.*, 2016, Kutu, 2014). The irresponsible usage of salted water in poultry has raised public concerns about the quality of meat in the poultry industry. Poultry abattoirs in South Africa use brine injection to add flavour, increase shelf life and quality of individually quick frozen portions and frozen whole birds. The brine process was introduced in the industry in the 1990s with the purpose of addressing the negative impact on organoleptic qualities of poultry portions that were marketed as quick frozen products (Christensen, 2014). According to the South Africa Poultry Association (2016), more than 900 million broiler chickens are processed annually in the country. It is also estimated that 2,2 million tonnes of poultry is consumed in South Africa. Therefore, spoilage is of great concern in the poultry industry owing to its potential to cause economic loss. While a nationwide study to determine the revenue losses suffered by poultry processors due to spoilage has not been conducted, concerns raised by some companies in the poultry industry following the Department of Agriculture, Forestry and Fisheries' exposure of irresponsible brine application by some abattoirs point to an industry that sometimes suffer economic losses due to spoilage. Psychrotrophic bacteria (*fluorescens*, *putida*, and *Shewanella putrefaciens*) are classified as internal bacteria and are largely

responsible for spoiling chicken meat. These bacteria are not measured easily during the processing of chicken (Murphy, 2009).

Russell (2009), in his study of spoilage bacteria, has stated that Psychrotrophic bacteria are lightly affected by chlorine solutions since they grow well under cold environment. The commonly used chlorinated antimicrobials are chlorine dioxide, acidified sodium chlorite and aqueous chlorine. Chlorine is the second highest halogen, the chemical element with symbol Cl and atomic number 17. At high concentrations, it is toxic and it dissolves in water to form a chlorinated solution that is used as sanitiser and disinfectant in the food industry (FAO/WHO, 2000). Its toxicity has raised some concern regarding its use in edible products. According to Mead *et al.* (1975) as cited in Barbut and Pronk (2014), the practice of using chlorinated liquid to control microbial contamination in chicken processing has been applied for several years in the South African poultry industry. The objective was to ensure the production of high-quality chicken.

There is a variety of laws that govern abattoir operations in South Africa. The existing legislation seeks to regulate different aspects in the meat processing value-chain such as hygiene, occupational health and safety, waste management, handling of condemned materials, animal product safety and waste-water management (Molapo, 2009). It is widely recognised that all chemical disinfectants form some potentially harmful by-products. Concerns the byproducts of chlorine when it's used as a disinfectant of equipment, environment and control of microbial contamination in food processing (Herrera & Donoghue, 2012).

In South African literature, there is not enough scientific information about the health effects of poultry disinfectants and by-products resulting from the use of chlorine disinfectants in food processing. Stephan (2012) states that chlorination can limit the growth of biofilms on food surfaces, thereby preventing the spoilage of the food and avoiding the generation of unpleasant odours. In the food industry, chlorine is used for reducing microbial contamination as a microbial safety control measure rather than a decontamination treatment. In the United States, USDA (1995) released a report in which most food processing industries were encouraged to reduce the sodium input in order to lower the blood pressure of consumers. They recommended chicken consumption owing to its low sodium content, leanness, low fat and low cholesterol. This would meet consumers' quality needs with less nutritional loss, which would be beneficial to the poultry processing industry and consumer health (Puolanne *et al.*, 2001; Demby and Cunningham, 1980; Guerrero-Legarreta, 2010; Bogosavljevic-Boškovic *et al.*, 2010). In South Africa, chicken is a very affordable source of protein, preferred by many although there is a high rate of hypertension among consumers.

In the context of consumer studies in terms of chicken quality, consumer perceptions are described in two ways, the daily context that includes buying, preparing and eating. The production context includes primary production, slaughtering and chicken processing. All of this is summed up as a value-adding process of poultry meat (Troy and Kerry, 2010; Korzen and Lassen 2010). In chicken processing industry, safety in the form of microbial and chemical contamination is very crucial in terms of product quality and consumer protection. Other factors of importance include the product yield, convenience and low input cost, to name but a few (Guerrero-Legarreta, 2010).

In the poultry industry, chlorine disinfectants are used primarily to reduce microbial contamination as a safety practice for microbial contamination rather than a decontamination agent (Khaled *et al.*, 2016). The amount of concentration and contact time of chlorine disinfectants solution with chicken samples determines the effectiveness of disinfectant (Bolton, 2014). The bacterial load reduction is greater in the chill system than in the spray system because of the greatly increased contact time. The initial use of chlorine was to extend the shelf life of poultry products (Purnell *et al.*, 2013).

However, it has little direct effect on carcass bacteria as attached or entrapped pathogens are not readily accessible to chlorine. The main benefit from chlorination of process water lies in its ability to control microbial contamination of the processing environment and equipment (Singh *et al.*, 2015; Burfoot *et al.*, 2015). There are public concerns about utilising chlorine as an oxidising agent in South Africa. Therefore, extensive research needs to be conducted in order to examine the risks and benefits from exposure to human beings when consuming products that have been treated with chlorine antimicrobials.

2.2 Brine

Brine is defined as a mixture of water with all additives often referred to as a pickle, marinade or cure (Feiner, 2006) as quoted by Kutu (2014). Schutte (2012) has described brine as a mixture of water, sea salt, maize starch, seaweed and maize extracts and sugar. It comprises 97% water and 3% solids, of which sea salt is 2% and thickeners and sugar making up the remaining 1%. The poultry industry has grown a culture of secondary processing which involves slaughtering, deboning and manipulating portion sizes as part

of value adding. This practice saves the consumers' time and presents consumers with a choice of purchasing only portions that they would like to have. Additionally, this practice is primarily responsible for significantly magnifying the poultry processing industry (Sams, 2001; Guerrero-LeGarrette, 2010).

According to Sams (2001), the practice of selling individual cuts rather than whole bird took place earlier in the 1960s in the United States and gained popularity in the year 2000. In South Africa, most of the individually quick frozen are brined during production. Table 2.1 below discloses the list of ingredients often labelled on packaging materials.

Table 2.1: Different brine formulations and ingredients applied to Individual Quick Frozen in RSA

Brands	Brine ingredients
Brand1	Water, phosphate, salt, dextrose, thickener (maize start), stabiliser
Brand2	Vitamin C, salt, starch, phosphate, stabiliser
Brand3	Water, phosphate, salt, dextrose, thickener, stabiliser
Brand4	Butter basting (water, salt, canola oil, butter phosphate, sucrose, spice extract, emulsifier)
Brand5	Brine, phosphate, thickener
Brand6	Water, sugar (dextrose and sucrose), emulsifier, salt, thickener and flavourant
Brand7	Brine (water, salt sodium citrate, citrate, citric acid, glucose, thickener, flavour enhancer, spice extract)
Brand8	Brine, phosphate, thickener

Source: Kutu 2014

The practice of brine injection has been practised for a few years. Brine is applied worldwide in the meat processing industry at different levels according to each country's regulations. It is not the case that companies in the same country apply the same

amounts, therefore, different levels are applied in South Africa by different producers depending on customers' needs and company practises.

Table 2.2: The ingredients of brine in South Africa

Ingredients	Concentrations (%)
Water	94
Solids	3
Sea salts	2
Thickeners	0.5
Sugar	0.5

Source: Schutte 2012

The amounts of salt used by South African poultry producers in the composition of brine solutions are at lower levels compared to those in countries such as the United States of America (US), but have higher water concentration. Poultry meat processors in the US have more salt in brine than South African producers, as shown in Table 2.2 and 2.3, respectively.

Table 2.3: The brine ingredients in the USA

Ingredients	Concentrations (%)
Water	75.5
Salts	17.5
Sugar	3.5
Phosphate	3
Na-ascorbate	0.35
Na-nitrate	0.15

Source: FAO 2010

In South Africa, the brining of chicken meat has raised some negative concerns mostly from regulators. Investigations conducted by and on behalf of the government organ responsible for regulating the poultry processing industry, the Department of Agriculture, Forestry and Fisheries, revealed that some producers were irresponsibly injecting as much as 60:40 ratio of brining (60% brine; 40 chicken). This has necessitated a need to determine brining levels that would not only ensure preservation of chicken meat, but also enhance the quality of the meat. High levels of brine injections pose a threat to human health, in particular hypertension or kidney sufferers, as this result in high salt levels in chicken meat (Kayode and Oyeteran, 2014). Scientific research concerning brining, the chemical composition of processed chicken, permissible additives, oxidative stability and sensory properties of processed meat is necessary to support evidence-based regulatory interventions. Science-based regulation would ensure no adulteration of processed chicken meat and prevent unnecessary chemical compositions, oxidative instability and unjustified weight. This is study aimed at investigating the most optimal antimicrobial for use in poultry processing and establish the necessary probable amount of brine to be applied in the processing of individually quick frozen chicken portions. Thus creating common ground among regulators, the poultry industry and consumers on what constitute best brine inclusion level and most optimal antimicrobial to ensure the best meat quality and most efficient preservative in poultry processing.

2.2.1 Salt

Salt, as an ingredient of brine, improves product flavour and water retention capacity in combination with tripolyphosphate (STP) to extract soluble protein in chicken (Alvarado and McKee, 2007). In the broiler processing industry, salts serve important functions

including the binding of chicken proteins as well as serving as a binding agent of meat and fat. It improves the texture, tenderness and palatability of poultry meat by increasing the water holding capacity (EU, 2012). Kutu (2014) has reported that the salt contained in brine improves meat texture by solubilising the myofibrillar meat proteins and improving flavour. Traditionally, salt is used as a preservative agent to enhance product shelf life. This practice of using salt as an ingredient is favourable owing to its affordability, availability and difficulty to replace (Desmond, 2006). According to Burfoot *et al.* (2015), water constituted approximately 70 percent of chicken, which is ionic in nature owing to the monovalent minerals present in muscle tissue as soluble salts and the ionised forms of these salts. However, the ionic strength of a brine injection is greater than that of muscle tissue fluid, and the brine solution will be absorbed by the chicken until a state of equilibrium is reached by osmosis process (Morrison *et al.*, 2011). There are concerns about reducing the salt added when producing the processed products owing to findings linking high sodium intake and its associated ailments such as hypertension.

According to EU (2012), salt content of both white and red meat is not a regulated ingredient but is self-limiting, because high concentrations will negatively affect the palatability of the product. Usually, the end product of chicken contains up to 2% salt on average. The levels of salts of finished products vary from 1.5 to 3% depending on product specificity. Depending on the products, salt levels can range from 1.5 up to 3%. In addition to salt levels, purity of the salt is also important, because impure salts may interfere with the quality of the product. The World Health Organisation does not put specific restrictions but has suggested a total of 1.5g daily intake for human beings (WHO, 2012). The National South African Health Department has on the 1st of March 2012 published a

labelling and advertising regulation (R146) under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972) defining brine as a solution of sodium chloride in water where the solution is used for flavouring and preservation of the food. No limitation has been set for salt injection in South Africa. The USDA has defined brine as an amount of water that contains salt as the main ingredients. The amount of salt injected into processed meat is not regulated in the United State of America (Alvarado and McKee, 2007) as cited by Kutu, 2014.

2.2.2 Nutritional Properties

A phosphate is one of the favoured ingredients for brine formulations owing to its advantages for reduced drip and cooking losses. It can also influence the fat and protein components negatively of raw meats (Kutu, 2014). This negative effect involves the dissolution of protein as it has been observed that chicken treated with brine containing phosphate have less protein as compared to the untreated. This protein dissolution is associated with higher moisture because of the phosphate's moisture retention capacity (Demby and Cunningham, 1980). According to a trial survey conducted with DAFF, it was found that thawing and cooking losses of commercially available, injected frozen chicken breast portions were significantly higher than those of non-injected and frozen control portions. Another observation made was a clear nutrient dilution effect in terms of protein and energy content and that is attributed to the injection of high levels of brine in processed chicken (Moholisa, 2011; DAFF, 2011). The protein content, fat content and energy content were negatively affected by the high injection levels (Moholisa, 2011).

2.3 Bacteriology

2.3.1 Bacteria responsible for spoilage

According to Burfoot *et al.* (2015) chlorine only treats the bacteria available on the skin not within the meat. These bacteria are known as Mesophilic bacteria (*Salmonella*, *Campylobacter*, *Escherichia coli* and *Vibrio*) which are associated with foodborne diseases. Mesophilic bacteria are those bacteria that do not multiply to an appreciable degree at refrigerator temperatures and are not a factor in spoilage. Psychrotrophic bacteria are those bacteria that are able to grow under cold conditions and are responsible for spoilage (Murphy, 2009). The Psychrotrophic bacteria (*fluorescens*, *putida*, *Shewanella* and *putrefaciens*) are classified as internal bacteria and are largely responsible for spoiling the carcasses. These bacteria are not measured easily during processing of chicken. According to Purnell *et al.* (2004) Psychrotrophic bacterial populations, under refrigeration (< 5 °C), have the capacity to multiply on broiler carcasses and spoil the chicken; however, the mesophilic bacteria that are found primarily in large quantities on the carcass remain constant or decrease in number. Every processed chicken is given an expiry date set owing to spoilage; most companies perform the aerobic plate counts (APC) on products. This method is not suitable for measuring the Psychrotrophic bacteria on chicken, as it does not indicate the levels of spoilage. Aerobic Plate Count is suitable for measuring mesophilic bacteria, which is found on the skin and furthers of chicken but does not contribute greatly to spoilage (ECR, 2005). This APC microbiological method may miss up to 99.9% (3 logs) of Psychrotrophic bacteria on the product surface (Elzamzamy, 2014). When measuring spoilage, samples should be plated and incubated at 7 Degree Celcius for 10 days. This will allow bacterial growth and

encourage colonial production on the plate, which is responsible for spoilage (Casaburi *et al.*, 2015). The Psychrotrophic bacteria are differentiated to mesophilic in a sense that they are able to grow under an environment with low temperature and spoil the product whereas mesophilic are unable to multiply at a lower temperature. Mesophilic bacteria are also not a factor in spoilage but are associated with human disease such as foodborne diseases (Adam *et al.*, 2010).

2.3.2 Microbiological quality

Kutu (2014) has stated that labelling of Individually Quick Frozen (IQF) chicken portions in South Africa preserved with brine that contains high amounts of water is important. Water is traditionally used in solutions added to chicken products. The use of excessive water in poultry processing is considered as one of the main concerns for the poultry industry since water is a favourable medium for bacterial growth in perishable products (Patsias *et al.*, 2008; ICMSF, 2011). The Psychrotrophic bacteria which are known as cold-loving will contribute greatly to this study as targets, which grow best at 0 degrees Celsius. The cold-loving organisms are Psychrotrophic bacteria which are defined by their ability to grow at 0 freezing temperatures. These bacteria are mainly responsible for spoilage in the refrigerators owing to their ability to multiply greatly and destroy chicken protein (Doulgeraki *et al.*, 2012).

2.3.2.1 Mechanism of Spoilage

The reaction of extracellular enzymes and accumulation of metabolic by-products secreted by Psychrotrophic spoilage bacteria as they multiply on chicken surfaces at

lower temperatures results in spoilage. Parts of these byproducts are characterised as off-odors and slimy nature since bacteria utilise the nutrients on the surface of the chicken. These off-odors are the result of the direct microbial utilisation of low molecular weight nitrogenous compounds such as amino acids that are present in skin and muscle. According to Ercolini *et al.* (2015), the amount of free amino acids increases as proteolysis occurs throughout the storage period. The measurement of these free amino acids, caused by the production of aminopeptidases and followed breakdown of protein, may be used to determine the bacteria quality of chicken. In the last stages of contamination, the meat begins to exhibit an ammoniac odour in addition to the dirty odour, which is associated with the breakdown of protein and the formation of ammoniac compounds (Adam *et al.*, 2010).

2.3.2.2 Potential of Hydrogen (pH)

This is one of the factors that directly affect the microbial activity in chicken meat and contributes towards chicken spoilage. The scale used to measure the acidity or alkalinity of the chicken is divided into three cardinals namely; the minimum pH (the organism cannot grow below this point) the maximum pH (the organism cannot grow above this point) and lastly the optimum pH, at which the growth of an organism is at exponential rate (Dilbaghi and Sharma, 2012). In this study, the Psychrotrophic bacteria were the targets as experimental output as cold loving microorganisms. Table 2.4 below demonstrate the classes of microbes and their temperature requirements for growth.

Table 2.4: The classification of microorganisms based on pH levels.

Microorganisms	Optimum	Maximum
-----------------------	----------------	----------------

Bacteria	4.5	9.0
Yeast	1.5 – 3.5	4.0 – 6.5
Molds	1.5 – 3.5	8.0 – 11.0

Source: Adams and Moss 2000

According to Khaled *et al.* (2016), the stress on the bird during slaughter causes high post-rigor meat pH. It has the ability to shorten the shelf life of chicken meat by six days and this is attributed to the fact that spoilage bacteria multiply rapidly on the chicken that is at pH of 6.2 than normal post-rigor pH of 5.4 – 5.6. According James (2005), chicken has higher pH (5.6) than fish (6.2 - 6.6), hence it has a longer shelf life than fish when refrigerated at the same temperature.

2.4 Legislations governing the poultry processing industry

2.4.1 Industry overview

The art of brining processed chicken is regulated differently in various countries and these regulations include labelling requirements, maximum injection levels, prescription of additives and nutritional compositions. According to Guerrero-Legarreta (2010) and Miller (2011) the ingredients added need to be listed and their specific amounts using correct measurements. The solution added into products greater than 10% should be labelled as marinated or deep blasted. The Department of Health (2010) issued a publication on regulations for labelling and advertising of processed products. This has led the South African broiler processors to disclose all the ingredients added to their processed products, starting from 01 March 2012. The regulations required declarations of the quantity of ingredients added in terms of percentages and water on the front part of the

label of processed meat. Government regulations have defined raw processed meat as raw meat products from all species of meat animals and chicken intended for human consumption in South Africa, that resembles a cut, joint, slice, portion or carcass of meat, cured or uncured, or a combination thereof, pre-packaged or not, that has not undergone any heat treatment and where any added ingredient and/or additive and added water, including brine, is retained in or on the product as sold, but excludes products covered by the SANS 885 standard. The quantities of additives added must be listed on the packaging materials (South Africa Department of Health, 2010).

According to New Zealand Food Safety Authority (2009), there must be strict regulations for injection pick-up and levels be set for specific additives for all food processing industry. The Brazilian government have banned the practice of brine injection as it has struggled with establishing effective control measures. The Brazilian Federal Inspection Committee has stated that brine is only allowed for chicken with a mass of 2.5kg or more, produced specifically for the festive season and for those chicken roasted before they are sold. The requirements of importing the processed birds guide product formulation.

2.4.2 South Africa

In South Africa, the Department of Agriculture, Forestry and Fisheries has introduced new regulations on acceptable maximum brine injection levels in terms of the Agricultural Products Standard Act 119 of 1990 as published under Government Notice R471 in Government Gazette 39944 of 22 April 2016. These regulations include, among others, the following major changes for producers of both fresh and frozen poultry meat:

- The total brine injection allowed for whole carcasses is limited to maximum 10% (versus the maximum of 8% that was previously prescribed).
- Total brine injection allowed for individual portions is limited to a maximum of 15% (no limit was previously prescribed).
- The product label shall include a true description of the added formulated solution.
- Producers must regularly perform tests to ensure compliance with the new water uptake and injection limits and must keep records thereof for at least 1 year for auditing purposes (Venter, 201).

The South Africa Poultry Association (SAPA), as well as the Association of Meat Importers and Exporters, as representatives of the poultry industry, were against the amendments arguing that (Venter, 2016):

- There was no scientific basis for the brine limits.
- Alternatively, the scientific basis relied on for the determination of the brining limits was fundamentally flawed.
- There was no consideration of the economic impact of the brining cap.
- There was no consideration of the reports that were submitted by SAPA.
- The regulations made arbitrary distinctions in respect of different categories of poultry; and that
- The regulations were incapable of proper enforcement.

The South African poultry industry has acts that govern meat-processing industry and all producers must be abided by. That is, the Meat Safety Act, 2000 (Act 40 of 2000) which

caters for implementation of hygiene management systems and involves the following regulations.

The processing plants must (NDA, 2007):

- Provide the provincial executive officer with a documented Hygiene Management System containing detailed information on control measures to monitor identified control points, including the methods of monitoring or checking these control points, for approval; provide relevant records of observations, checks, measurements or results.
- Provide sampling programs for laboratory analyses, as well as names of laboratories to do the required analyses
- Provide written accounts of decisions relating to corrective actions when taken, and assess the hygiene status of the abattoir by means of the Hygiene Assessment System (HAS) and
- Provide results to the provincial executive officer for verification as frequently as he or she may require.

2.5 Chlorine Antimicrobials

The high volumes of chicken contamination are associated with high levels of microbial loads and this has become an imperative issue as it is directly related to the safety of the product. The spoilage of chicken meat due to bacterial contamination is considered an economic burden as it may lead to huge financial losses to producers and cause health problem for consumers (Bolton *et al*, 2014; Duan *et al.*, 2016). Therefore, the strict

application of good hygienic decontamination practices to reduce the level of contamination during the processing of chicken depicts a major exercise for the poultry processing industry. Antimicrobial selection is the basis of decontamination interventions. Chlorine dioxide is the mostly used antimicrobial for commercial poultry processing due to its efficacy, availability, safety and affordability (Chen *et al.*, 2014).

Nonetheless, its high organic load decreases its effect (Purnell *et al.*, 2013). Furthermore, some researchers have reported some health concerns caused by the carcinogenic potential of its byproducts such as trihalomethanes (Burfoot *et al.*, 2015). Hence, it is advisable to look for alternative antimicrobials. Aqueous chlorine and acidified sodium chlorite were reported to have extensive bacterial effects with several advantages over chlorine dioxide and this presents an opportunity for them to be used as a potential replacement (Duan *et al.*, 2016). History shows that transport and process water apparatus have generally been treated with hypochlorite and chlorine dioxide. These chlorinated solutions are generated active in the prevention of unwanted microbial growth (Bolton *et al.*, 2014). Nevertheless, the demand of these chlorinated antimicrobials is very high since they tend to be rapidly consumed by the high organic load, which is included in fruit and vegetables.

Moreover, when consumed, the decomposition of compounds like chlorine dioxide secretes by-products such as chlorites and chlorates while hypochlorite produces trichloromethanes, which is toxic in very low amounts (Purnell *et al.*, 2013). According to a study conducted by Northcuth *et al.* (2005) when chlorine is added to the water at 50 mg/l inside-outside bird spray wash station it does not have any effect on the *E. coli*, *Salmonella* or *Campylobacter* when compared to unchlorinated control. It was concluded

that physical removal from washing may be equally important as chemical inactivation for these bacteria. Moreover, Berrang *et al.* (2007) have reported that when the chlorinated spray is used before evisceration did not have an effect on the post-chill number of bacteria in commercial processing plants. However, chlorination by means of immersion in a chill-tank has resulted in lower numbers of bacteria on processed carcasses. Hinton *et al.* (2007) have conducted a study wherein electrolyzed oxidising, chlorine and tap water were used to treat poultry carcasses to reduce microbial load. It was found that fewer Psychrotrophic bacteria and yeast were recovered from carcass treated with EO than from carcass treated with tap water. Table 2.5 below gives an illustration of the findings.

Table 2.5: Bacterial counts (log₁₀ cfu/mL) recovered on plate count agar incubated at 4°C for 10 d from rinsates of carcasses sprayed with tap, chlorine, or electrolyzed oxidising (EO) water and stored at 4°C.

Storage time (d)	IOBW ² spray treatment		
	Tap water	Chlorinated water	EO water
0	1.54 ± 0.56 ^{c,x}	0.99 ± 0.01 ^{c,y}	0.99 ± 0.01 ^{b,y}
3	2.15 ± 0.92 ^{c,x}	1.02 ± 0.12 ^{c,y}	0.99 ± 0.01 ^{b,y}
7	4.12 ± 1.10 ^{b,x}	1.71 ± 0.64 ^{b,y}	1.17 ± 0.40 ^{b,y}
14	6.82 ± 0.68 ^{a,x}	5.08 ± 0.40 ^{a,y}	4.60 ± 0.46 ^{a,z}

^{a-c}Within columns, different superscripts indicate significant differences ($P \leq 0.05$).

^{x-z}Within rows, different superscripts indicate significant differences ($P \leq 0.05$).

¹Values are average log₁₀ cfu ± SD (n = 18).

²IOBW = inside-outside bird washer.

Source: Hinton *et al.* 2007

Therefore, it was concluded that EO might be used as an alternative disinfectant to chlorinated water for reduction of the bacterial population of poultry carcasses. However, Sarjit and Dykes, (2015); Northcutt *et al.* (2003) has reported that chlorination of water used to spray carcasses has the ability to reduce the population of total bacteria on

carcass unlike electrolyzed oxidising water, which reduces a few at the initial processing stage.

2.5.1 Chlorine Dioxide

Chlorine dioxide is the rarest antimicrobial. It is an oxidising agent with a low redox potential. As a disinfectant, it is added to water in an amount up to 50mg/L in order to maintain a residual concentration of 2.5 mg/L (USDA, 2002). The competency of this disinfectant is not affected by pH of the solution. It is used in both spraying and washing methods and in chiller bath for dipping, to reduce microbial cross contamination (SVCPPH). It is a very active disinfectant and is transformed into chlorite and chlorate ions (ratio 7:3). After processing and usage, only 2.5 mg/L remains as chlorine dioxide, which is equivalent to 5% of the initial content (Burfoot *et al.*, 2015). This chlorinated disinfectant is an oxidising agent that is greatly influenced by the pH of the environment. Chlorine dioxide mainly applied to carcasses in on-line sprays at levels up to 50mg/L, to maintain a residual concentration of 2.5 mg/L (SCVPPH, 2003).

2.5.2 Acidified Sodium Chloride (ASC)

This is one of most effective disinfectant used in poultry processing industry and is applied through spraying or dipping. It is activated with any acid approved to be applied in the food industry at levels sufficient to provide solutions with pH values in the ranging from 2.3 to 2.9 for either a 15-second spraying or 5-8 second dipping, at a concentration of 500-1200 mg/L. When applied in chilling water for immersion, the amount acceptable is 150 mg/L at pH between 2.8 and 3.2. The average time for poultry to spend in a chiller is an hour, and 3 hours is regarded as the maximum (USDA, 2002). One of the main active constituents of ASC solution is chlorous acid that is a very strong oxidising agent, stronger

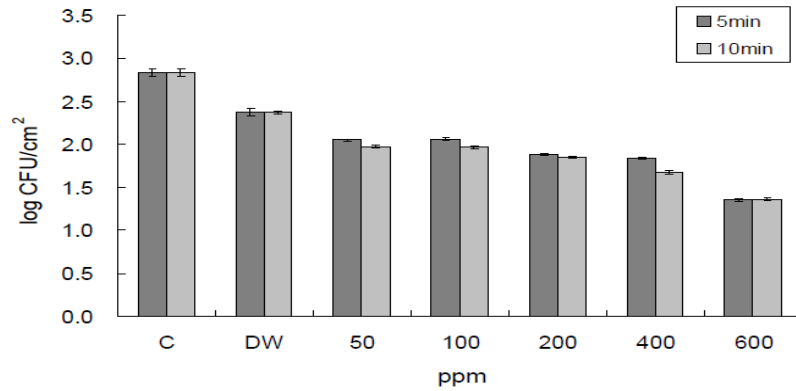
than both chlorine dioxide and chlorine. Its level is depended upon the pH of the solution. Purnell *et al.* (2013) reported that 31% is formed at pH 2.3, 10% at pH 2.9 and lastly 6% at pH 3.2. The production of chlorine dioxide is limited to less than 3mg/L in a solution (Duan *et al.*, 2016).

According to Schneider *et al.*, 2002 the currently used levels are as follows:

- 50-150 mg/L ASC for the whole carcass of poultry
- 500-1200 mg/L ASC for carcass parts of poultry
- Equivalent permissions are in place in USA and Canada (Food Standards Australia New Zealand, 2003).

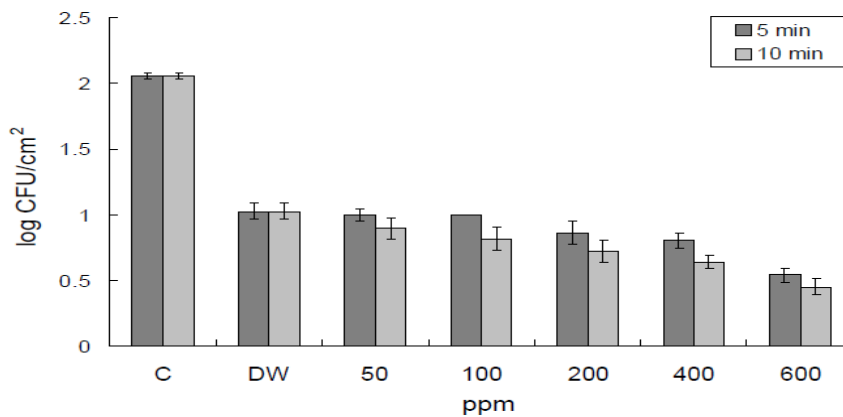
The USDA and FDA have approved ASC as a disinfectant to be used in meat industry owing to its use for antimicrobial intervention on red meat, poultry, seafood and fruit and vegetable through spraying or dipping. It works in ion-based solution by tearing sulphide and disulphide linkages and directly attaches the components of bacterial cells (Hosseinjeda and Jafari, 2016). Lee (2008) has conducted a study in which he determined the effectiveness of the ASC on Mesophilic and Psychrotrophic bacteria. It was found that ASC reduced the number of bacteria according to the amount of time the samples were dipped. Tables 2.6 and 2.7 below illustrate.

Table 2.6: Immersion tests against Mesophiles on whole carcass dipped into ASC solution for 5 or 10 minutes.



Source: Lee 2008

Table 2.7: Immersion tests against Psychrotrophs on whole carcass dipped into ASC solution for 5 or 10 minutes.



Source: Lee 2008

It was concluded that treatment of white meat with ASC has a direct effect on reducing naturally occurring microbial populations on the skin of the white meat. It is suggested that further investigation is done to determine the shelf life of ASC treated white meat and microbial quality (Sarjit and Dykes, 2015; Lee, 2008; Kutu, 2014; Hinton *et al.*, 2007).

2.5.3 Aqueous chlorine

Chlorinated water is used in poultry processing for spray washes of the carcasses during primary processing and for immersion chilling at the end of primary processing. The following measurements are used in commercials (Stephan, 2012):

- For spraying of carcasses with aqueous chlorine is 45-80 mg/L; and
- Aqueous chlorine for immersion chiller is 45-80 mg/L, with a residence time of 70-75 minutes.
- Aqueous chlorine of water exiting the immersion is 0.5-5 mg/L.

This chlorinated disinfectant has the following setbacks when used to prevent spoilage (Burfoot *et al.*, 2015):

- It is relatively ineffective against Salmonella
- Its ability to act as a corrosive on plant machinery
- Its ability to combine with organic matter to generate mutagenic substances.

CHAPTER THREE: MATERIALS AND METHOD

3.0. MATERIAL AND METHODS

3.1 Study area

This study area is divided into two phases, wherein phase one involves the purchasing of processed chicken from three selected commercial poultry abattoirs which are referred to as source A, source B and source C. All of the three abattoirs are high through-put and based in the Limpopo (Source A and B) and Gauteng (Source B) provinces of South Africa. The second phase of the study is microbial analysis, where the psychrotrophic bacteria load was measured in a private SANAS-approved laboratory in Gauteng, South Africa.

3.1.1 Source A

This processing plant is located in the northern part of South Africa and slaughters approximately 40 000 birds a day, with weekly placements of 210 000 day-old chicks in its broiler production division. Geographically, the abattoir is located in Polokwane Municipality, in Limpopo's Capricorn District, which covers an area of 106.84 square kilometres (41.25 sq. mi) with a population of 150,028 (Black African: 74.4%, Covered: 3.7%, Indian/Asian: 3.1 and White: 18.2). The ambient temperature around the study area ranges from 24.7 °C to 38.4 °C in summer and from -3.5°C to 17.7 °C in winter. The precipitation of the study area is about 478mm per annum.

3.1.2 Source B

This poultry abattoir is located in the Waterberg District of Limpopo. Geographically, the area covers 27.18km² (10.49 sq mi) and has a population size is 24 853 (Black African: 88.1%, White: 0.4%, Coloured: 16.16%, Indian: 0.2% and White: 10.9%). Annual precipitation of the study area is 450mm, with ambient summer temperature ranges of 23.9 °C to 34.5 °C and winter ranges of 5 °C to 23.9 °C.

3.1.3 Source C

This processing plant is located in central Gauteng (Ekurhuleni). Source C is an integrated business that slaughters 2.89 million broilers per week. The population size is 354.39 per square kilometres (black African = 64.65%, white = 18.18%, coloured = 16.16% and other = 1%). The annual precipitation of the area is 500mm and temperatures range as follows: summer 20 - 31.1 °C and winter 16.6 – 26.2 °C.

3.1.4. Laboratory

The tests for this study were carried out in a privately-owned biotechnology laboratory, located in central Gauteng. It is SANAS-accredited for a wide range of microbial tests. Its testing scope broadens from various microbiological, molecular and serological techniques, nutritional and chemical testing, and hygiene testing, analytical milk testing to food safety monitoring. The area covered is about 394.88 square kilometres with a population size of 396,580 (Black African: 29.3%, Coloured 2.3%, Indian/Asian: 8.4%, White: 59.0% and other: 1.0%). The annual rainfall is 500mm and ambient temperature ranges of 24.5 to 33.2 °C in summer and 1.2 to 15.2 °C during winter.

3.2 Experimental procedure

3.2.1 Brining: The injection process

The brine solution is prepared in two stainless steel tanks fitted with stirrers. These tanks are connected to a tumbling machine wherein the brine solution is deposited using a flexible hose. The brine solution is pumped through stainless steel to the injecting machine, referred to as a makeup tank. From the makeup tank, it flows into an open tank connected with a stainless steel mesh filter. From the filter, the brine solution is pumped by injection needles. The solution that is not well absorbed by the meat during the injection process is absorbed by stainless steel mesh belt to a tank underneath the belt and returns to the filtering tank through a flume that directs the filter. The filtered solution is recirculated through the equipment and the volume of solution is kept constant by addition of new solution from the make-up tank. The chicken portions are loaded into the injecting machine (tumbling) from stainless steel tubs. The injected portions are also collected in the same type of tub transfer to a tabling machine. The injecting machine is connected to

the computer whereby the amounts of injections are regulated at different levels according to the customers' needs. Figure 3.1 below is a picture of Fomaco brine injection machine used.



Figure 3.4: Fomaco brine injection machine

3.2.2 Sample preparation at the abattoir

Freshly slaughtered mixed portions were purchased from the three sources mentioned earlier. A basic brine solution was prepared in the following brining concentration levels of 0%, 15%, 20%, 25%, 30% and 35% as treatments. Each treatment was replicated 6 times comprising of 1.8kg freshly processed broiler from the abattoir. Each replicate was automatically injected with the appropriate brining level subject to the given level of brine treatment into the mixed portions. The 0% treated samples were taken as a control group and the experiment designed as a completely randomised design (CRD), with brining levels being the treatments, each replicated 6 times, and the Psychrotrophic bacteria load measured after the treatment as the experimental output. After 6 months of preservation at -5°C refrigeration, the chicken was thawed and each sample tested for Psychrotrophic bacteria, recorded and analysed for brining effect.

3.2.3 Sample preparation at the laboratory

After 6 months of refrigeration, the samples were thawed through cold water thawing. Prior to the thawing, the samples were placed in a leak-proof plastic bag, which served to stop direct contact with water from damaging the meat tissue and preventing bacterial infection. A large bowl was filled with water and the sample bag was submerged and left overnight, then the samples were subjected to the Psychrotrophic count test at the laboratory. The flowcharts below depict the this process.

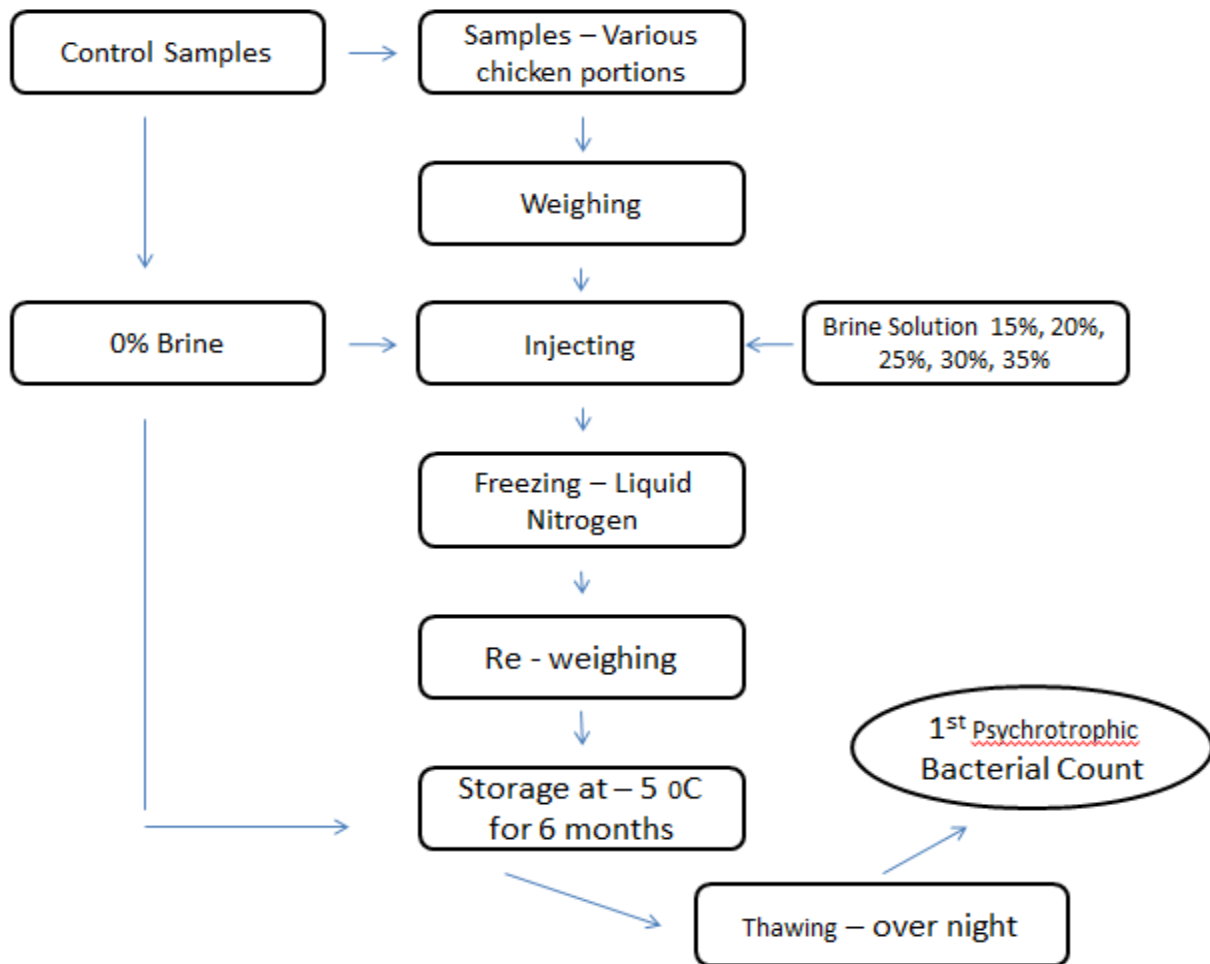


Figure 3.2: Flow diagram of sample preparation for the Psychrotrophic bacterial count.

3.2.4. Chlorinated antimicrobials:

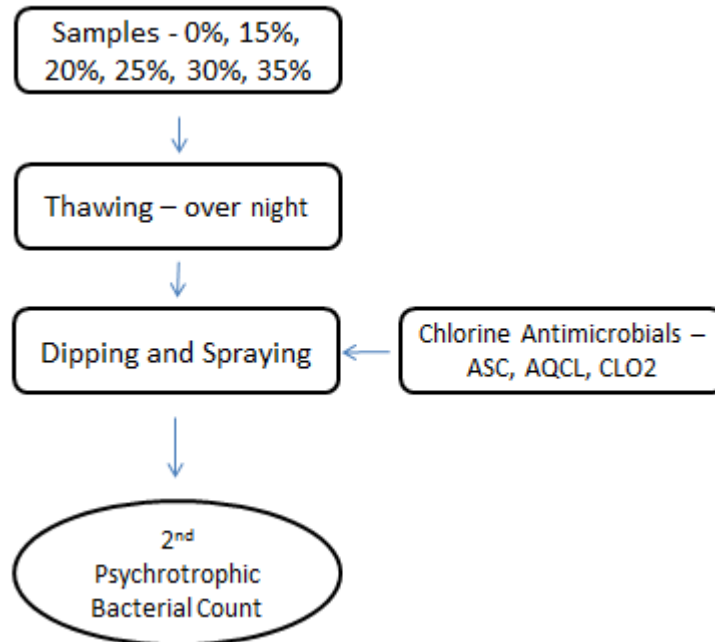


Figure 3.3: Flow diagram of sample preparation outline to second Psychrotrophic count.

Three chlorinated disinfectants viz Chlorine dioxide, Aqueous Chlorine, and Acidified Sodium Chlorite were tested on the thawed broiler packs after 6 months of preservation at -5°C refrigeration. The disinfectants were used in a CRD design with the 3 different disinfectant types as treatments, each replicated 2 times per treatment. This was achieved by sampling randomly two 1.8kg thawed broiler packs (replicates) out of 6 from each treatment earlier brined and assigning them to each disinfectant type. For each of the chlorinated disinfectants viz Acidified Sodium Chloride, Aqueous Chlorine and Chlorine Dioxide, each replicate was sprayed for 15 seconds at a concentration of 50mg/L. After treatment samples were allowed to dry and then subjected to Psychrotrophic bacterial test to determine the level of bacterial infection. This was recorded and analysed for chlorinated disinfectant effects.

3.3 Data Collection

3.3.1 Bacterial identification at the laboratory

The whole rinse procedure was used to recover the Psychrotrophic bacteria from the chicken portions according to Murad et al. (2017). Chicken portions (either drumstick, breasts or wings) were randomly and aseptically picked, weighed from each replicate (2 per replicate) and placed in a sterile plastic bag with 300ml of sterile, 0.1% Bacto peptone (Difco, Detroit, MI 48232) solution added and homogenized for 1 minute. Samples of rinsates were decanted from each bag for bacterial analysis. 0.1% peptone water was used for serial dilutions of the rinsates, and plated on Iron Agar (Atlas) with Pseudomonas Agar base supplemented with centrimide-fucidin-cephaloridine (C-F-C) SR103 (Oxoid, Basingstoke, Hamsphere, England). The Pseudomonas Agar plates (Iron Agar) were incubated at room temperature (25°C) for three days. The bacteria colonies were then isolated and subcultured onto an Agar media consisting of BBLTM Trypticase Soy Broth (BBL and Agar 2015) and 1.5% BBL Granulated Agar (BBL) at 28°C for 24 hours. After incubation of Agar plates at 28°C for 24 hours, the MIDI Sherlock Microbial Identification System (MIDI 2004) (MSMIS) was used to identify the isolates based on the number of bacteria per centimeter squared of rinsates on the colony forming units (cfus). The MSMIS functions by using gas chromatography for identification and quantification of fatty acids extracted from the cellular membrane of unknown bacteria. It also identifies the bacteria through comparison of bacterial profiles stored in the library of the MSMIS. The Psychrotrophic bacterial count due to different brining levels was identified and recorded, before the count due to chlorine antimicrobials treatment. The number of spoilage bacterial count identified was recorded and analyzed for brining and chlorinated antimicrobial effects. The MIDI Sherlock MIS functions by using the gas chromatography for identification and quantification of fatty acids extracted from the cellular membrane of unknown bacteria. The Sherlock program identifies the foreign bacteria through comparison of bacterial profiles stored in the library of the MIDI Sherlock MIS. The process was done before samples were treated with antimicrobials and repeated after samples were treated with antimicrobials. Below is the detailed experimental design of the study.

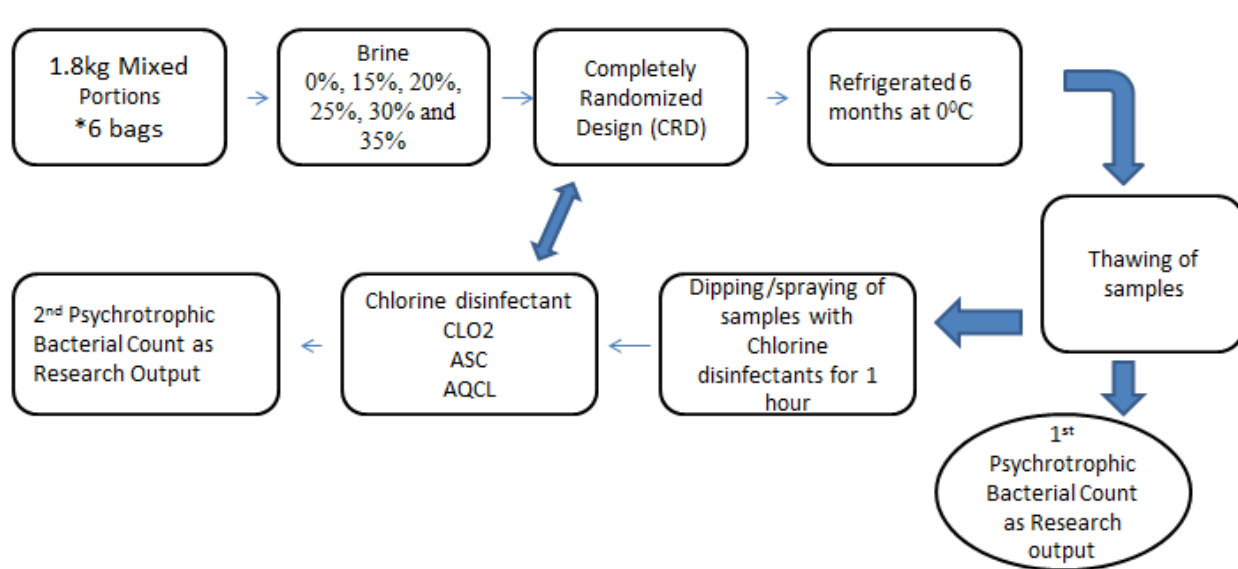


Figure 3.4: Flow diagram of the entire experimental design of the study.

3.4 Statistical analysis

The effects of brining levels and chlorine antimicrobials on the Psychrotrophic bacterial loads of chicken from abattoirs in South Africa were analysed with the general linear model (GLM) procedures of the SPSS (2017). The statistical model was:

$$Y_{ijk} = \mu + T_1 + \Sigma_{ijk}$$

Where: Y_{ijk} = the overall observation (Bacterial load)

μ = population means

T_1 = effect of brining and antimicrobial chlorine

Σ_{ijk} = residual effect.

Where there was a significant F-test ($P < 0.05$), the least significant difference (LSD) method was used to separate the means according to SPSS (2017).

The related responses in Psychrotrophic bacterial load to brine levels were modelled using the following quadratic model as stated below:

$$Y = a + b_1x + b_2x^2$$

Where Y = Psychrotrophic bacterial load; a = intercepts on Y axis; b = coefficient of the quadratic equation; x = brining levels in percentages and $b_1/2b_2 = x$ value for optimal responses. The quadratic model was fitted to the experimental data by means of the nonlinear model (NLIN) procedure of SPSS (2017). The quadratic model was used because it gave the best fit.

CHAPTER FOUR: RESULTS

4.1 Brine

The effect of the brining levels on bacterial load was the same across all three major abattoirs (Table 4.1). At 0% brine, the bacterial load was higher ($P < 0.05$) than any other levels in all three abattoirs. From Gauteng and Limpopo 1 abattoirs, there was no significant difference in bacterial load at 15% and 20% brining levels. However, from Limpopo 2 abattoir, there were differences ($P < 0.05$) of bacterial load between 15% and 20% brining levels. Furthermore, the result has indicated no significant difference of Psychrotrophic bacterial load between 20% and 25% across all samples from three abattoirs. The brining levels between 25% and 30% have different effects on the bacterial load ($P < 0.05$) across all three abattoirs. However, the brining levels of 30% and 35% do not have different ($P > 0.05$) effects in terms of reducing bacterial load (Table 4.8). This implies applying more brine (35%) does not guarantee a less load of Psychrotrophic bacteria but a likely increase in the weight of chicken meat, which in turn reduces the quality.

Table 4.1: Effects of different levels of brining on Psychrotrophic bacteria load ($\log_{10}\text{CFU}/\text{cm}^2$) of chicken from 3 commercial chicken abattoirs in South Africa.

Abattoirs	Brining Levels (%)						SEM	Std Dev.	P-Value
	0	15	20	25	30	35			
Gauteng	24.83 ^a	17.17 ^b	16.33 ^{bc}	15.83 ^c	14.67 ^d	14.17 ^d	0.619	3.715	0.0001
Limpopo 1	24.83 ^a	17.67 ^b	17.17 ^{bc}	16.33 ^c	13.83 ^d	13.33 ^d	0.652	3.912	0.0001
Limpopo 2	24.00 ^a	17.33 ^b	16.17 ^c	16.00 ^c	14.50 ^d	13.67 ^d	0.584	3.505	0.0001

^{a,b,c} : Means in the same row not sharing a common superscript are significantly different ($P < 0.05$)
 SEM= Standard error of Mean, P- value = Probability value, ClO_2 = Chlorine Dioxide, Aq. Cl = Aqueous Chlorine, ASC = Acidified Sodium Chlorite

4.2 Chlorine Antimicrobials

There was no significant difference found among the means of the three chlorine antimicrobials used in different abattoirs independently (Table 4.2). This implies that since all three antimicrobials were based on chlorine, their effectiveness in terms of reducing Psychrotrophic bacteria was somewhat similar. Acidified Sodium Chlorite has reduced slightly more bacteria during treatment but the difference was not statistically significant across all abattoirs when they were compared individually.

Table 4.2: Effects of chlorine antimicrobials on Psychrotrophic bacteria load ($\log_{10}\text{CFU}/\text{cm}^2$) of chicken from 3 commercial chicken abattoirs in South Africa

Chlorine Antimicrobials						
Abattoirs	ASC	Aq. <i>CI</i>	<i>ClO</i> ₂	SEM	Std Dev.	P-Value
Gauteng	12.17	14.92	14.17	0.667	4.003	0.225
Limpopo 1	12.33	14.50	13.58	0.589	3.533	0.330
Limpopo 2	12.33	14.67	13.92	0.621	3.728	0.302

^{a,b,c} Means in the same row not sharing a common superscript are significantly different ($P < 0.05$) SEM= Standard error of Mean, P- value = Probability value, Aq. *CI* = Aqueous Chlorine, ASC = Acidified Sodium Chlorite, *ClO*₂ = Chlorine Dioxide.

4.3. Overall effects of brining and chlorine antimicrobials on Psychrotrophic bacteria ($\log_{10}\text{CFU}/\text{cm}^2$).

The control samples which were not injected with brine (0% brine) had higher ($P < 0.05$) bacterial load than all samples injected with various injection levels (Table 4.11). Overall results indicated a difference ($P < 0.05$) in bacterial load among samples injected with 15% and 20% brine across all abattoirs. However, there was no difference ($P > 0.05$) between samples injected with 20% and 25% brine. Conversely, there was a difference ($P < 0.05$) in terms of Psychrotrophic bacterial load between the samples treated with 25% and 30% brine in all abattoirs. Furthermore, the result has also indicated a significant difference between samples treated with 30% and 35% brining levels. As depicted in the

results (Table 4.11), on average there was a significant difference in bacterial load across all three selected commercial abattoirs for different inclusion levels. However, there was no significant difference of bacterial load from samples treated with 20% and 25% across the selected abattoirs. Acidified sodium chlorite had significantly lower bacterial load than both aqueous chlorine and chlorine dioxide. On the other hand, aqueous chlorine had significantly higher bacterial load than both chlorine dioxide and acidified sodium chlorite. Chlorine dioxide had the second highest ($P < 0.05$) bacterial load, but a lower ($P < 0.05$) load when compared to aqueous chlorine.

Table 4.3: Effects of brining levels and chlorine antimicrobials on Psychrotrophic bacterial load (\log_{10} CFU/cm²) from abattoirs in South Africa

Psychrotrophic Bacteria Value	Brining Levels (%)						SEM	Std Dev.	P- Value
	0	15	20	25	30	35			
Load	24.56 ^a	17.56 ^b	16.56 ^c	16.06 ^c	14.33 ^d	13.72 ^e	0.621	3.726	0.0001
Chlorine Antimicrobials	Chlorine Antimicrobials								
	ASC	Aq. CI	ClO ₂						
Load	12.28 ^c	14.69 ^a	13.89 ^b						

^{a,b,c} : Means in the same row not sharing a common superscript are significantly different ($P < 0.05$) SEM= Standard error of Mean, P- value = Probability value, Aq. CI = Aqueous Chlorine, ASC = Acidified Sodium Chlorite, ClO₂ = Chlorine Dioxide

4.4 Analysis of optimal inclusion levels of brining using quadratic function

The results of the quadratic model regression analyses of the effect of the brine on the Psychrotrophic bacteria are presented in Table 4.3. The effect of percentage brining on average Psychrotrophic bacterial count had minimum quadratic values of $24.45 - 0.517$ brining + 0.805 brining² with $r = 0.995$; $r^2 = 0.989$ and optimum percentage brining dose of 43.08%. This is the most optimal brining level to achieve the lowest Psychrotrophic

bacterial load. The coefficient of determination r^2 is regarded as being high. This implies that high levels of brining have greater influence in reducing bacterial load.

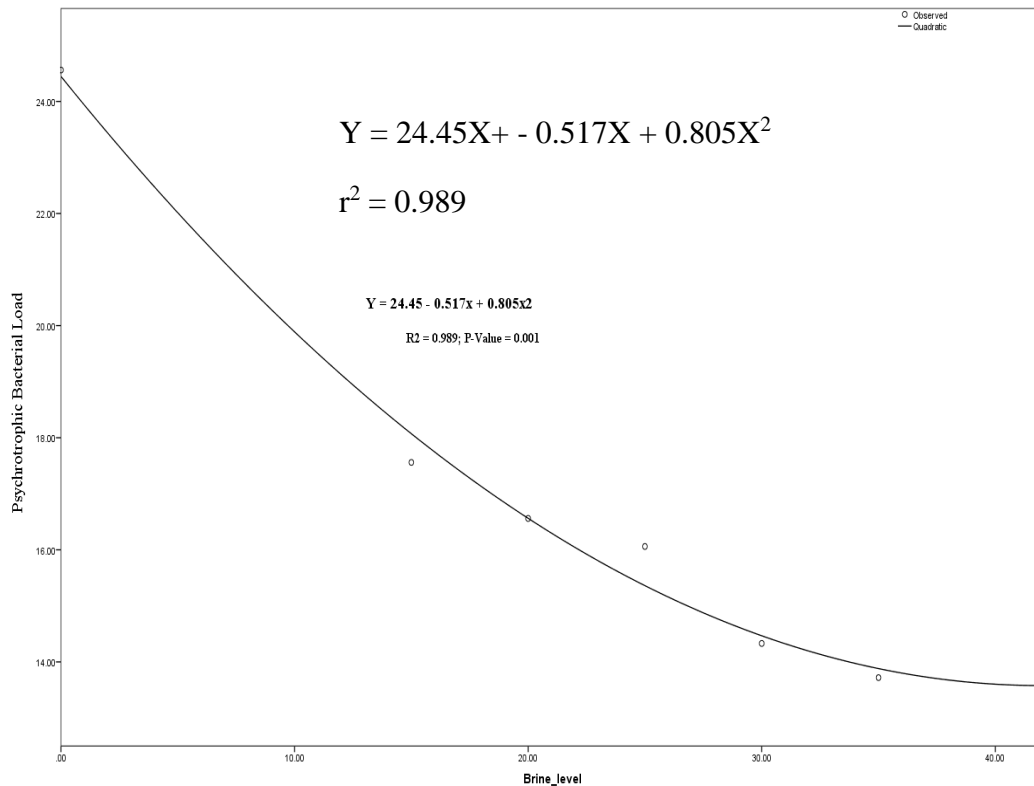
Table 4.4: The optimal brining levels using quadratic model

Trait	R ² values	Optimal Y levels	Optimal brining levels (%)	P - Value
P. Bacterial Load	0.989	1495.18	43.08	0.001

P. bacteria = Psychrotrophic bacteria load, R² = co-efficient of determination, P- Value = Probability value.

As indicated below in figure 1, the optimisation function shows that brining has an inverse effect on the number of bacterial infections that develop during refrigeration.

Figure 4.1: Quadratic function showing the optimum brining levels of quick frozen chicken of three selected commercial poultry abattoirs in South Africa



CHAPTER FIVE: DISCUSSION

5.1 Brine

The aim of this study was to determine the optimal brining levels for inclusion when producing individually quick frozen chicken portions. This was determined by counting the Psychrotrophic bacterial load ($\log_{10}\text{CFU}/\text{cm}^2$) found after treatment with different levels of brine. Therefore, the results of the current study show that brining has an effect on increasing the shelf life of individually quick frozen chicken portions by reducing the microbial load in the meat. The development of Psychrotrophic bacteria in non-marinated chicken increase rapidly when refrigerated (Knöchel *et al.*, 2009). However, brining chicken impedes the formation of a colony for spoilage bacteria and bacterial development remains below 103 cfu/g (Knöchel *et al.*, 2009; Vangelova and Dragoev, 2014). Current data shows that the control samples had significantly higher bacteria load than any other of the treated samples in the study (Table 4.1). Similar effects were demonstrated by Kutu (2014) and Perlo *et al.*, (2010) when they compared the cured chicken portions to uninjected control portions. The present data indicate that an increase in brine results in a decrease of microbial organisms within the chicken portions up to 43% brine inclusion level (Table 4.4). Beyond this, the efficacy of the brine is weakened and the quality of the chicken is compromised. Moholisa (2011) reported that high moisture content in samples treated with 60% brine resulted salt dilution. However, Kutu (2014) reported that portions treated with 60% brine were tenderer than those treated with lesser brine inclusion levels. The current study shows that different inclusion levels significantly affect the microbial load formation in chicken portions. This is associated with different composition and amounts of solutions on chicken microflora and gram-negative bacteria, as well as their sensitivity to acid conditions (Choi *et al.*, 2009). Similar findings were reported by Kutu (2014) on the effects of different brining levels on the chicken meat quality. The results of the present study have shown insignificant differences in Psychrotrophic bacterial load among samples from three commercial abattoirs under study (Table 4.1). This may be associated with environmental conditions of the origins of each sample and handling procedures of different processing plants (Bauermeister,

2015). Overall, the results indicate that brine has an effect on spoilage bacteria when the chicken is refrigerated for longer periods. The study reveals that the control samples (0%) have significantly greater bacterial load than all samples that were injected with brine (Table 4.1). The results also reveal a significant difference in Psychrotrophic bacterial load among samples injected with various levels of brine, i.e. 0%, 15%, 30 and 35%. However, there was no significant difference in terms of Psychrotrophic bacterial load found from samples injected with 20% and 25% (Table 4.1).

5.2 Salts

Anderson *et al.*, (2007) reported that salt within brine was introduced to reduce the rate of deterioration by reducing the growth of microorganisms in the chicken. High concentration of salt has proven to inhibit microbial spoilage in white meat. This is in line with the current study as high injection levels of brine results in greatly reduced loads of Psychrotrophic bacteria (Table 4.3). The primary preservation agents of brine are salt and sugar, which do not only prevent spoilage of the chicken but serve to inhibit the growth of pathogens and spoilage bacteria when applied correctly (Pichpol, 2009; Purnell *et al.*, 2013).

These preservative agents have antimicrobial mechanisms which capacitate them to disrupt microbial enzyme activity, weakening their DNA structure. Sugar's preservative mechanism is to accelerate the accumulation of antimicrobial compounds from the growth of spoilage microorganisms. This is achieved through, amongst others, the conversion of sugar molecules to organic acids by lactic acid bacteria (Oyarzabal, 2012; Vangelova and Dragoev, 2014.). Moreover, Vangelova & Dragoev (2014) reported that the development of spoilage bacteria depend on the presence of lactic acid bacteria solutions. This explains why the greatest reduction of Psychrotrophic bacteria is found at 43.08% brining level (Table 9). This also explains the strong relationship between brine and Psychrotrophic bacterial load, with a high coefficient of determination of 0.989. High levels of brine increase the salt content of the chicken portions. Kutu (2014) has reported that all samples injected with brine have had more salt than control samples not injected with brine, with NaCl ranging from 270mg/100g to 620mg/100g. This high level of NaCl in individually quick frozen chicken portions has made the chicken product to fall out of low

sodium content product category. In South Africa, a product containing any salt more than 120mg/100g sodium is regarded as a high sodium content foodstuff, which should be labelled as such on the product packaging material (South African Department of Health, 2010).

5.3 Antimicrobials

Chlorine is a widely used antimicrobial in poultry processing industry and is effective against a whole range of microorganism in poultry process water (Purnell *et al.*, 2013). It is regarded as the most effective antimicrobial used in commercial poultry processing industry to control bacterial, viral and protozoan pathogens (Duan *et al.*, 2016; Purnell *et al.*, 2004). However, it is reported that the efficacy of the antimicrobials is affected by the level of attachment of bacterial infection on the chicken skin. This factor might have a slide contribution to the results as the samples were selected randomly irrespective of their positions for analysis (Tamblyn & Conner, 1997). It is reported that chlorine dioxide, when used in commercial operations, has the potential to reduce 2 to 3 log reductions in microbial levels in poultry chiller (Mitchell *et al.*, 2010). Thus, the second objective of this research was to determine the most effective antimicrobial to be applied in the poultry processing industry.

The present study indicates that all three antimicrobials had insignificant differences in terms of microbial load when abattoirs are compared individually. This is associated with the fact that all three chosen antimicrobials originated from chlorine as a base. However, Acidified Sodium Chloride had reduced more bacteria than all other antimicrobials but the variation in load was not statistically significant (Table 4.2). This is attributed to the fact that ASC is in acidic format, a form which is very convenient to lactic acid bacteria that is capable of causing a great reduction of Psychrotrophic bacteria in white meat (Oyarzabal, 2012; Vangelova and Dragoev, 2014). Results of the study indicate that, when effects of antimicrobials are combined across all abattoirs to give overall effects, there were significant differences observed. Acidified Sodium Chlorite has significantly lower bacterial load than all other antimicrobials. Chlorine Dioxide was the second most effective antimicrobial which had significantly lower bacterial load than Aqueous Chlorine which proved to be the least effective antimicrobial (Table 4.3). This was in agreement

with the findings of Mitchell *et al.*, (2010) when they determined the effectiveness of Chlorine Dioxide against Aqueous Chlorine.

Chlorine dioxide is less affected by the pH (acidity or basicity) of the meat and organic matter. Chlorine dioxide is 20 times more effective than chlorine at reducing microbes, has more oxidising power and does not react with ammonia to form chloramines (Purnell *et al.*, 2013). Mitchell *et al.* (2010) reported that acidified sodium chloride has the potential to reduce 99.2% of bacterial load when applied on poultry carcass. Such reduction is found in post chill dip applications. The current data indicates that acidified sodium chloride is the most effective antimicrobial for application in the poultry processing industry.

The effect of percentage brining on average Psychotrophic bacterial count had minimum quadratic values of $24.45 - 0.517 \text{ brining} + 0.805 \text{ brining}^2$ with $r = 0.995$; $r^2 = 0.989$, with optimum percentage brining dose being 43.08%. (Table 4.4 and figure 4.1). Thus, it is clear from the results herein reported that brining levels above 43.08% will increase psychotrophic bacterial load on individually quick frozen (IQF) chicken portions and will have important consequences on the quality of the frozen chicken portions. However, although in disagreement with our findings, previous studies in frozen chickens on the optimum dose-response values of brining inclusion levels for optimizing different parameters have been dynamic, but trials in which parametric performance were achieved at variable brining inclusion levels predominate (Table 4.5). For example, studies by Kutu (2014) reported a value 5 % brine level when assessing the chicken meat for sensory properties. Similarly, the Department of Agriculture, Forestry and Fisheries, as the regulator of the poultry processing industry, proposed in draft regulations, that the brine content in frozen chicken products not exceed 15%. Futhermore, the South African

Poultry Association suggested 20% brine as the most accommodative amount for inclusion when producing individually quick frozen (IQF) chicken portions.

Table 4.5: Reported brining levels in South Africa

Brining levels (%)	Target	Study
5	Product Quality	Kutu (2014)
15	Neutralise	Venter, 2016 (DAFF)
20	Commercialisation	Venter, 2015 (SAPA)

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

This study has shown that increasing brining levels significantly reduces the rate of spoilage bacterial development in individually quick frozen (IQF) chicken portions and that different chlorine antimicrobials have different ($P < 0.05$) success rates at reducing Psychrotrophic bacteria in IQF chicken portions after 6 months of refrigeration. Precisely, it was observed that 43.08 % brine injection level is most optimal for inhibiting spoilage bacteria growth for refrigerated chicken. This implies that high levels of brine are very good for controlling spoilage bacteria and elongating product shelf life. However, as suggested by Kutu (2014) and Moholosi (2011) high levels of brine may reduce the quality of the chicken through dilution of nutrients such as proteins, fat and energy. Futherance to this, the current study has also shown that chlorine-based antimicrobials specifically Acidified Sodium Chlorite have the potential to reduce bacterial load. Its effectiveness, affordability and accessibility makes ASC a better preservative for chicken than other antimicrobials. These findings have a lot of implications on brining inclusion levels and the use of antimicrobials in frozen chickens. However, more studies should be done to ascertain these responses.

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