The effect of different sodium reduction strategies on the chemical, microbial and sensory quality of a traditional South African sausage

By

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Submitted in fulfilment of the requirements for the degree of

MAGISTER SCIENTIAE AGRICULTURAE

(FOOD SCIENCE)

in the

Department of Microbial, Biochemical and Food Biotechnology

Faculty of Natural and Agricultural Sciences

University of the Free State

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February 2019

DECLARATION

I declare that the dissertation hereby submitted by me for the M.Sc. Agric. degree in the Faculty of Natural and Agricultural Science at the University of the Free State is my own independent work and has not previously been submitted by me at another university/faculty. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

A. Rautenbach
February 2019

This thesis is dedicated to my parents

André & Elmeri

for making countless sacrifices in order for me to make every dream I ever had a reality

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The language, formatting and reference style of this thesis are in accordance with the requirements for the Meat Science journal

ACKNOWLEDGEMENTS

I would hereby like to express my most sincere gratitude and acknowledge the following persons and institutions for their assistance, contributions and continuous support and encouragement throughout the completion of this study:

My Supervisor, Prof. Arno Hugo, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for his guidance, never-ending patience, countless hours of work and exceptional knowledge and passion for the field of meat science.

My Co-supevisor, Prof. Celia Hugo, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for her guidance throughout the study, exceptional knowledge in the microbiological field, her keen eye for detail and her kind-hearted moral support when it was needed most.

My Co-supervisor, Dr. M. Cluff, for his insights and contributions in this study as well as BT Enterprises for the formulation and donation of the spice mixtures used in this study.

Dr. Carina Bothma and Mrs. Liezl van der Walt, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for their expertise and help with the sensory analysis.

Miss Eileen Roodt, the Department of Microbial, Biochemical and Food Biotechnology, for her kind assistance and mentoring in the Meat Science labs. Thank you for your friendship and endless support.

My lab colleagues and friends, the Department of Microbial, Biochemical and Food Biotechnology, Miss Stephani du Plessis and Miss Rita Myburgh for their friendship and support and for making even the dullest days better.

Mrs. Ilze Auld, for her always friendly assistance with any administrative task.

The Meat Industry Trust (MIT) and The National Research Foundation (NRF) for financial support.

My fiancée, Schalk, for love and support throughout my studies.

My parents, André and Elmeri, for their endless love, support and encouragement. Thank you for the countless sacrifices you made during my many years of study. I could not have done it without you.

Most important of all, my Heavenly Father for granting me the ability and opportunity. I have been blessed beyond measure. In Him I have the ability to achieve and overcome anything. *"When you pass through the waters, I will be with you; and through the rivers, they will not overwhelm you. When you walk through fire, you will not be scorched, nor will the flames burn you."* ~ Isaiah 43:2

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GLOSSARY OF ABBREVIATIONS

a*	Redness/greenness colour coordinate
AAS	Atomic absorption spectroscopy
$AgNO_3$	Silver nitrate
AgCl	Silver chloride
AMP	Adenosine 5'-monophosphate
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
a_w	Water activity
BPA	Baird-Parker agar
BPW	Buffered peptone water
°C	Degrees Celsius
*C**	Chroma
Ca	Calcium
$CaCl_2$	Calcium chloride
Cfu	Colony forming units
conc.	Concentration
Cl^-	Chloride
dH_2O	Distilled water
DoH	Department of Health (South Africa)
e.g.	Exempli Gratia; for example
EPS	Expanded polystyrene
et al.	*et alia;* and others
etc.	*et cetera;* and so forth
FSA	Food Standards Agency
G	Gram

*H**	Hue angle
HNO_3	Nitric acid
H_2O	Water
HVP	Hydrolyzed vegetable protein
i.e.	Id est; that is
IMP	Disodium inosinate
K	Potassium
KCl	Potassium chloride
Kg	Kilogram
K600	Treatment with potassium and 1.25% added NaCl
L^*	Lightness colour coordinate
LiCl	Lithium chloride
L600	Treatment with lactate and 1.25% added NaCl
M	Molar
MAP	Modified atmosphere packaging
MDA	Malondialdehyde
mEq	Milliequivalents
Mg	Magnesium
Mg	Milligram
mg/100 g	Milligrams per 100 gram
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
Min	Minute
mL	Millilitre
Mm	Millimetre
MSG	Monosodium glutamate
N	Normality

N	Population size
Na	Sodium
NaCl	Sodium chloride
NC	Negative control
NCSS	Number Cruncher Statistical System
nm	Nanometers
No.	Number
Nr.	Number
NS	Not significant
N600	Treatments with 1.25% added NaCl
O_2	Oxygen
P	Significance level
PC	Positive control
PCA	Principle Component Analysis
Ppm	Parts per million
PVC	Polyvinyl chloride
%	Percentage
R	South African Rand
RBCA	Rose-Bengal Chloramphenicol agar
rH	Relative humidity
Rpm	Revolutions per minute
s	Seconds
SA	South Africa
SANS	South African National Standard
ssp.	Subspecies
SPCA	Standard plate count agar
TAPC	Total aerobic plate count
TBARS	Thiobarbituric acid reactive substances
TVC	Total viable counts

UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
vs.	Versus
VRBM	Violet red bile agar + 4-methylumbelliferyl-β-D-glucuronide
WHO	World Health Organization
YE	Yeast extract

CHAPTER 1

INTRODUCTION

The primary source of sodium (Na) in the human diet is accepted to be sodium chloride (NaCl), better known as table salt (Ruusunen & Puolanne, 2005). Salt is the world's most well-known food additive, because of its excellent preservative effects, its sensorial properties and the functional properties it has on food during processing. Due to all of these factors, NaCl is now being used at much higher levels than necessary in most processed foods (Aursand *et al*., 2014).

Sodium helps with different physiological processes and, since the human body is not able to store large amounts thereof, the intake of Na is vital to human health (Bloch, 1963). Even though Na is essential in the human body, consumption far exceeds the necessary amount needed for physiological processes (Institute of Medicine, US, 2010). The consumption of NaCl has become a problem, since individuals generally have a fondness of salt and the consumption of a product is predicted by how much the product is liked (Schultz, 1957; Tuorila *et al*., 2008).

About a century ago, the correlation between the intake of Na and blood pressure was discovered. Studies done on different ethnic groups initially showed that changes in blood pressure might be linked to Na intake. It was found that the blood pressure of individuals in underdeveloped societies was in general lower and also did not increase with age, which is in strong contrast to more developed societies (Alderman, 2000). The direct link between the intake of Na and high blood pressure is undeniable. High blood pressure is one of the main risk factors for coronary heart disease and stroke (Sacks *et al*., 2001; Strazzullo *et al*., 2009; Aburto *et al*., 2013). Worldwide, high blood pressure accounts for 45% of all heart disease and 51% of deaths due to stroke (WHO, 2008). One way of controlling blood pressure and delaying the use of pharmacological methods is to reduce the amount of Na that is consumed through food (Appel *et al*., 1997).

In general, consumers are not very keen on changing their behaviour even though it may potentially have health benefits (Grunert, 2007; Mendoza *et al*., 2014). They are usually also not keen on compromising on the sensory experience (Verbeke, 2006). It is reported that the most effective way for NaCl consumption to be lowered is to lower the NaCl content of commercially produced foods, since it will not require consumers to change their behaviour (He & MacGregor, 2010). This led to a total of 83 countries planning or implementing NaCl reduction strategies, 38 countries establishing voluntary and/or mandatory Na content targets and two countries, namely Argentina and South Africa (SA) implementing compulsory targets for a wide range of food products (Webster *et al*., 2014).

Boerewors is a traditional South African fresh sausage that is produced in meat processing plants, butcher shops and at home all year round (Mathenjwa *et al.*, 2012). Boerewors falls under one of the categories that the Department of Health issued mandatory legislation for the reduction of the

Na levels (Department of Health (DoH) of South Africa, 2013). Sodium chloride has become one the most frequently used additives in meat processing because of its functionality in terms of flavour, texture and shelf-life (Ruusunen & Puolanne, 2005). This presents the industry with a challenge when NaCl levels need to be reduced because it seems that in order to reduce NaCl levels, a variety of functional ingredient combinations will have to be developed, since there is no single substitute for NaCl. The goal is to create a product that is still acceptable to the consumer but contains less Na (Cluff, 2016).

Purpose and objectives of the study

The purpose of this study was to evaluate NaCl replacers and partial replacement of Na in the production of Boerewors, in order to meet the Na reduction strategies that have been prescribed by South African legislation for processed meat products. This Na reduced Boerewors should have the same or comparable chemical stability, microbial stability and sensory properties as the currently produced Boerewors.

The first objective of this study was to establish the current general Na content of commercially produced (supermarkets and butcher shops) Boerewors in Bloemfontein, South Africa.

The following hypothesis was formulated:

Recent precautionary public health care improvements include current and upcoming regulations on Na content (DoH of South Africa, 2013) and product labelling requirements, in regard to the provision of nutritional information, including Na content (DoH of South Africa, 2014), of food. In light of these regulations it was suspected that the Na content of traditional South African sausages might be higher than the current Na limit in the case of butcher shops and within limits in the case of supermarkets. It was expected that all of the products would still exceed the next Na limit regulation to be implemented in June 2019 and would require reformulation for compliance.

The second objective of this study was to determine the effect of Na reduction and partial replacement of added NaCl on the microbial stability of Boerewors.

The following hypothesis was formulated:

The original purpose of NaCl, namely to prevent the growth of microorganisms, should be kept in mind when replacers are being considered (Dötsch *et al*., 2009). Microbial activity results in spoilage of food products, generally because of the composition of a product (Doulgeraki *et al*., 2012). Microorganisms present in large amounts may cause certain changes in a product that will make it unappetizing and inappropriate for human consumption (Gram *et al*., 2002; Fung, 2010). Meat product safety may also be negatively affected by growth of potential pathogens, such as *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus*) (Smith-Palmer *et al*., 1998). Different factors, such as microbial species, pH, temperature, oxygen levels and other components in food, such as moisture, fat and additives, all contribute to the concentration of NaCl necessary to limit the growth of pathogens (Doyle & Glass, 2010). Food systems are very complex and thus the addition of other additives may cause unforeseen changes in the sustainability and growth of microorganisms (van Boekel, 2008). The nul hypothesis would be that reducing NaCl or partially replacing the normally added amount of NaCl in Boerewors would allow for the survival and growth of more microorganisms than in sausage with a higher concentration NaCl.

The third objective of this study was to determine the effect of Na reduction and partial replacement of added NaCl in Boerewors on its chemical stability.

The following hypothesis was formulated:

Sodium chloride is a pro-oxidant and high levels of NaCl in fat-containing products will cause reduced oxidative stability of such products (Mariutti & Bragagnolo, 2017). Co-oxidation between lipid and myoglobin may also cause a deterioration in the colour stability of meat products (Møller & Skibsted, 2006). The nul hypothesis would be that reducing NaCl or partially replacing the normally added amount of NaCl in Boerewors would reduce fat oxidation and rancidity development, and would also improve the colour stability of the product during short term refrigerated display and long term frozen storage.

The fourth objective of this study was to determine the effect of Na reduction and partial replacement of added NaCl on the sensory quality of Boerewors.

The following hypothesis was formulated:

The first approach taken to reduce Na is mostly by using NaCl or Na replacers (Terrell, 1983). The addition of replacers and subsequent appearance of names of unfamiliar additives on food labels may not be positively welcomed by both consumers and retailers (Searby, 2006). Since NaCl is one of the most affordable food additives available, replacement of Na by other additives poses an obstacle in terms of costs (Desmond, 2006). It is crucial to maintain the salty taste of a meat product since consumers do not like the sensory experience to be compromised in return for possible health benefits (Verbeke, 2006). The null hypothesis would be that by using appropriate NaCl reduction and/or NaCl replacers it is possible to reduce the Na level of Boerewors to acceptable levels, without compromising sensory quality.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Sodium chloride is the world's most well-known food additive, because of its excellent preservative effects, sensorial properties and functional properties it has on food during processing. Due to all these factors, NaCl is being used at much higher levels than necessary in most processed foods (Aursand *et al.*, 2014). Even though NaCl is essential in the human body since it has several important functions, excess consumption thereof holds various health risks (Rodrigues *et al.*, 2016). There are well documented cases where high Na intake has adverse effects on blood pressure, leading to the risk of cardiovascular disease, as well as several other diseases (Aburto *et al*., 2013; Morgan, Aubert, & Brunner, 2001).

Consumers have become more concerned with their health and the harmful effect that high levels of Na in their diet could have and, therefore, it has become increasingly important for them to reduce the amount of NaCl in their food (Guardia *et al.*, 2006). Reducing NaCl levels in processed food has, thus, become a major goal in the food industry (Rodrigues *et al.,* 2016). Due to legislation recently passed by the South African government, the food industry now has to abide by new specified Na levels in food products (DoH of South Africa, 2013; Charlton, Webster & Kowal, 2014).

Sodium can be found in both plant and animal derived foods, as well as in drinking water. Sodium is added to food as salt during processing, cooking and at the table (Scientific Advisory Committee on Nutrition, 2003). Sodium chloride is added to meat products for several reasons, such as the effect it has on texture, flavour and shelf life. Since NaCl has many functions in meat products, reduction thereof is complicated, since it will affect water and fat binding, overall texture, the sensory quality and especially the taste (Ruusunen *et al.*, 2005).

From the early 1980's strategies have been developed to reduce NaCl in processed meat products. Initially, the complete replacement of NaCl with other chloride salts, such as calcium(Ca), lithium(Li), magnesium(Mg) and potassium(K), were evaluated, but results showed negative effects on important aspects such as texture, flavour, appearance, moisture retention and shelf life. Since then, the strategies have been adapted and the attention has shifted more to the formulation of NaCl replacers, flavour enhancers and also the addition of products that naturally have a salty taste, like yeast extracts and seaweed (Inguglia *et al.*, 2017).

Boerewors is a traditional South African fresh sausage that is produced in butcher shops and at home all year round (Mathenjwa *et al.*, 2012). It falls under one of the categories that the Department of Health issued mandatory legislation for to reduce the Na levels (DoH of South Africa, 2013). In a study to determine the Na content of the foodstuffs selected for reduction, it was found that, the category under which Boerewors falls (raw processed meat sausages), only 45% of the tested products were within the 2016 limit of 800 mg/100 g, while some even had Na levels as high as 2 213 mg/100 g of sausage (Peters *et al*., 2017).

The aims of this literature survey were firstly to get a broad overview of Boerewors, its origin, microorganisms involved in spoilage and shelf life. The second aim was to have a look at the role NaCl plays in Boerewors and what the legislation stipulates regarding NaCl levels in Boerewors. The final aim was to explore the alternatives to dietary NaCl that are currently being used and also the effect that reduced NaCl levels and NaCl replacers have on the characteristics of Boerewors, such as texture, taste and most importantly, consumer acceptability.

2.2. South African Boerewors

2.2.1. History

The word sausage is derived from the Latin word *salsus,* which means salted (Allen, 2015). According to the English Oxford Living Dictionary, the word Boerewors is derived from the Afrikaans word "boer," which means farmer and "wors" meaning sausage (https://en.oxforddictionaries.com/definition/boerewors Retrieved on 3 April 2017).

Boerewors is inherited from the South African founding forefathers and it was made in large quantities by the "Voortrekkers" during their trek. The remaining sausage that could not be eaten would be hung to dry, to become what is known as "droëwors" and it would be taken on explorations as food (http://www.biltongmakers.com/biltong16boeries1.html Retrieved on 15 June 2017).

Over the next few decades thereafter, this type of sausage progressively evolved and the term "Boerewors" became part of the South African culture. Boerewors was known only as Boerewors and no other name until the early 1960's. There was big competition between butchers to produce the best "boeries", as it was commonly known. The traditional Boerewors was experimented with when other flavours were added from the 60's onward. Barbecue spice, onion, tomato, garlic, cheese, chilies and peppers were some of the new flavours being added and consumers could buy quite a variety of sausage, such as garlic sausage, chilli sausage, cheese sausage and many others. Many consumers liked the different variations, while others still preferred the original flavour. The flavour variations were clearly successful, since they are still produced today. The secret of making the best Boerewors is said to lie in the quality of the ingredients - the better the quality of the meat, the better taste the Boerewors will have (http://www.biltongmakers.com/biltong 16_boeries1.html Retrieved on 15 June 2017).

2.2.2. Spoilage and shelf life

Fresh sausages, such as Boerewors, are prone to spoilage. Being manufactured from fresh ground meat, which provides favourable conditions for microbial growth of spoilage and pathogenic organisms, it has a high fat content, thus increasing the risk of lipid oxidation, it is stored in oxygen semi-permeable packaging and is kept at refrigeration temperatures. It is clear that such products, thus, have to be preserved in order to have a good quality product (Hugo & Hugo, 2015).

Processed meat products are one of the categories that contributes a significant amount to our daily NaCl and especially Na intake (Ruusunen & Puolanne, 2005) and, therefore, the meat industry faces major challenges because of salt reduction. For the industry to implement strategies to reduce Na, they need to anticipate the effect that these reductions will have on safety and sanitary aspects. The effect of NaCl reduction on the variety and ability of spoilage-causing microbial systems to grow, will have to be evaluated (Fougy *et al.,* 2016).

The shelf life of fresh sausages is mainly dependent on the quality of the sausage. Complex processes exist in the spoilage of food, which can lead to large amounts of food being wasted, not only resulting in economic losses, but also major health hazards. Consumer acceptability is a very important aspect and any changes in the colour, odour, flavour or texture due to spoilage will render the product unacceptable. Reasons for spoilage of fresh sausages can be anything from proteolysis, lipolysis or lipid oxidation in the absence of microorganisms. The factor mostly responsible for spoilage of fresh products is, however, microbial growth (Hugo & Hugo, 2015).

Fresh sausages have a pH value not lower than 5.5 and water activity (a_w) equal to or higher than 0.97. During storage at 4 °C, no fermentation takes place and the quality of the end product depends mostly on the hygienic quality of the raw materials used. The microbiological profile of the fresh sausage product is characterised by the presence of aerobes, facultative anaerobes, psychrotolerant bacteria and mesophiles, which are responsible for spoilage, and potentially pathogenic bacteria (Cocolin *et al.*, 2004).

It is of great importance to control the growth of pathogens to ensure public health safety, especially in the case of certain groups whom are more at risk, such as young children, pregnant women, elderly people and people who have compromised immune systems, due to either chronic disease, immunosuppressive therapy or chemotherapy (Doyle & Glass, 2010). When food is consumed that has been contaminated with foodborne pathogens, such as bacteria or toxins, viruses or parasites, it may cause illness in humans. Foodborne illnesses, associated with meat, are caused mostly by certain types of bacteria namely *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica* (Abdallah *et al.*, 2013). Yeasts also contribute a small part to the natural microflora of meat. Some are capable of growth at low temperatures, high NaCl concentrations and low oxygen levels. In some studies, it was shown that yeasts, such as *Debaryomyces hansenii, Candida zeylanoides, Candida vini, Cryptococcus curvatus* and *Rhodotorula mucilaginosa,* were found in fresh sausages and ground beef samples (Deak, 2008).

In live animals, parts of the animal such as the skin, hooves, and intestines, contain large numbers of bacteria. The extent to which the carcass is contaminated with these bacteria during slaughterhouse operations, depend on the slaughter hygiene. Skinning, scalding, evisceration, dressing and carcass transport are common contamination points. Most bacteria are transferred to the carcass via the butchers' hands, tools, contact with equipment and also through water, air and air (Rani *et al*., 2017). The main contributing factor that determines the bacteriology of ground meat is the quality of the meat used. During mincing, the bacteria that was initially present on the surface of the meat, will now be distributed throughout the meat and the grinding of the meat will also increase the temperature of the meat. The temperature increase can lead to growth of psychrotolerant bacteria and possibly also the growth of mesophilic bacteria, such as *E. coli* and Salmonellae. The grinder and other equipment used are also possible sources of contamination and should be adequately cleaned (ICMSF, 2005).

Depending on the amount of NaCl and fat added during the production of fresh sausages, a lowered a_w can inhibit the growth of *Pseudomonas*, *Psychrobacter* and *Moraxella* spp. and such sausages will have a longer shelf-life than regular ground beef (ICMSF, 2005). In a study to determine the total viable count (TVC) from samples of fresh beef (cut or minced), frozen beef (cut or minced) and sausages (fresh or frozen) on five consecutive days, Table 2.1 shows that for fresh sausage samples the TVC was 1.00×10^1 CFU/g on day 1 and then increased to reach 2.43×10^5 CFU/g on day 5 (Abdallah *et al.*, 2013).

Table 2.1: Total viable count (TVC) CFU/g of beef and sausage samples (Abdallah *et al.*, 2013).

Samples	**Fresh cut**	**Fresh minced**	**Fresh sausage**	**Frozen cut**	**Frozen minced**	**Frozen sausage**
Day 1	1.33×10^3	1.00×10^3	1.00×10^1	6.62×10^4	1.37×10^5	2.24×10^4
Day 2	1.70×10^5	4.44×10^2	5.11×10^3	5.33×10^3	6.67×10^2	1.95×10^5
Day 3	1.53×10^5	5.41×10^4	1.11×10^3	4.33×10^3	3.67×10^3	8.33×10^2
Day 4	1.22×10^3	1.03×10^4	5.67×10^4	6.39×10^4	1.56×10^3	3.71×10^5
Day 5	2.43×10^5	6.10×10^4	2.43×10^5	1.11×10^2	1.11×10^2	2.22×10^2

For fresh sausage, the upper limit, regarding the total aerobic plate count (TAPC), should be 10^7 CFU/g and the "end point" of the shelf life, in other words, where signs related with spoilage are found, is 6 days (Steyn, 1989; Shapton & Shapton, 1991). A good quality fresh sausage is considered to have a coliform count of 10^4 CFU/g or less, and a yeast and mould count of 10^2 CFU/g or less (Shapton & Shapton, 1991).

2.3. Dietary NaCl

2.3.1. Effect on health and reasons for reduction

Sodium chloride is the most significant contributing source of Na in our diets and therefore, when referring to Na reduction, it will in practical terms translate to NaCl reduction. There is a common misconception regarding NaCl and Na being the same thing, since these two terms are often used synonymously, when in fact NaCl comprises of 40% sodium and 60% chloride (Scientific Advisory Committee on Nutrition, 2003).

Sodium is almost always only seen for its negative effects on human health, but Na does in fact have a critical role to play in several functions in the human body. One of the functions of Na is to help with fluid balance and cellular homeostasis (Farquhar *et al.*, 2016). Sodium also maintains the acid-base balance, neural transmission, renal function, cardiac output and myocytic contraction (Liem *et al.,* 2011).

Sodium, in large quantities, is however, dangerous and holds several health risks. On average only 500 mg of Na is needed to maintain homeostasis in adults, which is significantly less compared to the average daily intake of most Americans, which is more than 3 200 mg (Farquhar *et al*., 2015). There is rather compelling evidence that the intake of high amounts of NaCl leads to elevated blood pressure or hypertension, which is a major risk factor in the development of cardiovascular disease (Scientific Advisory Committee on Nutrition, 2003). During the period between 1990 and 2010, there has been an increase in occurrence of hypertension, which correlates with high Na diets. The World Health Organization (WHO) identified NaCl reduction to be the best solution to public health problems and they aim to reduce the daily NaCl intake of an individual to less than 5 g/day. This reduction will help individuals to lower blood pressure and reduce the risk of cardiovascular disease (Charlton *et al.*, 2014).

There are many upsides to reducing the total amount of Na in food products and it has the potential to have a rather significant impact on public health. It can possibly prevent as many as 7 400 cardio vascular disease related deaths per year and prevent non-fatal strokes, which will relieve some of the pressure on the health system. Data available showed that the cost of treating a stroke adds up to R76 000, which excludes follow-up doctor visits and rehabilitation costs. When non-fatal strokes can be prevented, a rough sum of R300 million could be saved each year (Bertram *et al.*, 2012).

The effect of Na stretches beyond the increased risk of hypertension. There is compelling evidence that excess Na intake can cause damage to certain target organs and it may also have a direct effect on the brain, heart, kidneys and vasculature, as illustrated in Figure 2.1. These effects can be independent of changes in blood pressure (Farquhar *et al.*, 2016). The excessive intake of NaCl has been correlated with *Helicobacter pylori* infection and it is possible that these two factors may synergise to promote the development of stomach cancer. Additionally, NaCl may also increase the risk of stomach cancer by directly damaging gastric mucus, increasing temporary epithelial proliferation and the incidence of endogenous mutations, and inducing hypergastrinemia that leads to eventual parietal cell loss and progression to gastric cancer (Wang *et al.*, 2009; D'Elia *et al.*, 2013).

2.3.2. Sodium chloride intake of individuals and sources of NaCl

According to estimations, the global mean NaCl intake of an individual in 2010 was about 10 g/day, which is twice the amount recommended by the WHO (2012a). The number of countries currently implementing strategies to reduce dietary salt is increasing and they are looking to the food industry to voluntarily reformulate products to be lower in Na. South Africa is one of the countries which are currently trying to reduce NaCl by means of reformulations in the food industry.

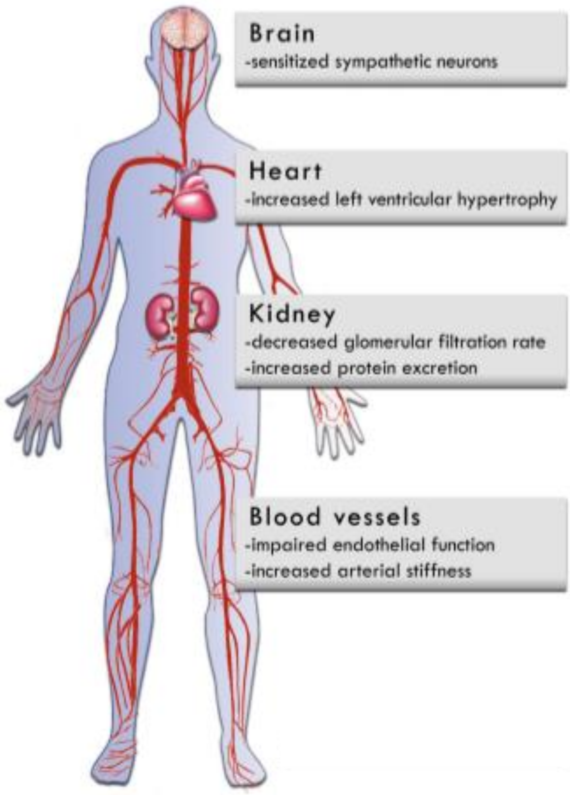

Figure 2.1: Blood pressure independent effects of high dietary Na (Farquhar *et al.,* 2016).

The current NaCl intake estimates are between 7.8 and 9.5 g Na/day for individuals living in SA, but these are also dependent on the ethnic group (Charlton *et al.*, 2014). The average daily Na intake is 7.8 g/day for black individuals, 8.5 g/day in mixed-race individuals and 9.5 g/day in white individuals. South African diets are high in salt and bread contributes 25 – 40% of Na intake (Bertram *et al.,* 2012).

Sodium can be found in both plant and animal derived foods, as well as in drinking water. Sodium is added to food as NaCl during processing, cooking and at the table (Scientific Advisory Committee on Nutrition, 2003). According to estimations, 15-20% of the total Na intake is either from NaCl added while cooking or added at the table. Natural occurring Na found in unprocessed food contributes 15% of the total Na intake, while manufactured foods contribute 65-70%. Meat and fish, especially processed meats, and bread are the two food groups that make up 50% of the Na intake, the remaining 50% comes from other processed food products that include milk products, soups and sauces, biscuits and cakes, and breakfast cereals (FSAI, 2005).

2.3.3. Legislation

Legislation has recently been passed by the South African government which specifies Na levels in various processed foods, as shown in Table 2.2. This legislation is implemented in more than one phase. The first Na level standard was made mandatory from 30 June 2016 and the second phase comprises of even more strict maximum Na levels and will become effective on 30 June 2019 (DoH of South Africa, 2013; Charlton *et al.*, 2014; Peters *et al*., 2017). According to the legislation of the South African DoH, Boerewors falls under the category "Raw-processed meat sausages" and the maximum allowed amount of total Na in boerewors is 800 mg/100 g by 30 June 2016 and 600 mg/100 g by 30 June 2019 (DoH of South Africa, 2013).

According to South African Regulations (DoH of South Africa, 1990), Boerewors must be made from meat from either cattle, sheep, swine, goat or a combination of two or more thereof. It is furthermore specified that it should be contained in edible casings. The total meat content may not be less than 90% and the fat content may not exceed 30%. No offal may be added, except for casings used and mechanically recovered meat is also prohibited. The Ca content may not exceed 0.02 g/100 g of sausage. With regard to other ingredients, only cereal products or starch, vinegar, spices, herbs, NaCl or other harmless flavourants, permitted food additives and water may be added. When manufacturing and selling Boerewors, it should be clearly labelled as Boerewors on the packaging.

Table 2.2: Maximum total Na levels allowed in certain foodstuffs in SA by June 2016 and June 2019 (Peters *et al*., 2017).

Foodstuff Category	**Maximum Total Na per 100 g by June 2016, mg**	**Maximum Total Na per 100 g by June 2019, mg**
Bread	400	380
Breakfast cereals and porridges	500	400
Fat and butter spreads	550	450
Savoury snacks, not salt and vinegar flavoured	800	700
Potato crisps	650	550
Savoury snacks, salt and vinegar flavoured	1 000	850
Processed meat, uncured	850	650
Processed meat, cured	950	850
Processed meat sausages, raw	**800**	**600**
Soup powder, dry	5 500	3 500
Gravy powders and savoury sauces, dry	3 500	1 500
Savoury powders with instant noodles, dry	1 500	800
Stock cubes, powders, granules, emulsions, pastes, or jellies	18 000	13 000

One year prior to the legislation being implemented, a study was done in SA on various packaged food products affected by the legislation, in order to determine if there has been progress in reducing the Na levels in foods and also to detect possible hurdles (Peters *et al.*, 2017). It was found that in the “raw processed meat sausages” category, only 45% of the tested products had Na levels below the 2016 upper limit as depicted in Figure 2.2. Table 2.3 it shows that within this category, 102 products were tested, of which the minimum value was 426 mg/100 g, the maximum value was 2 213 mg/100 g and the average was 851 mg/100 g (Peters *et al.,* 2017).

2.3.4. Functionality

All sausages, and in this case Boerewors, contain NaCl. Sodium chloride has been used to preserve meat products for hundreds of years and it is one of the most generally used additives when making processed meat products (Desmond, 2006). Sodium chloride is added to sausages to perform three functions: it helps to preserve the meat, it binds the proteins together; and it also helps to add flavour (Allen, 2015).

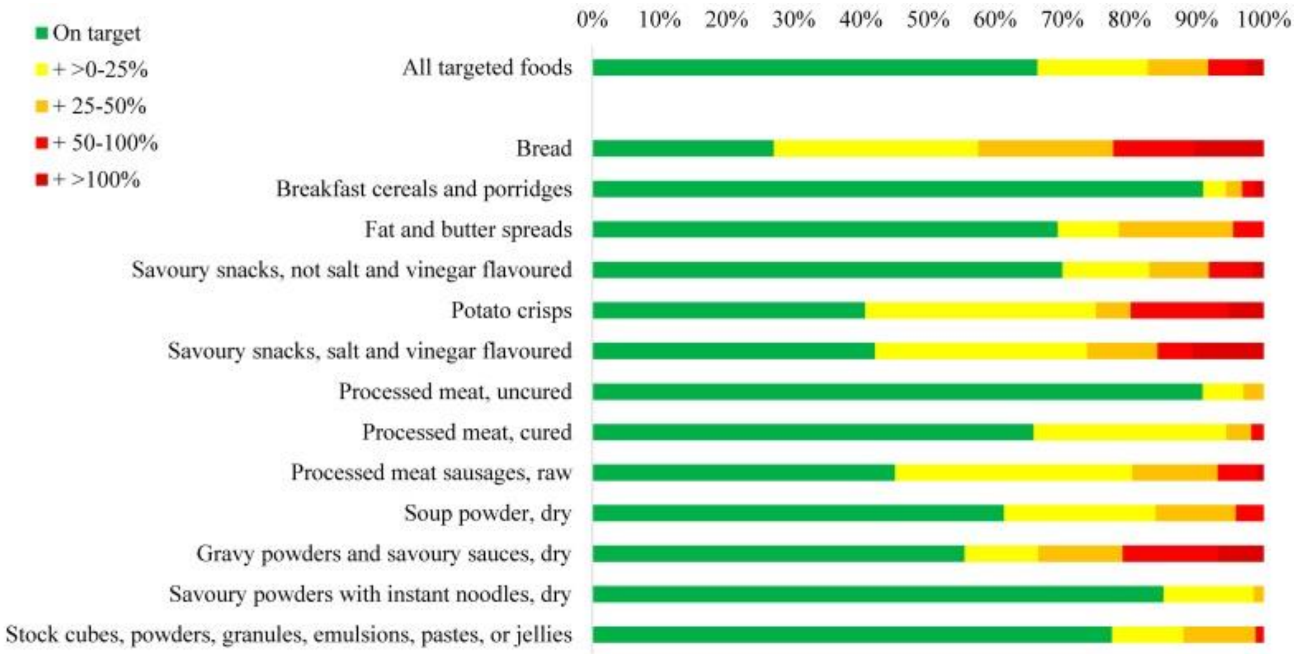

Figure 2.2: Foods targeted by the South African Na legislation according to the 2016 Na limits. Regions shaded in green are for foods with Na levels at or below the Na limit. The regions shaded in yellow, orange, red, and dark red are for foods with Na levels 0–25%, 25–50%, 50–100%, or more than 100%, respectively, above the Na limit (Peters *et al*., 2017).

Sodium chloride has an antimicrobial effect on meat, because it has the ability to reduce the a_w. Water activity is the amount of free water that is available in which microorganisms can grow. The amount of NaCl present in the aqueous phase of the food will determine the effect the NaCl has on the microorganisms.

When Na-ions are added to a food substance, it will cause water to flow out through the semipermeable membrane of the bacteria. The bacterial cells will experience osmotic shock due to the loss of water and this may lead to either cell death or severe injury to the cell, which in turn will substantially reduce the bacterial growth. For some microorganisms, NaCl may also limit oxygen solubility, interfere with cellular enzymes or force cells to use energy to exclude Na ions from the cell which can all reduce the rate at which microorganisms grow. When salt levels are increased in food, microbes have different ways of adapting, such as accumulating K, amino acids or sugars in the cell, in order to prevent the inflow of Na and resultant outflow of water. Other strategies include changing the cell morphology and fatty acids profile in the membrane, and producing specific stress response proteins (Inguglia *et al.,* 2017).

Table 2.3: Sodium levels in mg per 100 g for foodstuff categories targeted by the South African regulations (Peters *et al.,* 2017).

Foodstuff Category	**No. of Products**	**Minimum**	**25%**	**Median**	**Mean**	**75%**	**Maximum**
Bread	174	39	388	476	542	593	2 470
Breakfast cereals and porridges	376	0	46	171	262	346	4 180
Fat and butter spreads	88	0	339	400	428	625	826
Savoury snacks, not salt and vinegar flavoured	417	0	42	480	519	857	2 296
Potato crisps	96	175	554	702	721	802	1 670
Savoury snacks, salt and vinegar flavoured	19	510	807	1 094	1 173	1 258	2 851
Processed meat, uncured	33	44	500	638	618	784	1 065
Processed meat, cured	108	0	656	864	836	998	1 667
Processed meat sausages, raw	**102**	**426**	**708**	**826**	**851**	**914**	**2 213**
Soup powder, dry	168	123	2 842	4 782	4 505	6 366	9 180
Gravy powders and savoury sauces, dry	119	186	500	3 029	3 197	4 997	10 960
Savoury powders with instant noodles, dry	67	1	313	1 123	887	1 314	1 876
Stock cubes, powders, granules, emulsions, pastes, or jellies	84	217	1 252	3 075	9 122	17 270	27 010

Sodium chloride also helps achieve the texture which is desired in processed meat products through the functional properties it imparts in the meat products. Sodium chloride solubilizes the functional myofibrillar proteins in meat (Desmond, 2006). Proteins are activated by NaCl and it then increases the hydration and water-binding capacity of the proteins, which all contribute to texture (Terrell, 1983).

Sodium chloride does more than merely impart a salty taste to the overall flavour of a food product. Sodium chloride was found to improve the perception of product thickness, enhance sweetness, mask metallic or chemical off-notes, and round out overall flavour while improving flavour intensity in a variety of products (Gillette, 1985). In the modern meat industry, NaCl is added as a flavouring or as a flavour enhancer (Desmond, 2006). In addition to the perceived saltiness, NaCl brings out the distinctive taste of a meat product, which enhances the flavour (Gillette, 1985).

Because NaCl plays such an important role, it is essential that the effect of NaCl reduction be carefully considered from a scientific point of view and the effect on factors, such as water-holding capacity, fat binding, texture, sensory acceptability, stability and shelf life, should all be carefully examined (Desmond, 2006).

2.4. Factors affecting saltiness

One of the five primary senses is the sense of taste, which functions through taste receptor cells, which are located on taste buds in the oral cavity. The taste receptor cells are innervated by branches of the seventh, ninth, and tenth cranial nerves that synapse first in the brainstem, before sending messages to other parts of the brain (Breslin & Spector, 2008).

Five different taste sensations exist in humans namely: bitter; sweet; umami; sour; and salty (Hayes, Feeney & Allen, 2013). Tastes have numerous sensory characteristics that can be distinguished (Breslin & Spector, 2008). Each molecule identified by the sense of taste is characterised by one or more of the characteristics, such as salty, sweet and bitter. The NaCl molecule has the prototypical salty taste and conveys a very pure salt taste. Other molecules, such as potassium chloride (KCl) taste both salty and bitter. Potassium chloride is one of the substitutes often used in formulations of which the Na content is reduced. The bitterness may be one of the reasons why KCl is not as effective in replacing NaCl (Institute of Medicine, US, 2010).

The apparent saltiness of NaCl comes from the Na^+ cation in combination with the Cl^- anion. In meat products, NaCl acts as a flavour enhancer by increasing the flavour intensity. When NaCl is reduced in meat products, not only will the perceived saltiness be reduced, but the overall flavour of the product will also be less intense.

Many of the sensory properties which are characteristic of cooked sausages can be attributed to the combination of fat and NaCl. As NaCl levels are increased, the increase in saltiness is more perceptible in products with a higher fat content than in leaner products. The perceived saltiness of cooked sausages is, however, affected in different ways by the fat content, depending on the formulation. When the fat content is increased by replacing lean pork with pork fat, the protein content is also reduced and the perceived saltiness is increased. However, when the water is replaced with fat on an equal weight basis, the perceived saltiness of the sausage does not change. Therefore, any increase in meat protein content will reduce the perceived saltiness of cooked sausages (Ruusunen *et al.,* 2005).

2.5. Alternatives to dietary NaCl

When replacing NaCl in products, the most significant barrier is the cost thereof, since NaCl is one of the cheapest food additives available (Aursand *et al*., 2014). There are currently a few approaches to reduce the NaCl content of food (Desmond, 2006).

The first option is to reduce the amount of NaCl by stealth. Secondly, and probably the most popular one, is to use a NaCl substitute, like KCl. Masking agents are commonly used in these products. Thirdly, flavour enhancers can be used. These will enhance the saltiness of the product when it is used in combination with NaCl, which means that less NaCl needs to be added to the product. Lastly, the physical form of NaCl can be optimised, so that it becomes more taste bioavailable and ultimately less NaCl needs to be added (Desmond, 2006).

2.5.1. Reduction by stealth

One of the methods currently employed to reduce the consumption of Na is the reduction of NaCl by stealth. This method makes use of a stepwise reduction of NaCl in processed food products over a long period. Because of the gradual decrease in NaCl content, the change in saltiness is not detected by consumers. The significant outcome from using this strategy is the reduction of the perceived saltiness of a product, with no visible organoleptic differences detected by consumers. This strategy was successfully employed in the United Kingdom, where the Na content of different processed foods was reduced by 20-30% over a period of three years (Inguglia *et al*., 2017). A similar experiment was carried out in Sydney Australia, in a much shorter time period, using a group of 110 volunteers in a single-blind test. The volunteers were not able to detect a reduction of 5% Na per week in white bread for six weeks, which makes up a total reduction of 25% (Girgis *et al.,* 2003).

Even though this method can help to reduce the consumption of Na, there are still some limitations. It is firstly a very time-consuming approach and secondly, it needs to be applied on an industry wide scale. The other problem with this method is that even though consumers can adapt to a less salty taste, only a limited amount of NaCl can be reduced before the product becomes unpalatable and interferes with the function of NaCl in products (Inguglia *et al*., 2017).

2.5.2. Sodium chloride substitutes

When the NaCl content of a product needs to be reduced, one strategy is to reformulate the product and to partially replace the Na with other additives, such as phosphate and other mineral salts like K, Ca and Mg. The problem with these substitutes is that they often have undesirable tastes, such as bitter, sweet or sour, but when the optimal recipe reformulation is reached, it is possible to lower the Na content in many of today's products (Aursand *et al.*, 2014).

A food product of which the NaCl content has been reduced, relies on NaCl replacement additives to improve the palatability of the product. Sodium chloride substitute's primary function is to replicate the role of NaCl without affecting the saltiness of the product (Inguglia *et al.*, 2017). There are many different types of substitutes, but not all of them can be used in the manufacturing of food products, as shown in Table 2.4 (Institute of Medicine, US, 2010). Sausages are one of the products in which lower Na additives have been successfully used in manufacturing. In such products, the addition of additives, such as soy or milk proteins, gums and starches, are to replace the structural functions of NaCl soluble proteins (Inguglia *et al*., 2017).

The use of replacement additives and their impact on product taste depend on the type of replacer used, as well as on the meat product type and its formulation (Fellendorf *et al.,* 2016). There are currently a few commercially available products in the food industry to help reduce the amount of Na in food products (Table 2.5) (Inguglia *et al.*, 2017).

One of the most frequently used NaCl substitutes is KCl, but when a ratio of over 50:50 NaCl:KCl is used in a solution (Desmond, 2006), a loss of saltiness can be detected, as well as a bitter and metallic aftertaste and for this reason the replacement of NaCl by KCl should be limited to 30% (Rodrigues *et al.,* 2016). The primary limitation when using NaCl substitutes is the metallic flavour of KCl and, of course, the risks that are associated with higher intakes of K, which could hold health risks for individuals affected by type 1 diabetes, renal disease and adrenal insufficiency (Inguglia *et al*., 2017).

Table 2.4: Selected examples of proposed NaCl substitutes (Institute of Medicine, US, 2010).

Substitute	Applications	Comments
Potassium chloride (KCl)	Many foods, including cheeses, bread, and meat;-may be mixed with NaCl in up to a 50:50 ratio	Bitter to many people; many patents to reduce KCl bitterness exist, because K intake of the U.S. population is low, increased intake of K may benefit some, but could harm certain subpopulations (e.g., those with certain medical conditions or taking certain medications)
Lithium chloride (LiCl)	None: toxic although almost perfectly salty	
Calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), and magnesium sulfate ($MgSO_4$)	Few foods	Somewhat salty, but with many off-tastes; bitter tastes of $MgSO_4$ are usually perceived only at high levels; $CaCl_2$ can cause irritations on the tongue
Sea salt	Many foods, also used in salt shakers	Usually contains substantial amounts of NaCl; benefits of use in reducing Na consumption are unclear
NaCl with altered crystal structure	Some foods	Porous and star-shaped structures, created by manipulating the NaCl drying process, allow greater salty taste with smaller amounts of NaCl; particularly useful in applications where NaCl is used on the surface of food products

Sodium chloride mixtures with low Na content have been developed. These products are now commercially available and some are shown in Table 2.5. Such products include Pansalt® which is a mixture of KCl, $MgSO_4$ and L-lysine hydrochloride. A study indicated that there was no negative effects, in comparison to NaCl-containing patties, on the technological and sensory properties of ground beef patties that were formulated with the Pansalt® mixture (Ketenoğlu & Candoğan, 2011). Another product is Sub4salt®, which is made of NaCl, KCl and sodium gluconate, and allows a Na reduction of up to 30%, without a significant taste difference in hams and emulsified sausages (Inguglia *et al.,* 2017).

Table 2.5: Commercially available NaCl replacers (Wallis & Chapman, 2012).

Product	Product function	Replacement claim made	Composition	Suggested use
Low-So Salt Replacer ™	NaCl reducer	25% reduction in Na for hams	Modified KCl, rice flour	Meat products
KCLean™	NaCl reducer	50% reduction in NaCl	The proprietary ingredient, NaCl, KCl	Meats, canned foods
Kalisel	NaCl reducer	Up to 30% less NaCl	KCl	Processed meat
Pansalt®	Sodium reducer	100% substitution of NaCl, resulting in ≈ 77% less Na	NaCl, KCl, $MgSO_4$, L-lysine hydrochloride	All applications
Sub4salt®	NaCl reducer	100% substitution, resulting in 35% less Na	Sodium gluconate, NaCl, KCl	Meat
AlsoSalt	NaCl replacer	100% substitution, 100% reduction in Na	KCl, lysine	All applications
Soda-Lo™	Physically modified NaCl, NaCl replacer	Up to 50% reduction in non-physically modified NaCl	NaCl, gum Arabic	Processed meat

2.5.3. Flavour enhancers and masking agents

Flavour enhancers are substances that do not have a distinct taste and, therefore, do not change the taste of a product, but it instead increases the intensity of how the smell and taste of food are perceived. Taste enhancers work by activating receptors in the mouth and throat, which helps compensate for NaCl reduction. Taste enhancers stimulate receptors linked to the umami taste by improving the balance of taste perception in foods. They also help mask undesirable tastes (CTAC, 2009). Flavour enhancers include amino acids, such as arginine and monosodiumglutamate (MSG), disodium inosinate (IMP), yeast extract (YE), hydrolyzed vegetable protein (HVP), lactates, nucleotides or herbs and spices (Table 2.6), and they can reduce the Na content of the final product by extending the perception of the salty taste (Aursand *et al.,* 2014; Rodrigues *et al.*, 2016). Citric and lactic acid may also be added to enhance the perceived saltiness (Inguglia *et al.*, 2017).

In a study by Dos Santos *et al.* (2014), it was found that when 50% of NaCl was replaced with KCl and with addition of MSG in combinations with lysine, taurine, IMP and disodium guanylate, it was possible to produce fermented cooked sausages which had acceptable physicochemical and sensory qualities. By adding these compounds, it was possible to replace 50% and 75% NaCl with KCl, which resulted in a total Na reduction of 68% in sausages.

Although these flavour enhancers help to improve the flavour of products, they are associated with adverse health aspects. For example, the use of MSG is associated with health problems like headache, hyperactivity and metabolic changes, that may result in serious disorders (Insawang *et al.,* 2012).

A company in America, by the name Linguagen, patented a bitter blocker, namely adenosine 5'-monophosphate (AMP). Gustducin is a G protein that plays a role in the transduction of bitter, sweet and umami stimuli and AMP works by blocking the activation of the gustducin in taste receptor cells, which prevents stimulation of the taste nerve. This product is available under the name Betra and can improve the taste of NaCl/KCl mixes. Another product, known as NeutralFres naturally neutralises the undesirable metallic and bitter tastes of KCl. Many other such masking products are commercially available, such as Magifique Salt-Away, Mimic and SaltTrim, which all mask the unwanted flavours of KCl (Desmond, 2006).

2.5.4. Optimising the physical form of NaCl

Optimising and changing the physical form of NaCl, in order for it to become more taste bioavailable will mean that less NaCl will have to be added to products. The efficiency of NaCl will, therefore, be increased by changing the structure and modifying the perception of the NaCl (Angus *et al.,* 2005).

The crystal size and shape of NaCl in solid form affects the taste perception of NaCl. Research has been carried out where NaCl had been investigated in flaked and granular form, to see if it can be used as a method of reducing NaCl content in meat products. It was found that the flaked is more functional in terms of binding, increasing pH, increasing protein solubilisation and improve cooking yield in emulsion systems. Flaked NaCl can to solubilise faster than granular NaCl and this can be critical where formulations are used where no water is added (Desmond, 2006).

There are several modified NaCl crystal products commercially available, such as Alberger® Flake Salt and Star Flake® salt (Figure 2.3). Flake Salt crystals provide better solubility, blendability and adherence when compared to cube-based salt, because of its larger surface area and low bulk density. It has also been noted that flake shaped crystals have much better fat and water binding properties than granular NaCl (Desmond 2006; Inguglia *et al.*, 2017).

Table 2.6: Selected examples of proposed NaCl enhancers (Institute of Medicine, US, 2010).

Ingredient	Applications	Comments
Monosodium glutamate (MSG) and other glutamates	Many foods; can replace some NaCl	No pleasant taste in itself, but enhances salty tastes; imparts the taste of umami; MSG contains Na; other glutamate salts, such as monopotassium glutamate or calcium diglutamate may further reduce Na; synergises with 5′-ribonucleotides; may replace bitter blocking and oral thickening characteristics; often contained in hydrolysed vegetable protein and yeast extracts
Yeast extracts and hydrolysed vegetable protein	Some foods	Often contains MSG, but is seen as a “natural” alternative to MSG use; meaty and brothy tastes limit potential uses
Nucleotides including inosine- 5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP)	Some foods	Imparts the taste of umami; found to act synergistically with glutamates to enhance salty tastes in some foods
Amino acids, especially arginine and related compounds	Not known	L-Arginine is reported to enhance the saltiness of foods with low to moderate levels of NaCl; practical uses are not clear
Dairy concentrates	Many foods	Reported to allow moderate Na reductions in a variety of products
Lactates (potassium lactate, calcium lactate, and sodium lactate)	Few foods	May enhance the saltiness of NaCl, but not widely used; calcium lactate can impart a sour taste
Herbs and spices	Many foods	Herbs and spices provide other flavouring characteristics and may, for some people, help alleviate blandness following NaCl removal
Compounds that reduce bitterness including adenosine- 5′-monophosphate, DHB (2,4-dihydroxybenzoic acid), lactose, sodium gluconate, and mixtures for use in combination with KCl	Many foods	Designed to mask the bitterness of KCl or reduce bitterness from other food components that are usually masked by NaCl; allow partial reduction of total Na content
Mixtures of NaCl substitutes and enhancers	Many foods	Proprietary mixtures are produced by many companies; mixtures consist of a number of additives such as non-Na salts, yeast extracts, KCl, Na, and sodium gluconate

Another NaCl reducing ingredient, known as Tate & Lyle's SODA-LO® Salt microspheres, are made from free-flowing crystalline microspheres and this physical form of NaCl delivers a higher salty taste, by maximising the surface area relative to volume. This physical form of NaCl can reduce NaCl levels by 25-50%, depending on the application and product.

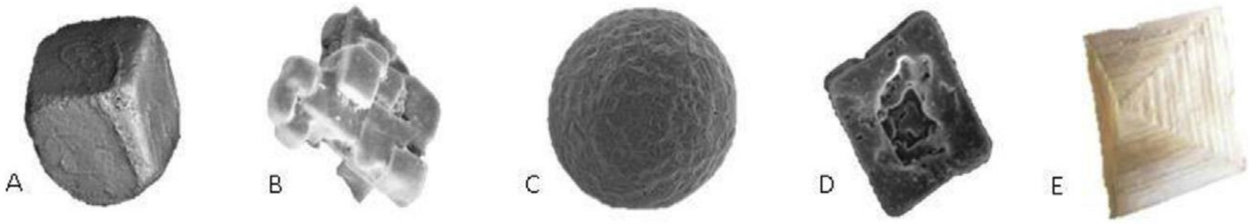

Figure 2.3: The modified shapes of the NaCl crystal. A) Regular shape table NaCl; B) Cargill Alberger Fine Flake NaCl; C) Tate & Lyle SODA-LO NaCl Microspheres; D) Cargill Star Flake Dendritic NaCl; E) Cargill, Alberger Flake NaCl (Inguglia *et al.*, 2017).

The fact that these products are comprised of 100% NaCl is a huge advantage, since it has no chemical aftertaste and is more acceptable to consumers. The downside to these products is that they are expensive and they have a limited commercial application (Inguglia *et al.*, 2017).

2.6. Effects of NaCl reduction and replacement on:

2.6.1. Physical properties and quality

Sodium chloride has several functions in sausages and it plays a vital role during manufacturing. Firstly, NaCl has a rather significant influence on the taste. Secondly, NaCl plays an essential technological role during production. Sodium chloride helps to extract muscle proteins and acts as a binder to make sure the grains of meat coheres and also to retain water and fat. When the NaCl content is reduced, the texture will be affected, and the water and fat retention in the sausage will be reduced. This will eventually lead to moisture loss in packaging and also during cooking. When reformulating, all of these aspects need to be taken into account (CTAC, 2009).

During a study it was found that mineral substitutes and certain binding additives seemed to be useful for reducing NaCl in meat products, such as sausages (CTAC, 2009). The tests done evaluated the NaCl substitutes individually in the prototypes, in which Na was reduced by 57%. The functional ingredient, i.e. the binders, were added to minimise cooking losses. Potassium chloride, used as a NaCl substitute, gave the best results in fresh sausages. Not only did it provide a salty taste, but it was also able to replicate the texture (grain cohesion and firmness) of the positive control better than the other substitutes. It is essential that the addition of KCl should be adjusted, in order to minimise bitterness. For example, a prototype with a KCl dose of 1.70% had a taste profile more similar to the positive control, than another prototype with a dose of 2.20%. Cooking losses can also be limited by adding oat fibres or milk proteins to the binder (CTAC, 2009).

Myofibrils and myofilaments are structural protein elements found in meat proteins. Some of the structural myofibrillar proteins are dissolved when NaCl is added, in order for the gel matrix (colloid structure) to be formed, trapping small drops of water and fat. The gel matrix helps to bind muscle fibres to one another and it becomes the suspension framework between them. When this framework is not formed in ground meat products, the water and fat would not be retained and it will lead to yield loss, texture and flavour degradation, as well as an undeliverable visual appearance (https://cargillsaltinperspective.com/approaches-to-sodium-reduction-in-meat-products/ Retrieved on 8 August 2017).

The presence of NaCl in meat will affect the colour of the meat negatively during storage (Andersen *et al.*, 1990; Trout, 1990). In fresh sausages, the colour of the meat will fade from a red colour to a brownish-grey colour in a short time, when exposed light, NaCl and oxygen. It has been found that when fresh sausages are made from pre-rigour meat, the red colour of the meat will be kept for up to 5 to 6 days when refrigerated, even with the addition of NaCl and light (Savić, 1985). When measuring the meat colour with instruments, the *a** value is usually the most important, since it represents the red colour of the meat, which is the most important visual aspect for consumers. The colour of meat has been studied by various authors and all found that oxidation will cause a decrease in the *a** value (Banon *et al*., 2007; Shan *et al.,* 2009).

2.6.2. Chemical properties

Sodium chloride has a pro-oxidative effect on a variety of meat products when it is included at a certain level. Oxidation directly influence the quality of food products, since oxidation reactions cause damage to lipids and proteins (Decker, 1998). Thiobarbituric acid reactive substances (TBARS) analysis showed that when NaCl is added at 0.5-2.5% in ground beef, it has a pro-oxidative effect (Rhee & Ziprin, 2001). The development of rancidity in ground pork was accelerated by the addition of NaCl, in comparison when replaced by KCl or $MgCl_2$. In both raw and cooked products, NaCl and $MgCl_2$ increased rancidity, while products that were treated with KCl had lower rancidity values (Rhee *et al.*, 1983a; Rhee *et al.*, 1983b). In a more recent study on fresh pork patties, it was found that TBARS values were successfully lowered when 50% of the NaCl was replaced with KCl (Cheng *et al.*, 2007).

The replacement of NaCl with other salts and the resulting decrease in lipid oxidation has also been found in various other studies (Hernández *et al.*, 2002; Zanardi *et al*., 2010). In another study, NaCl, KCl and $MgCl_2$ were used in different combinations in fresh sausage and it was reported that there were no differences between the different salt types concerning TBARS values, despite each one of them having pro-oxidative abilities (Triki *et al*., 2013b). The antioxidant potential of endogenous enzymes, like catalase and glutathione peroxidase, is thought to be compromised by the addition of non-meat additives, such as NaCl (Rhee *et al*., 1983a; Rhee & Ziprin, 2001). When other additives are added, the different effects that various NaCl combinations have on lipid oxidation rates in products may become irrelevant. Various factors, such as the presence of antioxidants in spices, use of sodium metabisulphite and the conditions under which products are refrigerated, may all work together to cause such an effect (Ruiz-Capillas & Jiménez-Colmenero, 2009; Triki *et al*., 2013a).

One of the causes of rancid and off-flavours in boerewors can be ascribed to the fat that is added which oxidises, especially when it is frozen. TBARS analysis is a method of evaluating lipid stability and is expressed as mg malonaldehyde (MDA)/kg meat. The higher the TBARS value, the higher the rancidity of the product. When a TBARS value of ≥1 mg MDA/kg meat is obtained, the rancid off-flavours will become detectable by consumer panels (Buckley & Connolly, 1980; Boles & Parrish, 1990). More recent studies have shown that a TBARS value of ≥ 2 mg MDA/kg meat is considered to be rancid (Campo *et al*., 2006; Suman *et al*., 2010).

2.6.3. Microbial properties

Raw meat contains a great diversity of microorganisms, but by means of processing, the organisms present may be limited to a group classified as well-adapted organisms. Although processing may help inhibit these organisms, it may also lead to pathogens emerging or re-emerging if there is any problem in the control system (Miller *et al.*, 1998). When NaCl content is reduced in a meat product, it may cause a change or failure in the control system. Each meat product is different, since they all go through different processing steps, have different packaging, storage and delivery characteristics, which cause each product to have a different microbial profile. This has to be taken into consideration when these products are reformulated (McDonald & Sun, 1999).

Sodium chloride inhibits the multiplication of pathogenic microorganisms. Foods, such as cold meat, can be at high risk for microbiological spoilage, due to reformulations aimed at reducing the Na. Microorganisms require the following to survive and multiply in food: 1) available water; 2) nutrients; and 3) favourable environmental factors. In order to prevent the growth of pathogenic microorganisms, it is essential to have one or more hurdles present in the food product. These hurdles may concern formulation (water activity, pH, acidity, NaCl, preservative agents, the competition between micro-organisms) or not (thermal processing, high-pressure processing, the absence of oxygen, preservation temperature, etc.). When these work synergistically, they can minimise the risks of spoilage-causing microorganisms and the growth of pathogens. Water availability (a_w) is essential for microorganism survival and growth, and the main effect of NaCl on microorganisms are to reduce the a_w in food and therefore, limit the multiplication of microorganisms (CTAC, 2009). Reduction of a_w by adding NaCl in foods is used to decrease the growth of pathogens and spoilage organisms. Reduction of NaCl concentration will, thus, probably lead to other preservatives being added in higher concentrations (Fulladosa *et al*., 2009).

The microbial species present in a product will determine the NaCl concentration needed to inhibit pathogens. Other factors, such as pH, temperature, oxygen levels, as well as other components in food, like moisture, fat and other additives, will also have a determining effect (Doyle & Glass, 2010). When the NaCl concentration of a particular product is reduced, the present bacterial load will initially not be changed, but the survival and growth of microorganisms will be affected over time. Microorganisms' ability to tolerate NaCl stress is dependent on several factors such as target microorganism, environmental conditions such as pH, temperature, redox potential and nutrient availability, presence of other antimicrobial agents and processing variables. From different studies it has been shown that the rate of growth of foodborne pathogens was higher in products with reduced NaCl (Inguglia *et al.*, 2017). The main problem with NaCl reduction in food products is to ensure that the quality is maintained without affecting the shelf-life and other essential characteristics (Desmond, 2006).

Staphylococcus aureus is seen as the most halotolerant, non-halophilic bacterium that can grow at a_w values as low as 0.86. *Staphylococcus aureus* is a common cause of food poisoning. It is one of the significant causes of gastroenteritis worldwide and the presence of *S. aureus* is an indicator of bad hygiene during processing (Soriano *et al.,* 2002).

The growth of most anaerobes is reduced when NaCl is added at a concentration of at least 3.5% in cured whole meat pieces (Jensen, 1944). The likelihood of growth and toxin production of *Clostridium botulinum* has shown to be reduced at a concentration of 5% NaCl, together with lower pH and storage temperatures. A concentration of 2% NaCl did not influence the growth from spores of nonproteolytic *C. botulinum* in many foods (Whiting & Oriente, 1997).

Various statistical models can describe the growth of pathogenic bacteria and the effect that different combinations of environmental factors have on them. To name an example, the USDA (USA), Food Standards Agency (UK), Institute of Food Research (UK) and the Australian Food Safety Centre of Excellence have all worked together to create the ComBase model. Factors like NaCl concentration and the effect that it has on the growth of pathogens and spoilage microorganisms has been evaluated and large quantities of data have been generated. The ComBase model can predict how microorganisms will respond when three or more environmental factors are changed, by making use of a quantitative method. The fact that most of the primary data have been retrieved by growing microbes in laboratory conditions, does make the usefulness thereof limited. Exact predictions in food products can, thus, not be made by these models, but it can indicate possible interactions among different factors that should also then be tested in food products (Doyle, 2008).

There are some microorganisms that can survive in very high concentrations of NaCl even though they are not able to grow or multiply, such as *Salmonella* and *Escherichia coli,* which can survive at an a_w of 0.85 for 8 days but need an a_w of at least 0.95 to be able to grow (Wijnker *et al.*, 2006).

A study was carried out on laboratory media, using a variety of pathogenic bacterial species, such as *Aeromonas hydrophila, Enterobacter sakazakii, Shigella flexneri, Yersinia enterocolitica* and three strains of *Staphylococcus aureus,* to test the preservative effect of KCl. It was found that KCl has a comparable antimicrobial effect on these organisms, when compared to NaCl (Inguglia *et al*., 2017).

The arising question, of whether or not predictions can be made on what the impact of Na reduction will be on the microbial impact on food, remains. Existing published data could be used to make predictions. One method may be to compare the typical NaCl concentration in products with average shelf-life and foodborne illness data during a specific time frame (Taormina, 2010). Microbial modelling may also be a useful tool, but in a complex system, such as a meat product, all the different possible interactions may be difficult to all be accounted for (Cluff, 2016).

2.6.4. Consumer acceptability

Sodium chloride reduction, by means of stealth, is a method which relies on gradually reducing the amount of Na over time in processed foods, so that consumers do not notice the reduction (Kuo & Lee, 2014). The demand for NaCl to be reduced in processed food products keep being a matter which is essential, but proves to be challenging, since NaCl can only be reduced up to a certain point before the decreased salt content will affect the taste and overall likeability of these products (Dötsch *et al*., 2009; - *et al*., 2012; Zandstra *et al.*, 2016). The optimum NaCl curve is used to show the relationship between liking and NaCl intensity, and indicates where NaCl intensity is most favoured, and higher and lower levels are less favoured (Zandstra *et al*., 2000; Bolhuis *et al.,* 2010).

Differences, such as previous food experience and dietary habits, contribute to people's individual NaCl concentration preferences (Bobowski *et al.*, 2015). These include habits, such as routinely adding NaCl and frequent consumption of products that contain high levels of NaCl (Shepherd *et al.*, 1984; Farleigh *et al.*, 1990; Mattes, 1997; Herbert *et al*., 2014). The goal is to shift the curve to a lower NaCl concentration. It has been proven that it is indeed possible to change the preferred saltiness, by means of recurring exposure (Bertino *et al.*, 1982; Blais *et al*., 1986; Huggins *et al.*, 1992), such as continually giving people a particular product of which the NaCl has been reduced, in order to make them used to the taste. This proved to be effective, even though the NaCl levels of the total diet were not included (Bolhuis *et al*., 2011; Methven *et al.*, 2012; Bobowski *et al*., 2015; Janssen *et al*., 2015).

Consumers should be able to adapt and get used to the lower NaCl concentration products, but it is still unclear how long it will take for consumers' NaCl preference to be shifted and how long it will last (Bobowski, 2015; Zandstra *et al.*, 2016). One of the identified problems that may decrease the impact of reduced NaCl products is the possibility that consumers may merely add NaCl using the NaCl shaker on the table (Zandstra *et al.*, 2016).

In a Western diet, it is estimated that on a daily basis, 15-20% of total NaCl intake is from NaCl added at the table, while 75% is from foods that are commercially prepared (Mattes & Donnelly, 1991; Sanchez-Castillo *et al*., 1987). When the total NaCl intake in the diet is reduced, it has been shown that people only partly compensate by adding NaCl by means of the NaCl shaker (Beauchamp, 1987). In more recent research, however, it indicated that consumers would add extra NaCl at the table, when one product has an unacceptable low NaCl content, even to the point of overcompensation (Liem *et al.*, 2012a; Liem *et al.*, 2012b; De Kock *et al*., 2016).

Saltiness is a very important sensory attribute in many foods and it contributes more than just saltiness. Reducing the Na intake of a population is a complex task when the population has adapted to a high Na diet and it will require several strategies. There were previous attempts to reduce the Na content in food, but they failed, because the food was less palatable. Potassium salts are one way of reducing Na, but they do not produce the same saltiness and when used at higher concentrations, they have a metallic and bitter taste, which is not acceptable to consumers. The other option is to reduce Na by means of stealth, where the consumers are gradually made used to lower levels of Na in food. This is arguably the best method, since there are no other added chemicals with possible health risks or any side effects or consumer dissatisfaction, due to after taste or change in taste (Liem *et al.*, 2011). Consumers have become used to NaCl in certain products and even though there are alternatives to NaCl in terms of functionality, some consumers may not be at ease with these new additives on the label (Desmond, 2006).

Products can be made more attractive to consumers who read the nutritional information on the label, when they deliberately meet specific food claims criteria (Appel *et al*., 2012). Depending on the awareness and understanding the consumer has regarding low Na or reduced Na content in products, it may be used as a marketing advantage. One way of promoting products in which the Na has been reduced, is to realign these products as functional meat products by decreasing unhealthy additives, such as NaCl while adding bioactive additives at the same time. Such products can be marketed as healthier processed meat products and it may lead to new market opportunities in the meat industry (Grasso *et al*., 2014). One way to have better control of the number of bioactives in the end product, as well as the cost involved, is to add the bioactives during processing, instead of adding it to the animals' diet. When in a fresh beef sausage, 50% of the NaCl was replaced by a mixture of KCl, $CaCl_2$ and $MgCl_2$ salts, giving a reformulated product with a 50% reduced Na content, it was found that there was no adverse effect regarding the sensory aspect. These reformulated sausages contained 10-15% of the K, 8-10% of the Ca and 10-20% of the Mg recommended dietary allowances (Triki *et al.,* 2013b), which can also be used as a marketing advantage, since it boosts the nutritional value of the product (Gimeno *et al.*, 1999).

2.7. Conclusions

The Voortrekkers first made Boerewors during the "Groot Trek," and since then became a very popular and traditional South African dish. Sodium chloride has been used to preserve food for several decades and its addition to Boerewors contributes more than just saltiness and preservation. It plays several crucial roles, such as binding of meat proteins, to give the desired texture and water retention, which helps inhibit the growth of microorganisms.

Although the intake of Na is vital to human health, intake far exceeds daily requirements and this excessive intake of Na (mostly as NaCl) has led to the development of health issues, such as hypertension and cardiovascular disease that is costing the Government large amounts of money each year in healthcare. Sodium chloride also contributes to other health problems, like gastric cancer. It has, thus, become a priority of the South African DoH to take specific measures to reduce Na intake. Lowering the Na intake of a population that has adapted to a high Na diet, is not easy and a number of strategies have been employed. The Government has implemented a program where the food industry needs to gradually reduce the NaCl added in products and this needs to be done according to specified dates. The first phase was already implemented in June 2016 and the second phase will become effective in June 2019. This reduction by stealth helps the consumers to gradually become used to less NaCl without realising it. Other approaches involve NaCl replacers and substitutes, but there is still much research that needs to be done on such additives, since they often have undesirable aftertastes. The physical form of NaCl can also be modified for a saltier taste, but this method is not always cost effective.

Producing high quality processed meats that contain reduced amounts of NaCl is a major challenge for the food industry, when keeping in mind the importance of NaCl toward the functional, microbial stability and sensory properties of these products. Since NaCl helps to preserve Boerewors and help to inhibit the growth of microorganism, the NaCl content cannot be reduced too much. There are many pathogenic bacteria associated with Boerewors, but when good quality meat and other additives are used and the right safety measures are in place, the risk is significantly reduced.

Since processed meat products are so popular in SA and especially boerewors, it is of great importance to make sure that consumers are sold good quality products that are not harmful to their health. Sodium reduction needs to be done without compromising food safety and consumer acceptance. Consumer acceptability is one of the most important aspects when Na reduction is being implemented. It is also essential to focus on educating consumers about the negative effects of a diet high in Na, which will better their understanding of why it is necessary for the government and food industry to implement these strategies. There is no doubt that Na reduction in foods will be difficult, but equally, there is no doubt that reducing the level of Na in foods is essential for population health.

CHAPTER 3

THE SODIUM CHLORIDE AND MINERAL CONTENT OF COMMERCIALLY AVAILABLE BOEREWORS – A SURVEY

ABSTRACT

The DoH of SA has published new regulations specifying the allowable amount of Na in a variety of processed food products, in an effort to improve public health. There is currently no information available as to how the products will be affected by the new regulations and, therefor, it is also not known to what extent these products will have to be modified. A list was compiled of the available supermarkets and butcher shops in the Bloemfontein area that sell Boerewors and a representative subset of products was bought and analysed for NaCl, Na, K and ash percentage and information of these, with regard to NaCl and Na content, were recorded. The tested Na content was used to compare the values of the products to the regulatory limits for the category of raw, processed sausages. All of the evaluated products, except for one, had Na values within the current Na regulation limit, while about one third were still above the next mandatory Na reduction limit and it was unclear what impact the next reduction will have and what Na reduction strategies will be applied to reach this goal.

Keywords: regulations; Na content; traditional South African sausage; NaCl

3.1. Introduction

The consumption of excessive amounts of dietary NaCl has been linked to elevated blood pressure, which is the main risk factor for cardiovascular diseases (He *et al.*, 2013; Peters *et al*., 2017). In 2010 an estimation was made that there were roughly 1.6 million deaths due to cardiovascular disease, of which one out of every ten cardiovascular deaths was ascribed to the intake of NaCl above the limit of 5 grams per day, as recommended by the WHO (WHO, 2012a; Mozaffarian *et al.,* 2014). The WHO has identified the reduction of NaCl as one of the best ways to minimise the burden of elevated blood pressure and cardiovascular diseases, and, thus, increase public health in a cost-effective manner (WHO, 2012a). There is an increase in the number of countries implementing NaCl reduction strategies, by means of food reformulations, different labelling techniques and educating consumers, to name a few (Webster *et al*., 2011; Trieu *et al.*, 2015). The strategies to reduce NaCl intake in many countries are limited to certain food products or even voluntary (Webster *et al*., 2014).

South Africa was the first country in the world to develop and implement wide-ranging mandatory legislation to reduce the Na levels in many different processed food products. This led to the need for cooperation from members of the food industry from various sectors (DoH of South Africa, 2013; Hofman & Lee, 2013).

Processed foods contribute an estimated 50% of SA's daily NaCl intake (Charlton *et al.*, 2014). The DoH of South Africa passed legislation regarding the maximum levels at which Na may be added to certain selected food products, such as bread, breakfast cereals, margarine, meat products, snack food and soup mixes. The legislation to reduce the Na levels in specified food products is implemented in two phases. For fresh South African sausage, the first limit of 800 mg Na/100 g sausage was made mandatory on the 30th of June 2016. The second limit comprises of 600 mg Na/100 g sausage and will be mandatory from the 30th of June 2019 (DoH of South Africa, 2013).

The strategy to reduce the Na in the specified food products is anticipated to annually save the lives of thousands of people and to considerably reduce the cost of the South African health service, should it be successful (Bertram *et al*., 2012; Watkins *et al.,* 2015).

The aims of the survey were firstly to create a representative pool of Boerewors from supermarkets and butcheries in Bloemfontein that could act as experimental material for this survey. Secondly, to collect labelling information on NaCl and Na content of these products, and lastly to establish how many products currently comply with the South African standard in terms of Na content targets for 2016 and 2019.

3.2. Materials and methods

3.2.1. Product selection methodology

Five supermarkets were identified and selected in the Bloemfontein city on the basis of being representatives of the major national supermarket retailers. Sausages were bought at these supermarkets at random up to a total of 20 samples. Twenty butcheries were also randomly selected in Bloemfontein and one sample was bought at each of the selected butcheries. The samples from supermarkets and butcheries made up a total of 40 samples. A list was compiled of all the samples and relevant information (name of supermarket/butchery; NaCl and/or Na content, if available) was recorded.

All the sausages bought were specifically labelled as Boerewors. The process of identifying and selecting supermarkets and butcheries, as well as buying samples and collecting labelling information, were done over a period of one month. When products were bought, they were placed in cooler boxes that contained refreezable ice packs to keep the samples cool and they were then transported back to the meat processing facility at the University of the Free State. The samples were individually minced through a small household electric mincer (LOGIK, Model RSH-011765-018), fitted with a 3 mm mincing plate, after which the subsequent mince was thoroughly mixed and placed in pre-numbered, tight-lidded plastic cuvettes (30 mL volume) and stored frozen at -18°C, until analyses were carried out.

3.2.2. Na and K content determination

Sodium and K content were determined using atomic absorption spectroscopy (AAS). For AAS analysis, the samples were reduced to a mineralised form free from any organic compounds, by dry ashing according to a modified method by Nielsen (2010). Samples were thawed at 4 °C the night prior to sample preparation. The next day, 10 g samples were weighed off into porcelain crucibles (ø = 30 mm). Up to 10 samples were placed in a muffle furnace (Heraeus, Model MR 170; Hanau, Germany) and ashed overnight, with increments in temperature of 100 °C per hour, up to a maximum temperature of 525°C. The next day the crucibles were removed, cooled to room temperature and weighed. Enough 55% nitric acid (HNO_3; Merck, Johannesburg, SA) was added to wet the sample. The crucible was then returned to the furnace for one hour at 550°C, which resulted in a cream-white coloured ash that served as indication that all carbonaceous material was completely broken down. The sample was again cooled to room temperature and weighed. Ten millilitres of an 1:2 HNO_3:H_2O solution was added to the crucible and this was placed on a heated sand bath, until bubbles started to form and all crystals were dissolved. The samples were removed from the sand bath, cooled to room temperature before being filtered through a Whatman® nr. 1 filter paper (Sigma-Aldrich, Johannesburg, SA) into a 100 mL volumetric flask, to prevent any possible contamination of the AAS with foreign bodies. The crucible and filter paper were rinsed three times with 10 mL type 1 distilled H_2O, each time allowing the filter paper to run dry. The volumetric flask was then filled with type 1 distilled H_2O. After carefully inverting the volumetric flask ten times, the solution was poured into a clean 100 mL pop-top container, three quarters of the way. The samples were stored at -18 °C until the day before AAS analysis were to be done, when it was thawed overnight at 4°C.

Atomic absorption spectroscopy was performed using a Varian SpectrAA-300 Atomic Absorption Spectrometer (SMM Instruments, Johannesburg, SA) with a GTA-96 graphite tube atomiser furnace fitted with an MKVI acetylene-air, single-slot burner head and four lamp manual positioning turret. The lamp turret was locked on the Na-containing, narrow beam hollow cathode lamp and measurements were taken for Na at 589.0 nm, with a maximum retention time of 10 seconds. For the determination of K, the AAS lamp turret was locked to the K-containing, narrow beam hollow cathode lamp and measurements for K-content were taken at 766.5 nm. Sample absorbance values were captured and processed, using the Varian SpectrAA-300/400 Plus software package.

3.2.3. NaCl content determination

For determination of NaCl content, the Volhard method was used to volumetrically and quantitatively determine the amount of Cl present in the sample, which was then used to calculate the amount of NaCl present in the sample (AOAC, 2005). For the NaCl analysis, 5 g of each finely comminuted and thoroughly mixed sample was weighed into a 300 mL Erlenmeyer flask and the weight was recorded to two decimal spaces. Then, 25 mL of 0.1 N $AgNO_3$ (Merck, Johannesburg, SA) solution was added to the weighed sample and swirled, until the sample and solution were in intimate contact, after which 15 mL of concentrated HNO_3 (Merck, Johannesburg, SA) was added. The Erlenmeyer flask was then put onto a sand bath and heated until the meat within the solution dissolved. Three times 5 mL portions of 0.1 N $KMnO_4$ (Merck, Johannesburg, SA) were added to the solution and left to boil until the colour disappeared and the solutions became almost colourless. Then, 25 mL distilled H_2O was added and the solution boiled for 5 minutes, after which flasks were removed from the sand bath, diluted to approximately 150 mL with distilled H_2O and left to cool. Five millilitres of diethyl ether (Merck, Johannesburg, SA) and 2 mL saturated ferric alum indicator (Merck, Johannesburg, SA) were added and the solution was shaken vigorously to coagulate the precipitated AgCl. The excess $AgNO_3$ was titrated with 0.1 N KCNS (Merck, Johannesburg, SA) solution to a permanent salmon-coloured endpoint. The recorded volume KCNS used to titrate the solution to its endpoint was then used to calculate the percentage NaCl with the following equation:

$$\%\ \text{NaCl} = \frac{(25\ \text{mL- mL KCNS})(0.1\ \text{N})(5.85)}{\text{sample weight}}$$

3.2.4. Statistical analysis

Labelling information on selected products from Supermarkets and Butcher shops were summarised with pie charts (XLSTAT, 2018). The effect of retail type on NaCl, ash, K and Na content of commercial Boerewors was determined with one-way Analysis of Variance (ANOVA) (NCSS, 2016). The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to identify significant differences between Supermarket and Butcher shop means (NCSS, 2016). Inconsistencies in the ash, salt, Na and K content of the 40 products were demonstrated with Walter A. Shewhart's control charts, which were developed in the 1920's (Mohammed *et al*., 2011). The Na chart has been adapted to accommodate the 2016 and 2019 limits for Na in this product category.

3.3. Results and discussion

3.3.1. Na and NaCl content on product labelling

Labelling information was collected from all the samples used in the survey, to establish how many of the products described the Na or NaCl content in the nutritional information table on the packaging. It was found that 60.0% of the labels did not indicate the addition of these additives, while 40.0% did indicate the addition thereof (Figure 3.1). Out of the 40.0% of products that indicated the addition of Na or NaCl, a mere 37.5% indicated the amount of either NaCl or Na added (Figure 3.2). Prior to 2014, there were no regulations regarding product labelling information, but this was amended and all products, except for a few (e.g., 100% tea, coffee, and herbs), are now by law required to put nutritional information, such as NaCl and Na values, in a table on the product label (DoH of South Africa, 2014). The high number of products that omitted to indicate either the addition of Na/NaCl or the amount thereof, shows that there is a major problem with regard to providing consumers with important nutritional information. Since it is required by law for information such as added preservatives to be available to the consumer on the product label (DoH of South Africa Act No. 54 of 1972), it is a violation of the consumer's right to be able to make an educated choice when buying and consuming products.

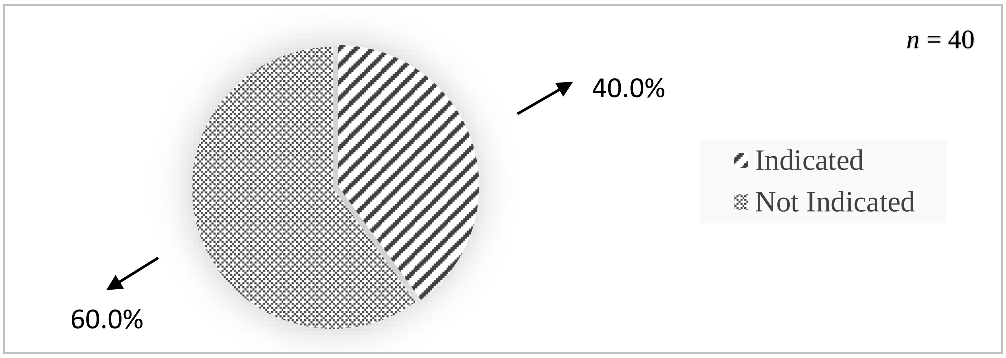

Figure 3.1. Boerewors products from butcheries and supermarkets in the Bloemfontein district that indicated the addition of Na or NaCl on the label

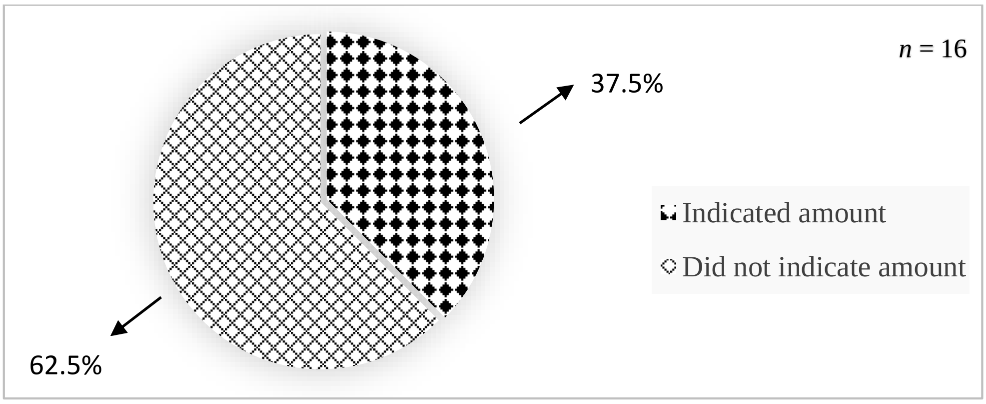

Figure 3.2. Boerewors products from butcheries and supermarkets in the Bloemfontein district which indicated the specific amount of Na or NaCl addition on the labels

3.3.2. Effect of retail type on NaCl, ash, K and Na content of commercial Boerewors samples

The effect that the retail type (butchery or supermarket) had on the NaCl, ash, K and Na content of commercial Boerewors samples was evaluated (Table 3.1). Sodium reduction translates to NaCl reduction, since Na is added as NaCl. From the survey results, it was found that the percentage NaCl did not differ significantly between supermarkets and butcher shops (Figure 3.3). The maximum value was 2.91% NaCl and the minimum value of 0.63% NaCl. For the current Na inclusion limit of 800 mg Na/100 g sausage, a total of 1.8% NaCl may be added (DoH of South Africa, 2013). The values, as seen in Figure 3.3, were relatively close to one another and except for one product, they all clustered around 1.5% NaCl. The ability of NaCl to lower the a_w leads to a reduction of the growth capacity of several bacterial species and, therefore, the food product's safety is increased (Hutton, 2002; Taormina, 2010). Some of the samples had unexpected low NaCl levels, when taking into consideration the functional properties of NaCl and also the fact that fresh sausages generally have a short shelf-life of not more than 6 to 9 days.

Table 3.1. The effect of retail type on NaCl, ash, K and Na content of commercial Boerewors samples

	Supermarkets n=20	**Butcher shops n=20**	**Sign. Level**
% NaCl	1.66 ± 0.35	1.49 ± 0.38	p = 0.157
% Ash	2.93 ± 0.81	2.75 ± 0.44	p = 0.377
K (mg/100 g)	239.05^{b} ± 38.58	215.60^{a} ± 19.25	p = 0.020
Na (mg/100 g)	587.10 ± 154.72	572.61 ± 105.66	p = 0.731

*Means with different superscripts in the same row differed significantly

The % ash did not differ significantly between the two retail types (Table 3.1). In Figure 3.4 it can be seen that the values are centred around 2.9% ash. The percentage ash is an indication of the mineral content. Higher percentages of ash correlated directly with higher percentages of added NaCl (Figure 3.3), as the inorganic matter is left behind after ashing, because organic compounds are ignited and oxidised by air at elevated temperatures (~ 500 °C) (Honikel, 2008; Munoz *et al.*, 2013).

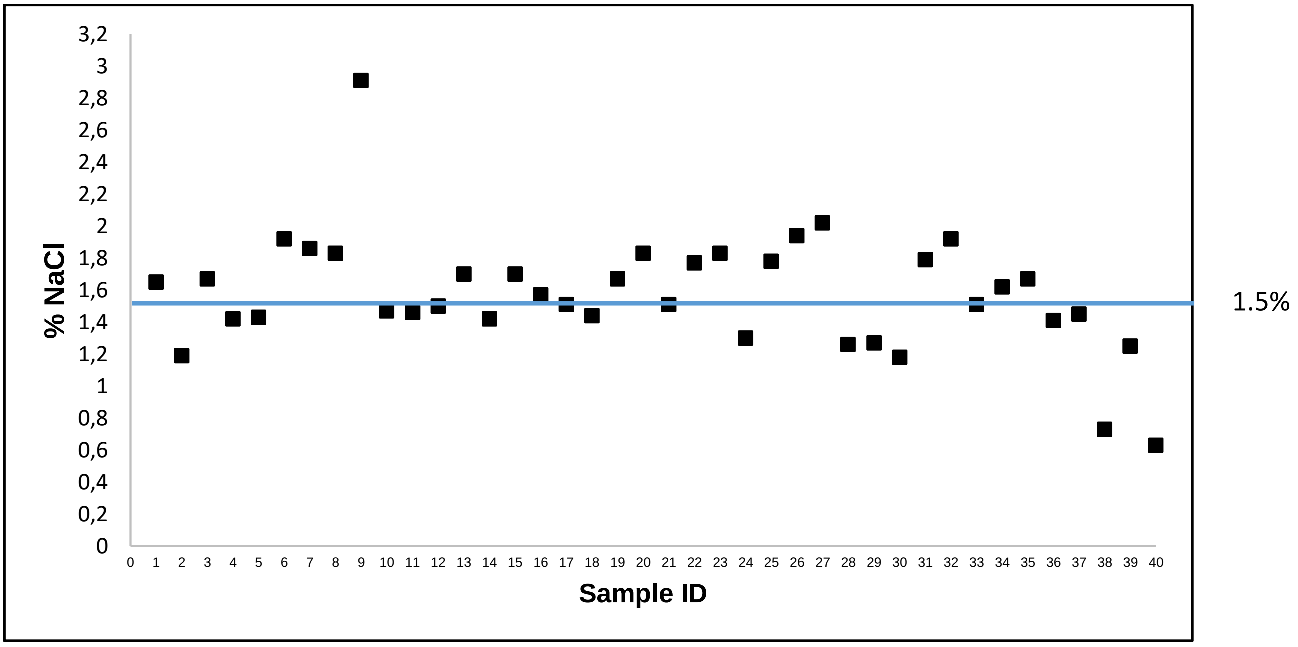

Figure 3.3. Variation in percentage NaCl of Boerewors samples from butcheries and supermarkets in the Bloemfontein Municipality.

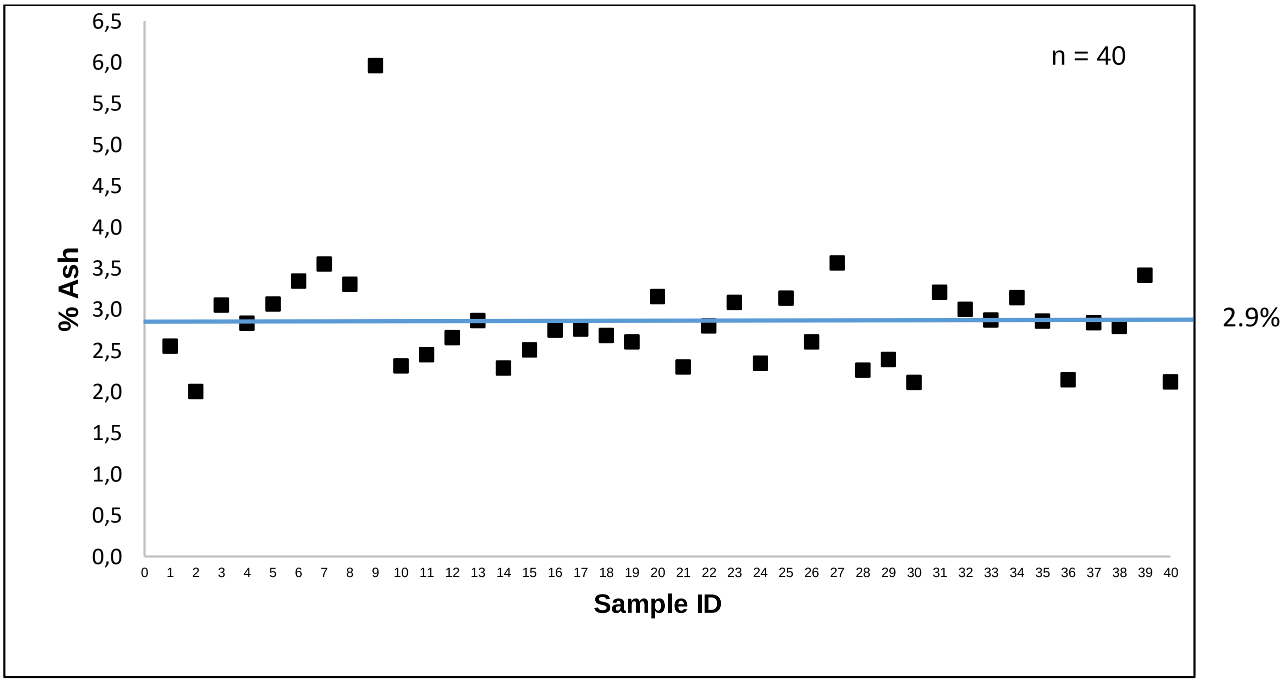

Figure 3.4. Variation in percentage ash of Boerewors samples from butcheries and supermarkets in the Bloemfontein Municipality.

The K differed significantly and the samples from supermarkets had an overall higher value than butcher shops (Table 3.1). The reason as to why the K value of supermarkets are higher, may be because supermarkets make more use of batch packs that contain KCl as a replacer. Potassium chloride is currently one of the most popular NaCl replacers, because of its ability to perceive a salty taste in food products (Murphy *et al.*, 1981; Van Der Klaauw & Smith, 1995). The average K level was around 240 mg/100 g sausage (Figure 3.5). The recommended dietary intake of K by the WHO is at least 3 510 mg/day for adults (WHO, 2012b). So, for every 100 g of sausage consumed, it makes up about 7% of the recommended minimum daily intake. Because of the high consumption of processed foods, K intake is generally too low (He & MacGregor, 2001). This was confirmed during a study done in SA, where it was found that K levels among different population groups differed and that only a few of the participants met the ideal dietary intake (Charlton *et al*., 2005). The use of KCl as a replacer for NaCl could help the population to take in the needed K and increase K levels, that are currently too low.

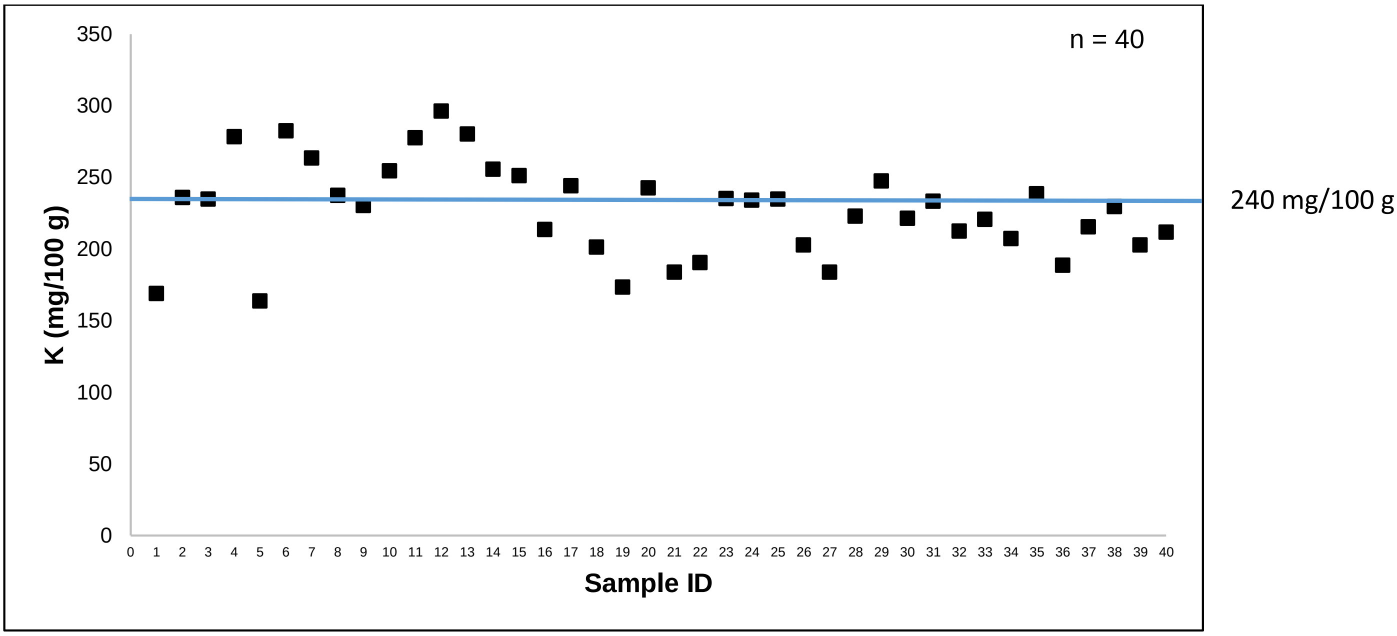

Figure 3.5. Variation in K content of Boerewors samples from butcheries and supermarkets in the Bloemfontein Municipality.

There were no significant differences in Na levels between supermarkets and butcher shops (Table 3.1). In Figure 3.6, the 2016 specified Na inclusion level of 800 mg is shown and it can be seen that all, but one sample is within this limit. This shows that the food industry is cooperating to reduce the Na level in food products. The 2019 limit of 600 mg is also shown in Figure 3.6 and 33% of the samples are above this limit. The fact that about one third of the samples had values above the 2019 inclusion limit indicates that it is important to evaluate the effect of further Na reduction to know what the implication of the 2019 limit will be.

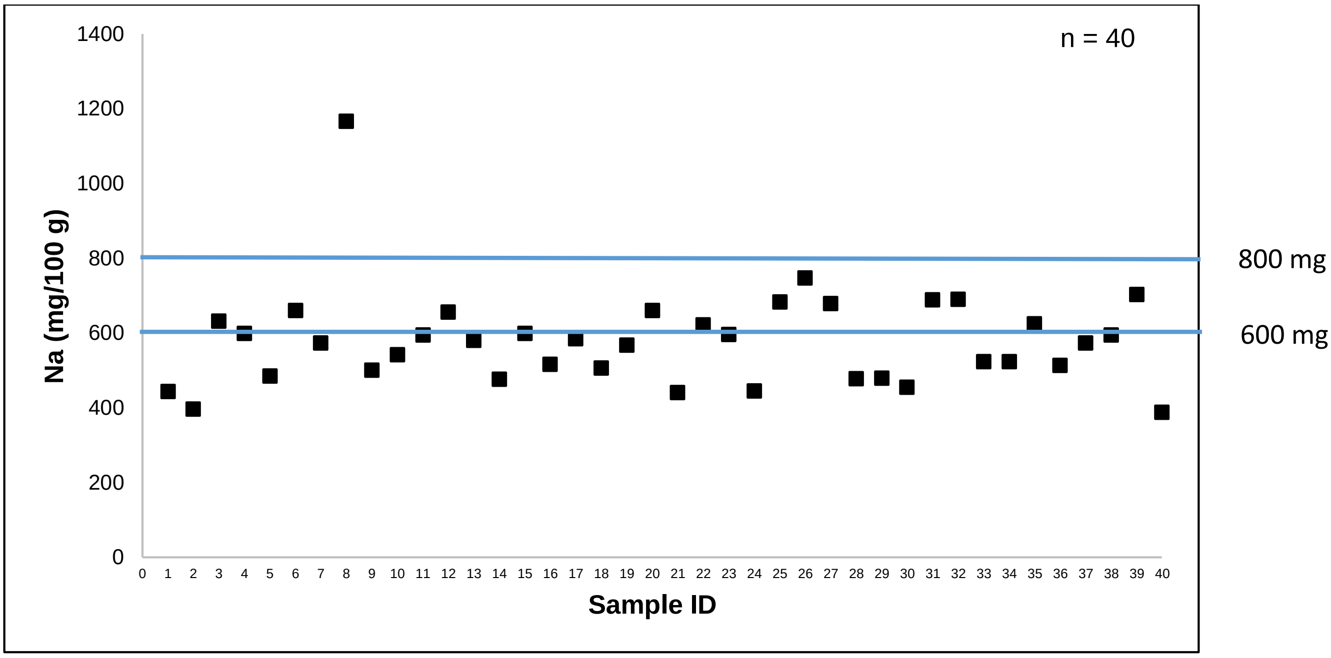

Figure 3.6. Variation in Na content of Boerewors samples from butcheries and supermarkets in the Bloemfontein Municipality.

3.4. Conclusions

The legislation implemented by the South African government to reduce the amount of Na in certain food products suggests positive progress for SA. By improving public health, it will lead to less money being spent on health care and, there for, a better economy. The fact that most of the tested products, except for one, were within the current Na limit shows the willingness of the food industry to cooperate and to help with reaching the goal of improving public health. In spite of this, there is however, a problem with the labelling of products. Product labels do not indicate necessary dietary information and there is still a large margin for improvement in providing consumers with important information. Since it is required by law to have a nutritional table with specific information on all products sold, it is very important to address this issue, to make sure that consumers can make an educated and informed decision when buying food products and, in this case, traditional South African sausage.

There were no significant differences between sausages from supermarkets and butcher shops regarding the percentage NaCl, percentage ash and Na content. The difference in K levels could be attributed to supermarkets using batch packs formulated by spice suppliers, who probably already make use of KCl as a NaCl replacer. The use of KCl as a NaCl replacer can be beneficial for the South African population, because by using KCl, the Na content in food will be lower, because less

NaCl is added and also because it will increase the intake of needed K which, according to research, is currently too low in the average person's diet.

The Na inclusion values were overall satisfactory with all of the samples, except for one, being within the current legislative limit of 800 mg Na/100 g sausage. It is, however, necessary to investigate the effect of the current limit, as well as the next inclusion limit of 600 mg Na/100 g sausage, on the overall quality of traditional South African sausage. It is important to know the effect of these inclusion levels on the chemical, microbial and sensory quality of these products. This information is critical in order to know what the implication of the following inclusion limit will be and to what extent it will affect the food industry. It will give clarity to questions, such as shelf-life, product safety and overall acceptability, which is important to the food industry and the consumer, since both parties are affected.

CHAPTER 4

THE EFFECT OF DIFFERENT SODIUM REDUCTION STRATEGIES ON THE CHEMICAL, MICROBIAL AND SENSORY QUALITY OF BOEREWORS

ABSTRACT

Sodium reduction in practical terms translates to adopting alternative methods and use of different kinds of replacers. There has, thus, far not been any assessment on the effect that Na reduction will have on fresh sausages, such as Boerewors. Sodium reduction will require the NaCl inclusion level to be reduced and possibly the addition of replacers to maintain the quality and stability of fresh sausage products. In this study, NaCl inclusion levels in Boerewors models were adjusted to contain the Na inclusion limits of regulations for 2016 and 2019, respectively, in combination with different replacers (KCl and K-lactate, respectively) in different treatments. The treatments with different Na levels and replacers were evaluated against a negative control that contained no added NaCl. It was found that the treatments with different Na inclusions and addition of KCl and K-lactate as salt replacers were effective without having a negative effect on product quality or stability. None of the treatments stood out to the extent that it gave significantly better results overall in terms of parameters tested. These findings will provide a starting point for further research on Na reduction in different processed meat products.

Keywords: Na reduction; processed meat products; microbial stability; sensory acceptability; fresh sausage

4.1. Introduction

Besides sugar, NaCl is the second most used food additive (Seligsohn, 1981). In meat processing, besides the meat, NaCl is the most used component (Forsythe & Miller, 1980; Sebranek *et al.,* 1983). In comparison to other foods, the Na content in processed meat products are usually higher, because of the significant role it plays in key factors, such as functionality, microbial stability and sensory quality. One of the major roles of NaCl is to bind water and fat (Ruusunen & Puolanne, 2005). Sodium chloride activates meat proteins, which lead to higher water binding, which in turn also affects the texture. This process happens when NaCl is dissolved, which causes the isoelectric point of meat proteins to shift towards a lower pH value (Hamm, 1961). It also acts as a preservative, since it decreases the microbial growth by lowering the a_w. Another very important factor of NaCl is the saltiness that it produces, which increases the perception of meat flavour. This plays a vital role in the overall acceptably of any meat product (Ruusunen & Puolanne, 2005). It is, therefore, prominent that the overall quality of processed meat can be negatively impacted by reducing Na (Pietrasik & Gaudette, 2014).

In an effort to create meat products that contain less Na, a number of alternative methods have been used. One approach was to substitute NaCl with KCl, but it was, however, found that the K cation causes bitterness at a certain level, which is undesirable (Bartoshuk *et al*., 1988). Using K as replacer may, therefore, be limited to products where only a small amount of K is added (Desmond, 2006).

Sodium chloride consumption provides humans with a hedonic sensation (Mattes, 1997). Meat and meat products are some of the world's most critical dietary components and even though consumers have on occasion indicated health concerns, in general they do not seem to take on healthier eating habits (Grunert, 2006). Diets that are unbalanced and include high amounts of NaCl intake were identified by the WHO as some of the causes of certain chronic diseases, because of their negative effect on blood pressure (CTAC, 2009). Currently, the health costs around the world for cardiovascular diseases make up 11% of the total health expenditures, making it the most expensive (Australian Institue of Health and Welfare, 2005). In combination with additional cardiovascular related expenses, it is predicted that the average Na reduction strategy will only amount to 0.3% of the current costs, concerning hypertension control (Asaria *et al*., 2007). In most of the developed countries, individuals consume far more Na than what is recommended, so lowering Na intake is a priority in terms of public health and will be beneficial (Cordain *et al.,* 2005; Neal *et al.*, 2006). In order to encourage the consumption of meat, the meat industry must produce healthier and more convenient products (Font-i-Furnols & Guerrero, 2014).

When NaCl is reduced, it may lead to the need for other parameters to be changed, in order to maintain the required texture (Doyle & Glass, 2010), as well as good chemical and microbial shelf life. Reduction of NaCl in large amounts is a major challenge in terms of upholding consumer acceptance (Bobowski *et al*., 2015). It is important to avoid higher production costs that will cause the price-sensitive consumers to be affected (Cluff, 2016).

From the results obtained from the survey on the current Na content of commercially available Boerewors in the Bloemfontein area, it was found that most of the products comply with the current Na legislation for this particular product (DoH of South Africa, 2013). Even though there are products that already comply with the 2019 inclusion limit, it is necessary to know what the implications will be (Chapter 3).

This chapter aimed to examine the effect of using the 2016 and 2019 regulations for Na limits in Boerewors, by adjusting the added NaCl, which reduces the total amount of Na in combination with different replacers. This was applied using five different formulations, consisting of different NaCl inclusion levels, in combination with different replacers. The effects of these treatments were examined on chemical and microbial stability, sensory quality, as well as thawing and cooking losses.

4.2. Materials and methods

4.2.1. Sourcing of lean meat, fat, additives and spices

All the meat and fat used during this trial were sourced from an owner-operator meat processing plant in Bloemfontein, SA. Fresh pork, consisting of a minimum of 70% lean meat and 30% fat (70/30), fresh lean beef (80/20) and good quality pork back fat were used to manufacture the sausages. The meat was collected less than 24 h before required and transported to the meat processing facility of the University of the Free State for further processing, where it was kept at 4 °C until used. The spices and additives were obtained from BT Enterprises in Johannesburg, SA.

4.2.2. Formulation of Boerewors

The basis of the formulation was the two Na limits as set out in the South African Regulations (Department of Health of South Africa, 2013) (Chapter 3, section 3.3.2, Table 3.1). The limits are 800 mg Na per 100 g product by 30 June 2016 and 600 mg Na per 100 g product by 30 June 2019. Five different treatment formulations were used.

Treatment 1 (Tables 4.1 and 4.2) which is the negative control (NC), were formulated with 0% NaCl, and contained 110.03 mg Na/100 g. Treatments 2, 3 and 4 were formulated in accordance with the next Na limit of 600 mg Na/100 g sausage (mandatory from 30 June 2019) and they were treated with different NaCl replacers. Treatment 2 (K600) was formulated to contain 600.12 mg Na/100 g (1.25 % NaCl) and KCl was added as replacer (Tables 4.3 and 4.4). Treatment 3 (L600) was formulated to contain 599.78 mg Na/100 g (1.25 % NaCl) (Tables 4.5 and 4.6) and lactate was added as replacer. Treatment 4 (N600) was formulated to contain only 600.38 mg Na/100 g (1.25 % NaCl) and no replacers were added (Tables 4.7 and 4.8). Treatment 5, the positive control (PC), was formulated in accordance with the current Na limit of 800 mg/100 g sausage, which was made mandatory on the 30th of June 2016. Treatment 5 (Tables 4.9 and 4.10) was formulated to contain 816.69 mg Na/100 g (1.80 % NaCl).

4.2.3. Manufacturing of Boerewors

Three separate replicates of Boerewors were manufactured at least one month apart. This was done to compensate for variations in raw materials and processing, as well as environmental conditions. A single replicate consisted of 3 kg batches, made for each of the five treatment groups.

Table 4.1*:* Treatment 1: Negative control spice formulation

Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Fine NaCl	**0.00**	0.000	39330.000	0.000
Coriander coarse roasted	0.12	1.200	35.000	2.211
Coriander coarse	0.16	1.600	35.000	2.947
Coriander ground roasted	0.16	1.600	35.000	2.947
Coriander ground	0.18	1.800	35.000	3.316
Clove stems ground	0.08	0.800	242.000	10.189
Nutmeg ground	0.09	0.900	16.000	0.758
Black pepper ground	0.03	0.300	20.000	0.316
White pepper ground	0.04	0.400	5.000	0.105
Mustard powder	0.04	0.400	13.000	0.274
MSG	0.25	2.500	11850.000	1559.211
Dextrose	0.19	1.900	0.000	0.000
Medium rusk	0.50	5.000	11.800	3.105
Sodium metabisulphite	0.06	0.600	24187.000	763.800
Total	**1.90**	**19.000**		**2349.179**

Table 4.2: Treatment 1: Negative control sausage formulation

Meat block Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Beef 80/20	61.420	614.200	72.000	44.222
Pork 70/30	30.680	306.800	69.000	21.169
Ice water	6.000	60.000	0.000	0.000
Spice pack	1.900	19.000	2349.179	44.634
Total:	**100.000**	**1000.000**		**110.026**

Table 4.3: Treatment 2: K600 spice formulation

Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Fine salt	**1.249**	12.490	39330.000	13276.532
Potassium chloride	**0.551**	5.510	25.000	3.723
Coriander coarse roasted	0.120	1.200	35.000	1.135
Coriander coarse	0.160	1.600	35.000	1.514
Coriander ground roasted	0.160	1.600	35.000	1.514
Coriander ground	0.180	1.800	35.000	1.703
Clove stems ground	0.080	0.800	242.000	5.232
Nutmeg ground	0.090	0.900	16.000	0.389
Black pepper ground	0.030	0.300	20.000	0.162
White pepper ground	0.040	0.400	5.000	0.054
Mustard powder	0.040	0.400	13.000	0.141
MSG	0.250	2.500	11850.000	800.676
Dextrose	0.190	1.900	0.000	0.000
Medium rusk	0.500	5.000	11.800	1.595
Sodium metabisulphite	0.060	0.600	24187.000	392.222
Total:	**3.700**	**37.000**		**14486.591**

Table 4.4: Treatment 2: K600 sausage formulation

Meat block Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Beef 80/20	60.200	602.000	72.000	43.344
Pork 70/30	30.100	301.000	69.000	20.769
Ice water	6.000	60.000	0.000	0.000
Spice pack	3.700	37.000	14486.591	536.004
Total:	**100.000**	**1000.000**		**600.117**

Table 4.5*:* Treatment 3: L600 spice formulation

Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Fine salt	**1.249**	12.490	39330.000	15599.609
Coriander coarse roasted	0.120	1.200	35.000	1.334
Coriander coarse	0.160	1.600	35.000	1.778
Coriander ground roasted	0.160	1.600	35.000	1.778
Coriander ground	0.180	1.800	35.000	2.001
Clove stems ground	0.080	0.800	242.000	6.148
Nutmeg ground	0.090	0.900	16.000	0.457
Black pepper ground	0.030	0.300	20.000	0.191
White pepper ground	0.040	0.400	5.000	0.064
Mustard powder	0.040	0.400	13.000	0.165
MSG	0.250	2.500	11850.000	940.775
Dextrose	0.190	1.900	0.000	0.000
Medium rusk	0.500	5.000	11.800	1.874
Sodium metabisulphite	0.060	0.600	24187.000	460.851
Total:	**3.149**	**31.490**		**17017.024**

Table 4.6: Treatment 3: L600 sausage formulation

Meat block Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Beef 80/20	60.200	602.000	72.000	43.344
Pork 70/30	30.100	301.000	69.000	20.769
Ice water	6.000	60.000	0.000	0.000
K-lactate	**0.551**	**5.510**	**0.000**	**0.000**
Spice pack	3.150	31.490	17017.024	535.866
Total:	**100.000**	**1000.000**		**599.979**

Table 4.7*:* Treatment 4: N600 spice formulation

Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Fine salt	**1.249**	12.490	39330.000	15599.609
Coriander coarse roasted	0.120	1.200	35.000	1.334
Coriander coarse	0.160	1.600	35.000	1.778
Coriander ground roasted	0.160	1.600	35.000	1.778
Coriander ground	0.180	1.800	35.000	2.001
Clove stems ground	0.080	0.800	242.000	6.148
Nutmeg ground	0.090	0.900	16.000	0.457
Black pepper ground	0.030	0.300	20.000	0.191
White pepper ground	0.040	0.400	5.000	0.064
Mustard powder	0.040	0.400	13.000	0.165
MSG	0.250	2.500	11850.000	940.775
Dextrose	0.190	1.900	0.000	0.000
Medium rusk	0.500	5.000	11.800	1.874
Sodium metabisulphite	0.060	0.600	24187.000	460.851
Total:	**3.149**	**31.490**		**17017.024**

Table 4.8: Treatment 4: N600 sausage formulation

Meat block Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Beef 80/20	60.570	605.700	72.000	43.611
Pork 70/30	30.280	302.800	69.000	20.893
Ice water	6.000	60.000	0.000	0.000
Spice pack	3.150	31.490	17017.024	535.871
Total:	**100.000**	**999.990**		**600.376**

Table 4.9*:* Treatment 5: Positive control spice formulation

Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Fine salt	**1.800**	18.000	39330.000	19133.514
Coriander coarse roasted	0.120	1.200	35.000	1.135
Coriander coarse	0.160	1.600	35.000	1.514
Coriander ground roasted	0.160	1.600	35.000	1.514
Coriander ground	0.180	1.800	35.000	1.703
Clove stems ground	0.080	0.800	242.000	5.232
Nutmeg ground	0.090	0.900	16.000	0.389
Black pepper ground	0.030	0.300	20.000	0.162
White pepper ground	0.040	0.400	5.000	0.054
Mustard powder	0.040	0.400	13.000	0.141
MSG	0.250	2.500	11850.000	800.676
Dextrose	0.190	1.900	0.000	0.000
Medium rusk	0.500	5.000	11.800	1.595
Sodium metabisulphite	0.060	0.600	24187.000	392.222
Total:	**3.700**	**37.000**		**20339.849**

Table 4.10: Treatment 5: Positive control sausage formulation

Meat block Component	Final product inclusion (%)	Final product inclusion (g/kg)	Na content (mg/100 g component)	Na content (mg/100 g final product)
Beef 80/20	60.200	602.000	72.000	43.344
Pork 70/30	30.100	301.000	69.000	20.769
Ice water	6.000	60.000	0.000	0.000
Spice pack	3.700	37.000	20339.849	752.574
Total:	**100.000**	**1000.000**		**816.687**

A fourth replicate was manufactured specifically for sensory analysis and a fifth replicate was manufactured for determination of cooking losses and yield. The sausage models were manufactured, by using representative industrial procedures (Hugo, Roberts & Smith, 1993) and in compliance with the South African regulations for Boerewors (Government Notice No. R.2718 of 23 November, 1990; Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972). Tables 4.1 to 4.10 show the spice and sausage formulations for the five different groups. Fresh meat (beef [80/20] and pork [70/30] were comminuted through a 13 mm mincing plate, fitted to a number 32 Okto mincer. The meat was thoroughly mixed to obtain a homogenous raw material mixture. The spice pack, together with the additives, was mixed with the ice water and left to stand for five minutes, to allow for hydration and the water-soluble additives to dissolve properly. This spice mixture was thoroughly mixed with the meat mixture, before being minced through a 4.5 mm mincing plate. Natural hog casings with a diameter of 28-32 mm were filled with the sausage mixture, using a manual sausage filler (Trespade, Crown National, Johannesburg, SA). This resulted in a single, continues roll of sausage that had a weight of 3 kg per treatment. Individual sausages were cut from each roll. Each treatment's roll of sausage was cut in individual 150 g pieces for day 0 samples, 100 g pieces for days 3, 6 and 9 samples and 50 g pieces for the 90 and 180 day samples. This resulted in 120 sausage samples per replicate.

Each individual sausage was placed in an expanded polystyrene (EPS) tray, containing an absorbent pad and was then over-wrapped with polyvinyl chloride (PVC) film. The sausages that were sampled on days 0, 3, 6 and 9 were stored at 4 °C under retail refrigeration-type conditions, including fluorescent lighting, for fresh product shelf-life determination (20 sausages per treatment for each day of sampling). The other sausages for the 90 and 180-day analysis, were, in addition to being over-wrapped with PVC, also vacuum packed and stored at -18 °C for frozen product lipid stability determination (20 sausages per treatment for each day of sampling). For the fourth replicate a whole 3 kg sausage roll of all five treatment groups were stored at -18 °C, until sensory analysis was carried out shortly after the sausages were made. The sausages of the fifth replicate, for the cooking losses, were weighed after manufacturing in 100 g pieces, placed in individual EPS trays containing absorbent pads, over-wrapped with PVC film and stored at 4 °C for one day and then at -18 °C for 9 days, before analyses was carried out.

4.2.4. Sampling

In South Africa, Boerewors are typically sold as fresh sausage. It is also common practice for fresh Boerewors to be bought and frozen at home. To this end, both a fresh product shelf-life evaluation of up to 9 days, as well as a frozen product shelf-life evaluation of up to 180 days, were used, during which sampling took place. The fresh product shelf-life evaluation sampling took place on days 0, 3, 6 and 9, and the frozen product shelf-life evaluation took place on days 0, 90 and 180. For each sampling interval, four sausages per treatment group per replicate were collected for quadruplicate chemical and microbial analyses. For thaw, cooking and total losses, 12 sausages per treatment group per replicate were used, with individual sausages weighing about 100 g.

4.2.5. Chemical analyses

Various chemical analysis techniques were used across all five different treatments. After samples for microbial analyses were taken aseptically; the remaining sausage sample was used to fill three plastic cuvettes with tight fitting lids. Two cuvettes were frozen at –18 °C for chemical analyses, that were conducted on days following the sampling day. The third cuvette was used for same-day chemical analyses. The same process was repeated for each of the four samples per treatment group, per time interval, per replicate.

4.2.5.1. NaCl, Na and K content of Boerewors samples

Sodium chloride and Na content were determined on day 0 samples, as representatives of finished products. For NaCl, the Volhard method was used to volumetrically and quantitatively determine the amount of Cl present in the sample (as discussed in Chapter 3, section 3.2.3), which was then used to calculate the amount of NaCl present in the sample (AOAC, 2005). Sodium content was determined (as discussed in Chapter 3, section 3.2.2) by atomic absorption spectroscopy (AAS), using a Varian SpectrAA-300 spectrometer (SMM Instruments, Johannesburg, SA). For AAS analysis, the samples were reduced to a mineralised form free from any organic compounds, by means of ashing (Nielsen, 2010).

4.2.5.2. pH measurements

The pH was measured directly using a direct pH measurement probe (Model MA920, Milwaukee Instruments, Rock Mount, USA), coupled to a pH meter (Thermo Scientific, Orion 3-Star Plus Model, Labotec, Midrand, SA) to record quadruple pH measurements, per treatment group, per replicate, at room temperature. Each day before use, the pH meter was calibrated with standardised buffers (Merck, Johannesburg, SA) with pH values of 4.01 and 7.00 respectively.

4.2.5.3. Water activity

A homogenously mixed sample was filled into a a_w container (height of 5 mm and diameter of 39 mm) to the appropriate level and covered with a container lid. The a_w was determined, using a Novasina Thermoconstanter TH 200 (Labotec, Midrand, SA) water activity meter. After equilibrium was reached with deionised distilled water, quadruplicate measurements per treatment group, per replicate, were made at a temperature of 25°C. The results were reported as percentage relative humidity (% rH) and converted to a_w values, by dividing each value by a factor of a 100.

4.2.5.4. Lipid oxidative stability and moisture content

A 5 g sample was taken at each sampling interval, per treatment group, per replicate and used for thiobarbituric acid reactive substance (TBARS) analysis, using the aqueous acid extraction method of Raharjo *et al.* (1993), to determine the effect of the different added NaCl levels and NaCl replacers on lipid oxidation. Frozen samples were thawed overnight at 4°C. The TBARS results were quantified in terms of milliequivalents (mEq) malondialdehyde (MDA) per kg sample, as other TBARS may also be present (Beltran *et al*., 2003). The analysis took place after days 0, 3, 6 and 9 of storage at 4 °C, as well as after 90 and 180 days of storage at -18 °C. Moisture content (%) was determined by oven drying overnight at 121 °C and used as a second establishing parameter (AOAC, 2005) as it is required in the calculation of TBARS.

4.2.6. Microbial analyses

For the microbial analysis, first the natural casing around the sausage was removed aseptically with a pair of flame-sterilised scissors and forceps. Some of the sausage sample was then transferred into a sterile petri dish, after which 10 g sample from each product was aseptically weighed and placed into a sterile 207 mL WhirlPak™ bag (Lasec, Bloemfontein, SA), after which 90 mL of sterile 0.1 M buffered peptone water (BPW) solution was added to create a 10^{-1} dilution. The sample was homogenised using a stomacher (AME Stomacher Lab-Blender 400, JHB) for 1 minute. Further dilutions (10^{-2} to 10^{-6}) were made by adding 1 mL of the 100 mL sample (10^{-1} dilution) in McCartney bottles, containing 9 mL sterile 0.1 M phosphate buffer solution (Harrigan, 1998). One millilitre volumes of each dilution were then plated on different selective media using the pour plate technique. All media were sourced from ThermoFisher (Pty) Ltd (Randburg, SA).

Standard plate count agar (SPCA; Oxoid 0463) was used to enumerate the total viable counts and the plates were incubated at 32 °C for 48 h. After incubation, the colonies were enumerated using a colony counter.

Violet red bile agar + 4-methylumbelliferyl-β-D-glucuronide (VRBM; Oxoid CM0978) was used for total coliform counts, as well as for detection of *E. coli*. The VRBM plates were incubated at 37 °C for 48 h and fluorescence under ultraviolet light (366 nm, CAMAG Universal UV Lamp) was used as an indication of the presence of *E. coli*.

Yeast and mould enumeration were done using Rose-Bengal Chloramphenicol agar (RBCA; Oxoid CM0549), with chloramphenicol supplement (Oxoid SR0078), incubated at 25 °C for four days.

Baird Parker agar (BPA; Oxoid CM0275), with egg yolk tellurite supplement (Oxoid SR0054), was used for the detection of *S. aureus*. Incubation was at 37 °C for 24 h. *Staphylococcus aureus* typically forms colonies that are 1.0-1.5 mm in diameter, black, shiny, convex with a narrow white margin and surrounded by clear zones, extending 2-5 mm into the opaque medium (Harrigan, 1998).

4.2.7. Physical analyses

4.2.7.1. Colour

Colour measurements were performed only on the refrigerated Boerewors samples on days 0, 3, 6 and 9 of storage at 4 ºC, after the sample for microbial analysis was taken and samples were left to stand for 30 min. Four sausage samples of each of the five treatment groups were used and measurements per sausage were done in sextuplicate. Colour measurements were made using a Minolta CR 400 chromometer (8 mm measuring area). The *CIE L*a*b** colour scale was used for comparison, where L^* represents lightness, a^* represents redness and b^* represents yellowness. The chroma ($\sqrt{a^{*2}+b^{*2}}$) and hue angle [$\tan^{-1}(b^*/a^*)$] were also calculated (Ripoll, Joy, & Muñoz, 2011; Tapp, Yancey, & Apple, 2011).

4.2.8. Thaw and cooking losses

Twelve sausages of each treatment group were kept frozen at −18 °C for 9 days, to simulate a short-term home-freezing scenario. After 9 days, the samples were kept at 4 °C for 24 h, to allow it to thaw gradually, after which the samples were removed from their packaging and weighed. The sausages were then dry-cooked in a convection oven, pre-heated to 160 °C, until an internal temperature of 72 °C was reached. During cooking, the baking tray was rotated 90° every 2 min, for even cooking conditions. Afterwards, the sausages were removed from the oven and air-cooled to room temperature before being weighed again.

Thaw loss was calculated with the formula:
Thaw loss (%) = [(initial weight – weight after thawing) / initial weight] x 100

Cooking loss was calculated with the formula:
Cooking loss (%) = [(weight after thawing – weight after cooking) / weight after thawing] x 100

The total loss percentage was calculated as the sum of the thaw and cooking losses.

4.2.9. Consumer sensory evaluation

Samples of each treatment group were defrosted overnight at 4 °C. The sausages were then dry-cooked in a convection oven, pre-heated to 160 °C, until an internal temperature of 72 °C was reached. During cooking, the baking tray was rotated 90° every 2 min, for even cooking conditions. The cooked sausages were cut into pieces each with a length of ~ 2 cm and placed individually in small glass bowls that were covered with squares of aluminium foil. The bowls were kept warm at 55 °C until just before serving. Each container was marked with a randomised, three-digit code unique to each sample. Five glass bowls representing one of the five treatment groups were arranged from left to right on a serving tray, in ascending order of the three-digit codes, ensuring that the samples were evaluated in a random order from one consumer to the next.

A 75-member consumer panel of staff and students from the Agriculture Building of the University of the Free State was used. The panel consisted of 63 % females and 37 % males; ranging from 19 to 61 years of age, with an average age of between 20 and 29. The sensory evaluation was performed in individual booths of the sensory laboratory and the booths were fitted with three overhead light fittings with three red coloured bulbs emitting only red light to mask any possible colour variations between different samples.

Respondents were first expected to taste a wood ice-cream stick dipped in a weak NaCl solution and indicate the taste without prior knowledge as to what should be tasted. This was used as a screening method to exclude non-tasters from the sensory panel. Each respondent received a printed, 5-page questionnaire consisting of five nine-point hedonic rankings per page, ranging from 1 = dislike extremely to 9 = like extremely (Table 4.11). The respondents were then expected to rank each sample individually for the following attributes: appearance; flavour; saltiness; texture; and overall liking. Bottled water was presented at 20 °C as a palate cleanser between samples. Consumers generally have a more favourable attitude towards nutritional improvements made to meat products, as is the case with reduced NaCl content (Guardia *et al.,* 2006). At the same time, they might react negatively when a supposedly healthier product is regarded as unpalatable (Guerrero *et al.,* 2011). For these reasons, the members of this consumer panel were not specifically informed of the differences in added NaCl content of the four models, before sensory evaluation.

Table 4.11. A simplified example of the hedonic ranking used for consumer sensory analysis.

Nine-point ranking scale for taste, saltiness, texture and overall liking								
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

4.2.10. Statistical analyses

All results were captured in multiple spreadsheets in Microsoft Excel 2016. The experimental design consisted of five treatments and three replicates, for most methods of analysis. Exclusions to this included: sensory analysis; and thaw and cooking losses, where only one replicate was used. An ANOVA (NCSS 11 Statistical Software, 2016) was used to determine the effect of added NaCl and/or replacer levels had on various quality parameters of Boerewors treatments. The Tukey-Kramer multiple comparison test ($\alpha = 0.05$) was carried out to identify significant differences between the treatment means (NCSS 11 Statistical Software, 2016). Statistically significant differences were visualised in a 2-dimensional space by principal component analysis (PCA) (XLSTAT, 2018).

4.3. Results and discussion

4.3.1. Ash, NaCl, Na and K content

Ash was determined as a preparation step in the determination of minerals, before measurement with AAS (Table 4.12). The different ($p < 0.001$) percentages of ash content is a result of the different amounts of NaCl added in each of the different treatment formulations. The treatments with higher percentages of ash correlated directly with treatments that had higher amounts of added NaCl and added replacers, because the inorganic matter that was left behind mostly consisted of metal oxides, which are enhanced by the amount of NaCl added (Honikel, 2008).

Sodium chloride was present at a base level percentage of 0.21 ± 0.04 in the NC, with all contributions made by formulation components, other than added NaCl. The NaCl content of each treatment group following NaCl addition increased ($p < 0.001$), with increasing amounts of added NaCl. The K600 treatment did show a higher NaCl percentage (Table 4.12) and this could be attributed to the Volhard method of analysis that has a limiting factor in terms of accuracy, because the amount of NaCl was calculated from the amount of Cl present in the sample. This entirely omitted the Na contributed to the final product. Potassium in the K600 treatment was added as KCl, which then gave a higher value. The NaCl content was converted to Na content values, for the Na content per 100 g of product, using the percentage Na usually contributing to the molecular weight of pure NaCl (39.33%). Comparisons were drawn between the calculated Na content, that was converted from the Volhard results and the actual Na content, as determined by AAS analysis. All the values for the Na calculated from NaCl was underestimated, except for the value of the K600 treatment which had a higher value and was ascribed to the Cl^- interfering with the Volhard method, as previously discussed. The values of the Na content in each treatment correlated with the added Na in each treatment, which verified that formulations were correct (Figure 4.1). With regard to the K values, the K600 and L600 had significantly ($p < 0.001$) higher values than the rest of the treatments (580.87 ± 87 and 401.23

Table 4.12: Effect of NaCl replacer treatment on chemical properties of fresh Boerewors directly after manufacturing

Treatment	NC	K600	L600	N600	PC	Sign. level
Formulated Na (mg/100 g)	**110.03**	**600.12**	**599.78**	**600.38**	**816.69**	
% Ash	$1.03^{a} \pm 0.04$	$3.07^{c} \pm 0.11$	$2.73^{b} \pm 0.27$	$2.63^{b} \pm 0.22$	$3.69^{d} \pm 0.15$	$p < 0.001$
% NaCl	$0.21^{a} \pm 0.04$	$1.78^{d} \pm 0.04$	$1.44^{c} \pm 0.04$	$1.40^{b} \pm 0.04$	$1.92^{e} \pm 0.06$	$p < 0.001$
Na (mg/100 g)	$131.89^{a} \pm 16.63$	$607.68^{b} \pm 46.95$	$610.49^{b} \pm 122.22$	$628.02^{b} \pm 111.06$	$855.21^{c} \pm 45.25$	$p < 0.001$
Na calculated from NaCl	$81.26^{a} \pm 14.02$	$698.28^{d} \pm 14.69$	$566.04^{c} \pm 14.10$	$552.53^{b} \pm 14.50$	$755.38^{e} \pm 21.93$	$p < 0.001$
K (mg/100 g)	$227.03^{a} \pm 9.60$	$580.87^{d} \pm 42.89$	$401.23^{c} \pm 70.12$	$302.11^{b} \pm 56.66$	$340.73^{bc} \pm 14.84$	$p < 0.001$

NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts in the same row differed significantly.

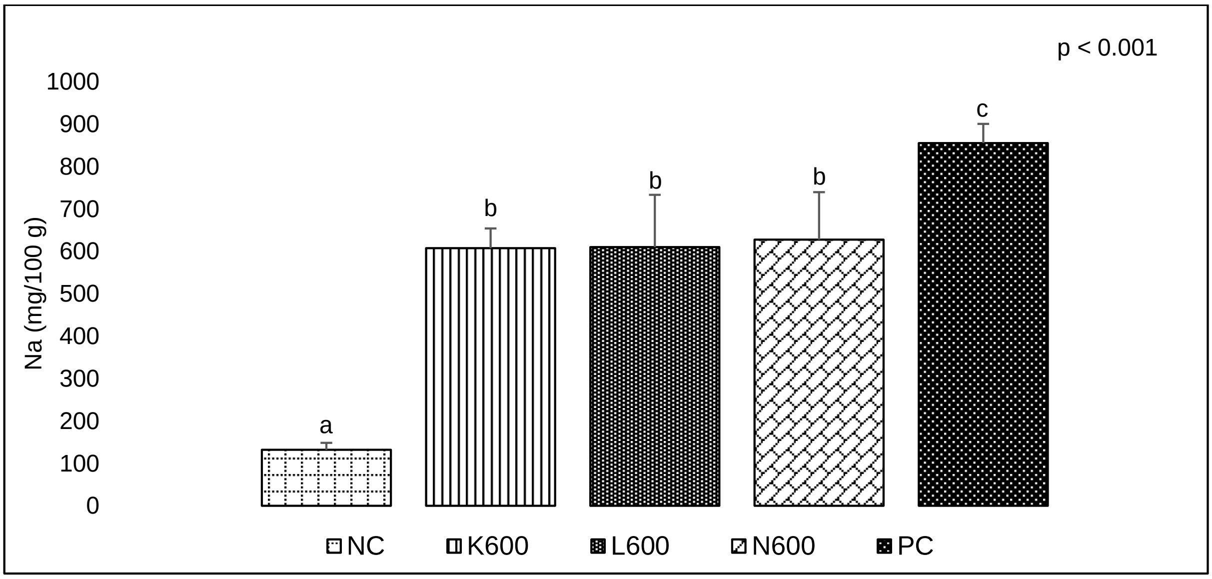

Figure 4.1. Sodium content of each of the five Boerewors formulations based on different added NaCl and/or replacer levels. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts differed significantly. Error bars represent standard deviations of means.

± 70.12, respectively). The reason why the L600 treatment had a significantly higher value was because lactate was added as K-lactate in the formulation.

4.3.2. pH, a_w and moisture content

The pH values showed significant differences for the different formulations (Table 4.13). On days 0, 3 and 9, the NC had significantly higher ($p < 0.001$) pH values and differed significantly from the rest of the treatments. On day 6 the PC had the lowest value of 5.54 ± 0.12 and the NC had the highest value (5.75 ± 0.10). The PC, L600 and K600 differed significantly ($p < 0.001$) from both the NC and the N600 treatment. The dissociated Na^+ and Cl^- ions of NaCl determine the functions thereof in meat mixtures. When NaCl is added to minced meat, the negative charge on the proteins is increased by the Cl^- ions.

The isoelectric point is shifted to a lower pH when Cl^- ions are adsorped onto positively charged groups of myosin (Gregory, 1998). From the results obtained, it seemed that the addition of NaCl lowered the pH, which correlated with results obtained in a study done on the effect that NaCl and storage time had on the physicochemical and sensorial properties of beef meatballs, where it was found that the pH of meatballs decreased as NaCl concentration increased (Tanny *et al.,* 2014). These results were in contrast with results obtained in another study, where it was found that the pH increased as the NaCl concentration increased (Kuo & Ockerman, 1984).

Table 4.13: Effect of NaCl replacer treatment on chemical properties of fresh Boerewors directly after manufacturing

Day	Treatment	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level
	NC	5.67[b] ± 0.05		0.9606[ab] ± 0.0135		63.09 ± 2.93	
	K600	5.52[a] ± 0.05		0.9609[ab] ± 0.0084		62.61 ± 2.67	
0	**L600**	5.57[a] ± 0.14	$p < 0.001$	0.9576[ab] ± 0.0165	$p = 0.036$	62.47 ± 3.01	$p = 0.470$
	N600	5.50[a] ± 0.11		0.9632[b] ± 0.0102		63.18 ± 3.49	
	PC	5.51[a] ± 0.05		0.9491[a] ± 0.0053		61.75 ± 2.63	
	NC	5.73[b] ± 0.08		0.9641[b] ± 0.0088		62.66 ± 2.86	
	K600	5.57[a] ± 0.08		0.9621[ab] ± 0.0075		61.95 ± 2.26	
3	**L600**	5.55[a] ± 0.14	$p < 0.001$	0.9528[a] ± 0.0110	$p = 0.020$	62.69 ± 2.15	$p = 0.277$
	N600	5.51[a] ± 0.05		0.9569[ab] ± 0.0097		62.81 ± 3.52	
	PC	5.48[a] ± 0.11		0.9549[ab] ± 0.0091		61.43 ± 1.71	
	NC	5.75[c] ± 0.10		0.9645[b] ± 0.0079		62.76 ± 2.45	
	K600	5.65[abc] ± 0.06		0.9503[ab] ± 0.0176		61.27 ± 1.86	
6	**L600**	5.59[ab] ± 0.09	$p < 0.001$	0.9486[ab] ± 0.0157	$p < 0.001$	62.18 ± 2.56	$p = 0.079$
	N600	5.65[bc] ± 0.11		0.9607[b] ± 0.0094		63.05 ± 2.87	
	PC	5.54[a] ± 0.12		0.9380[a] ± 0.0180		61.66 ± 2.51	
	NC	5.76[b] ± 0.08		0.9597[c] ± 0.0083		62.60 ± 2.06	
	K600	5.62[a] ± 0.07		0.9461[ab]± 0.0109		61.78 ± 2.73	
9	**L600**	5.60[a] ± 0.12	$p < 0.001$	0.9443[a] ± 0.0030	$p = 0.002$	61.96 ± 2.84	$p = 0.590$
	N600	5.58[a] ± 0.10		0.9578[bc] ± 0.0117		62.85 ± 2.83	
	PC	5.60[a] ± 0.08		0.9502[abc] ± 0.0155		62.39 ± 2.35	

NC = negative control (0.00% added NaCl); K600 = treatment with potassium as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts in the same column and for the same day differed significantly

On day 0, the PC, which also had the highest NaCl content (Table 4.13), had the lowest a_w value. This can be attributed to the fact that NaCl lowers a_w by binding available free water (Forsythe & Miller, 1980; Sebranek *et al.,* 1983). On day 3, all four treatments with added NaCl differed significantly ($p = 0.02$) from the NC, which had the highest a_w value of 0.9641 ± 0.0088. On days 6 and 9, the a_w differed significantly ($p < 0.001$ and $p = 0.002$) between the treatments, with the NC having the highest a_w with a value of 0.9645 ± 2.45 for day 6 and 0.9597 ± 0.0083 for day 9. It could be seen that the addition of replacers also helped to reduce the a_w throughout the 9-day storage period, since the K600 and L600 treatments overall had low values.

The moisture content remained constant throughout the 9-day storage period and there were no significant differences between the five different treatments (Table 4.13). Even though the sausage samples were stored in trays wrapped with semi-permeable film, it did help to keep moisture and prevent samples from drying.

4.3.3. Lipid oxidative stability

The lipid oxidative stability was determined by quantification of TBARS over time, using MDA as an indicator of the level of secondary lipid oxidation in the five different treatment groups (Figure 4.2.). Lipid oxidation in meat is accelerated through catalytic reactions by the synergistic working of NaCl with heme and non-heme iron in meat, because of its pro-oxidant reactions (Rhee & Ziprin, 2001; Kiliç *et al*., 2014). Food quality is negatively affected when NaCl causes damage to lipids (Decker, 1998). There was no significant difference between treatments on days 0, 3 and 9. On day 6 there were significant differences between the treatments, with the N600 treatment having a significantly ($p = 0.020$) higher value than the rest of the treatments. The L600 treatment had the lowest value and could be ascribed to the fact that lactate can reduce the pro-oxidant effect of NaCl on meat products (Tan & Shelef, 2002). As seen in Figure 4.2, the TBARS values gradually increased over time and the peak for shelf life, in terms of secondary lipid oxidation, which translates to fat stability, was probably reached around day 6, where significant differences could be found between treatments. All the TBARS values were below that of an organoleptic threshold of 0.5 mg MDA/kg (Wood *et al*., 2008).

A first threshold of 0.50 mEq MDA/kg (Wood *et al.,* 2008) and a secondary threshold of 1 mEq MDA/kg (Gray & Pearson, 1987) exist for the detection of rancidity. As seen in Figure 4.2, there were no significant differences between the treatments regarding TBARS values on day 0 and all five different treatments had values below the 0.50 mEq MDA/kg threshold on day 9, which means at this point no rancidity was present due to secondary lipid oxidation.

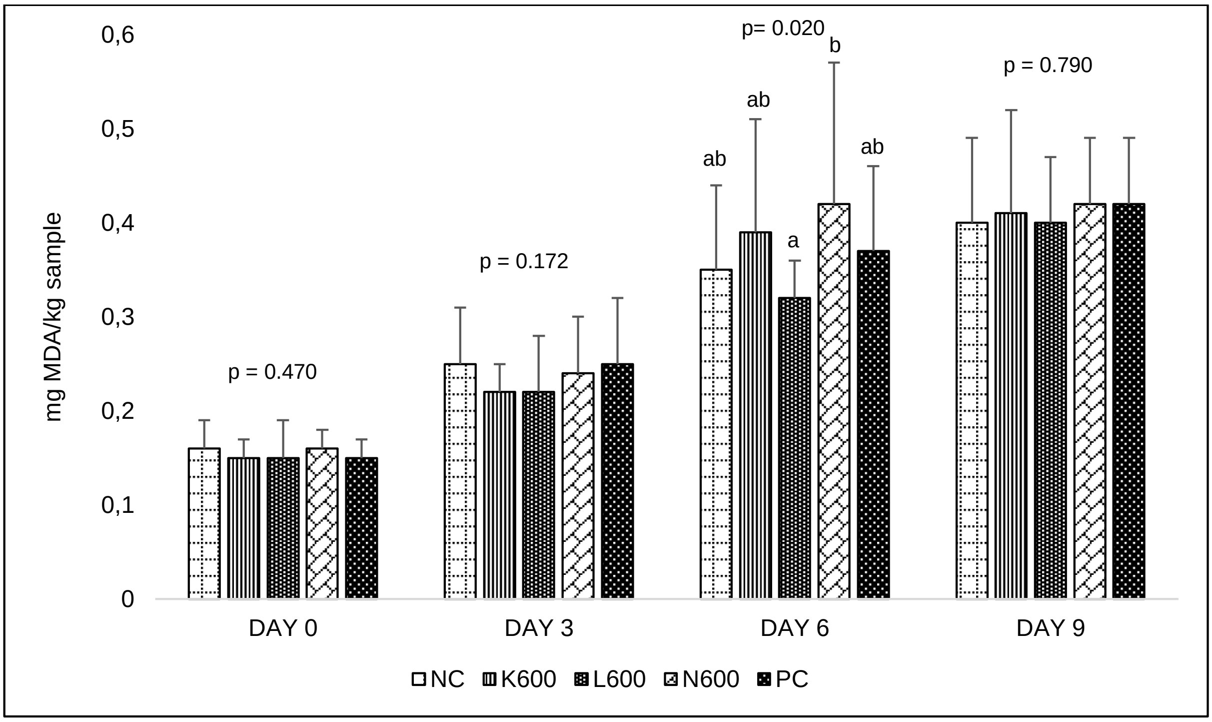

Figure 4.2. The effect of added NaCl level on the TBARS of Boerewors stored at 4 °C for up to 9 days. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

Sausages that were kept frozen at -18 °C for 90 days showed increased TBARS values (Figure 4.3). The N600 and PC treatments differed significantly ($p < 0.001$) from the rest of the treatments and their values also exceeded the 0.50 mEq MDA/kg threshold. The NC had the lowest secondary lipid oxidation. After 180 days of storage at -18°C, the secondary lipid oxidation had progressed to such an extent that only the NC and K600 treatments were below the one mEq MDA/kg threshold, with 0.80 ± 0.08 and 0.92 ± 0.08 values respectively. Even though the K600 treatment was below the limit, it was very close to the threshold. All the treatments differed significantly ($p < 0.001$) from one another. The PC that had the highest NaCl concentration also had the highest TBARS value, in contrast with the NC that had the lowest value. After 180 days of storage at -18 ºC sausages from the L600, N600 and PC treatments were above the one mEq MDA/kg threshold.

All the treatments showed that the TBARS values increased from day 90 to day 180. These results from products that were kept either under refrigerated (Figure 4.2) or frozen (Figure 4.3) storage, confirmed the previously stated pro-oxidative nature of NaCl (Rhee & Ziprin, 2001; Kiliç *et al*., 2014) which leads to the formation of secondary lipid oxidation products. Once again the K600 and L600 treatments that contained K and lactate as respective replacers, helped to reduce the secondary lipid oxidation, since it had lower values on days 90 and 180, compared to the N600 treatment, that contained the same amount of NaCl, but had higher TBARS values.

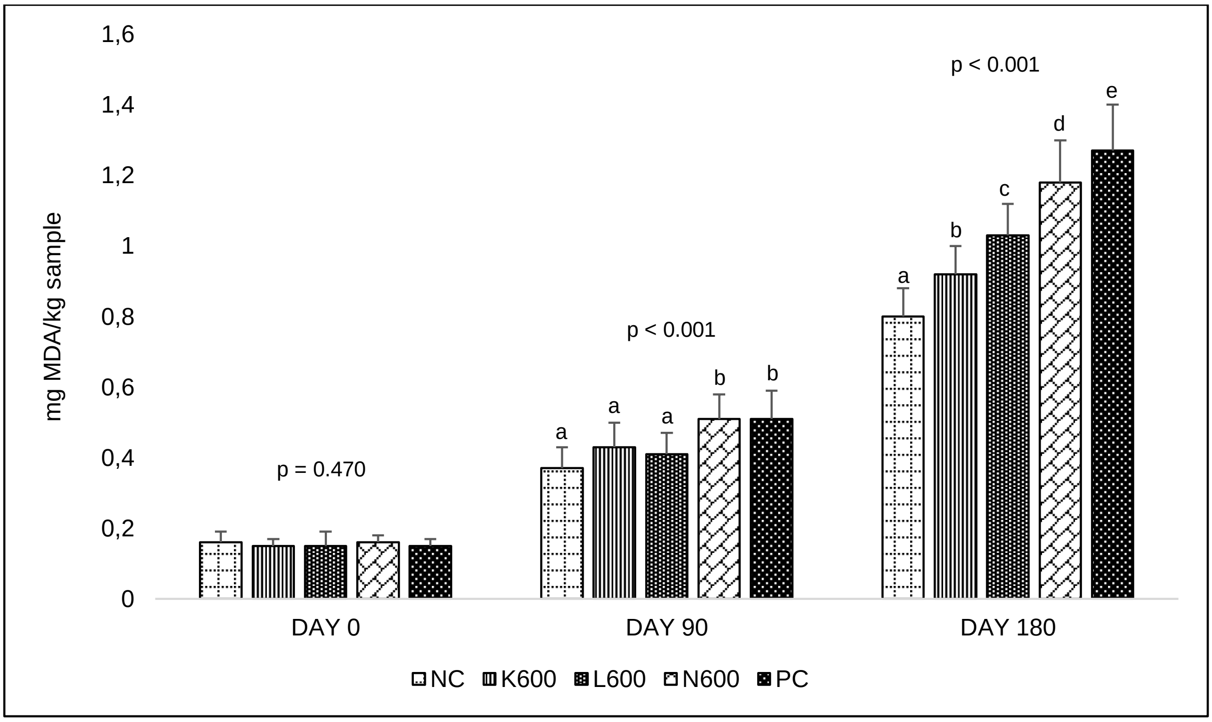

Figure 4.3. The effect of added NaCl and/or replacer levels on the TBARS values of Boerewors stored at -18 °C for up to 180 days. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sample day differed significantly. Error bars represent standard deviations of means.

4.3.4. Microbiological analyses

The different treatments did not show any immediate effect on the TVC and there were no significant differences between treatments on days 0 and 3 (Table 4.14). On days 6 and 9, however, there were significant differences ($p = 0.049$ and $p = 0.020$, respectively) between the NC and the rest of the treatments. The significant higher value of the NC showed that the addition of NaCl had an inhibitory effect on microbial growth. Even though the NaCl had an inhibitory effect, it did, however, not have any significant differences on the TVC between the treatments with different NaCl concentration and addition of replacers. From the data it is clear that all the different replacer combinations had similar inhibitory effects on microbial growth.

The storage time influenced the microbial growth, as seen with increasing TVC values. There are currently no standards for fresh South African sausage regarding maximum allowable counts. Therefore, the criteria for ground meat, suggested by Shapton & Shapton (1991), was followed ,

Table 4.14. Effect of NaCl replacer treatment on microbiological parameters of Boerewors stored at 4^{o}C for 9 days.

Day	Treatment	TVC (log cfu/g)	Sign. level	Coliforms (log cfu/g)	Sign. level	Yeasts (log cfu/g)	Sign. level	Moulds (log cfu/g)	Sign. level
	NC	6.12 ± 0.38		3.53b ± 0.14		3.47 ± 0.20		1.17 ± 0.22	
	K600	5.87 ± 0.31		2.84^{a} ± 0.15		3.41 ± 0.09		1.26 ± 0.21	
0	**L600**	5.90 ± 0.22	p=0.232	3.11^{a} ± 0.39	p<0.001	3.38 ± 0.15	p=0.324	1.28 ± 0.34	p=0.641
	N600	5.83 ± 0.36		3.04^{a} ± 0.24		3.34 ± 0.18		1.32 ± 0.25	
	PC	5.88 ± 0.35		2.99^{a} ± 0.21		3.45 ± 0.20		1.24 ± 0.20	
	NC	5.51 ± 0.57		2.93^{b} ± 0.11		4.12 ± 0.53		1.79 ± 0.45	
	K600	5.45 ± 0.62		2.52^{a} ± 0.31		4.16 ± 0.37		1.59 ± 0.44	
3	**L600**	5.44 ± 0.55	p=0.967	2.71^{ab} ± 0.16	p<0.001	4.07 ± 0.47	p=0.959	1.89 ± 0.22	p=0.371
	N600	5.34 ± 0.67		2.52^{a} ± 0.29		4.05 ± 0.48		1.68 ± 0.43	
	PC	5.48 ± 0.53		2.61^{a} ± 0.29		4.16 ± 0.31		1.72 ± 0.32	
	NC	6.17^{b} ± 0.31		2.94^{d} ± 0.15		5.59^{b} ± 0.19		1.76 ± 0.44	
	K600	6.00^{a} ± 0.37		2.33^{ab} ± 0.19		5.42^{ab} ± 0.17		1.49 ± 0.37	
6	**L600**	6.11^{a} ± 0.27	p=0.049	2.67^{c} ± 0.15	p<0.001	5.40^{a} ± 0.20	0.025	1.65 ± 0.31	p=0.322
	N600	5.76^{a} ± 0.50		2.51^{bc} ± 0.14		5.55^{ab} ± 0.10		1.58 ± 0.37	
	PC	5.92^{a} ± 0.35		2.25^{a} ± 0.26		5.46^{ab} ± 0.13		1.42 ± 0.58	
	NC	6.71^{b} ± 0.29		2.91^{b} ± 0.13		6.19^{c} ± 0.11		1.81^{b} ± 0.40	
	K600	6.32^{a} ± 0.39		2.40^{a} ± 0.28		5.95^{bc} ± 0.35		1.56^{ab} ± 0.48	
9	**L600**	6.69^{a} ± 0.29	p=0.020	2.43^{a} ± 0.23	p<0.001	5.96^{bc} ± 0.37	p<0.001	1.44^{ab} ± 0.44	p=0.037
	N600	6.36^{a} ± 0.41		2.43^{a} ± 0.20		5.64^{ab} ± 0.32		1.32^{a} ± 0.22	
	PC	6.50^{a} ± 0.35		2.32^{a} ± 0.28		5.49^{a} ± 0.53		1.45^{ab} ± 0.35	

NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts in the same column and within the same day differed significantly.

which suggests an upper limit of log 7 for TVC. The TVC of all the treatments in this study over the 9-day storage period remained beneath this upper limit (Figure 4.4).

The addition of NaCl and replacers in treatments were found to have significant effects on the coliform counts on days 0, 3, 6 and 9 (Table 4.14). On days 0, 3 and 9 the NC had a significantly ($p < 0.001$) higher value than the rest of the treatments. Even though the four treatments with added NaCl did not differ significantly from each other, it could be seen that the K600 and PC treatments had lower values than the rest. On day 6, the PC and K600 treatments once again had the lowest values and differed significantly ($p < 0.001$) from the other three treatments, which also differed significantly from one another. From the graph (Figure 4.5) it is clear that the NC had predominantly higher values than the rest of the treatments and also that the K600 and PC treatment had lower values than the other treatments. The coliform count is a hygiene indicator that gives an indication of the microbiological condition of a food product and exposes unsanitary conditions (Martin *et al*., 2016). The values obtained were also lower than the specified criteria that specifies a maximum limit of log 4 (Shapton & Shapton, 1991). This suggested sanitary conditions and good hygienic practices during and after production of the sausages.

Yeasts showed a steady increase over the 9-day period (Table 4.14). No significant differences were found between treatments on days 0 and 3. On days 6 and 9 the NC differed significantly ($p = 0.025$ and $p < 0.001$, respectively) from the other treatments, probably because of no Na that was added. The N600 and PC had the lowest values, the NC had the highest value and the K600 and L600 had intermediate values. It is clear that the NaCl had an inhibitory effect. Yeasts in high volumes are known to cause spoilage (Fleet, 1992). It has, however, more recently been found that yeasts do not contribute significantly to the spoilage of meat products (Nielsen *et al.,* 2008).

The moulds did not show any significant differences on days 0, 3 and 6 (Table 4.14). On day 9 however, it was found that the NC differed significantly ($p = 0.037$) form the other treatments. The mould increased from day 0 to day 3 and after that only the mould count of the NC increased from the count on day 3 of 1.79 ± 0.45 log CFU/g to 1.81 ± 0.40 log CFU/g on day 9.

There were no *S. aureus* or *E. coli* found in any of the samples, which is an indication of good quality raw ingredients used, as well as sanitary manufacturing practices.

It should be made clear that it is highly unlikely to have a shelf-life of 9 days for this type of product. It was decided to evaluate a 9-day shelf life in order to demonstrate an abusive storage time for a product like Boerewors. Even though there are numerous factors, such as pH, temperature, oxygen, fat and other additives (Doyle & Glass, 2010), that determine the amount of Na needed to inhibit microbial growth, it is clear that NaCl does play a very significant role in the control of microbial growth.

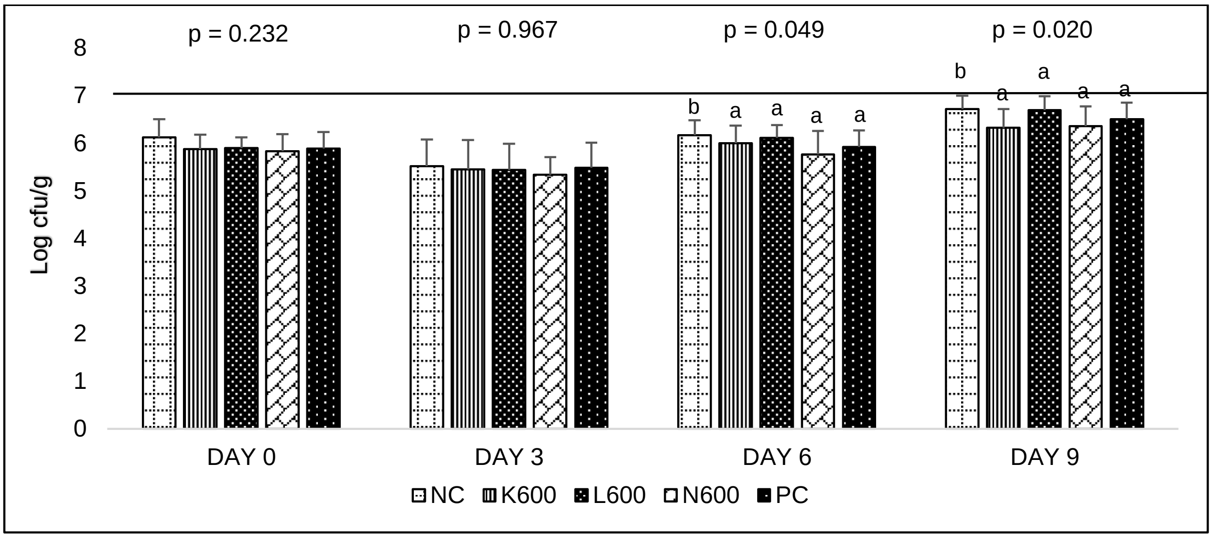

Figure 4.4. TVC counts (log cfu/g) of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). NS = not significant. Means with different superscripts for the same sampling day differed significantly. Error bars represent standard deviations of means.

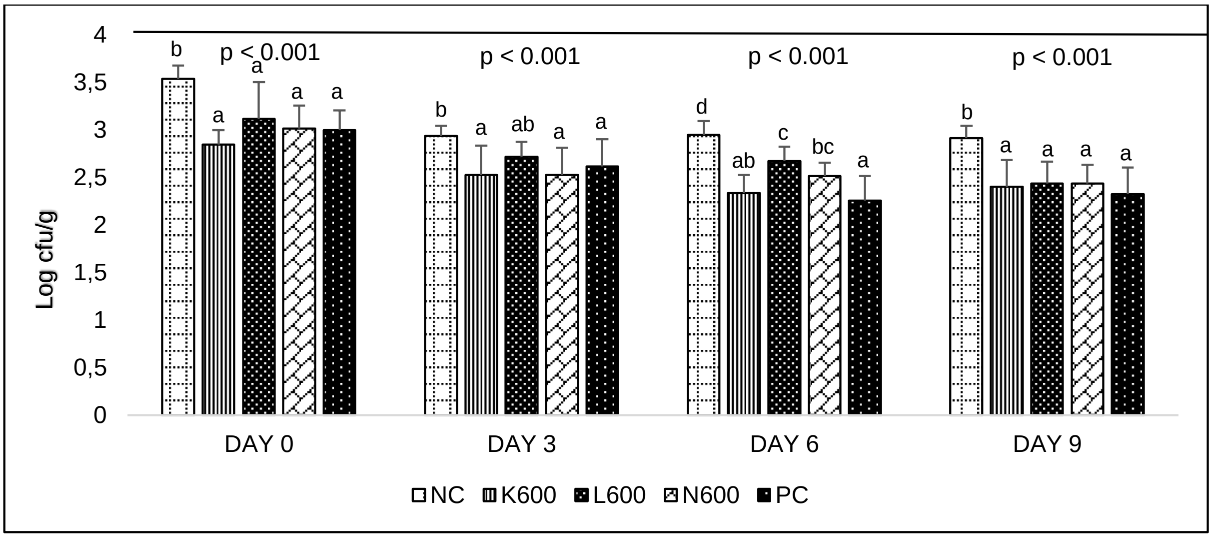

Figure 4.5. Coliform counts (log cfu/g) of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

4.3.5. Physical analysis: colour

The results of the colour stability of the sausages treated with different NaCl levels and replacers are presented in Figures 4.6, 4.7, 4.8, 4.9 and 4.10. The values can be seen in Table 4.15. The lightness (L*) values of the different treatments are shown in Figure 4.6. It can be seen that the L* value of the NC was significantly ($p = 0.006$, $p < 0.001$ and $p = 0.006$, respectively) higher than the rest of the treatments on days 0, 6 and 9. On day 3, the three treatments with the same amount of NaCl and different added replacers differed significantly ($p < 0.001$) from the PC, which in turn also differed from the NC. Over the 9-day period, there was a gradual decrease in the L* value, which could be ascribed to the loss of water holding capacity (WHC) of myofibril proteins, due to mincing that leads to a reduction in the ability to scatter light (Offer & Trinick, 1983).

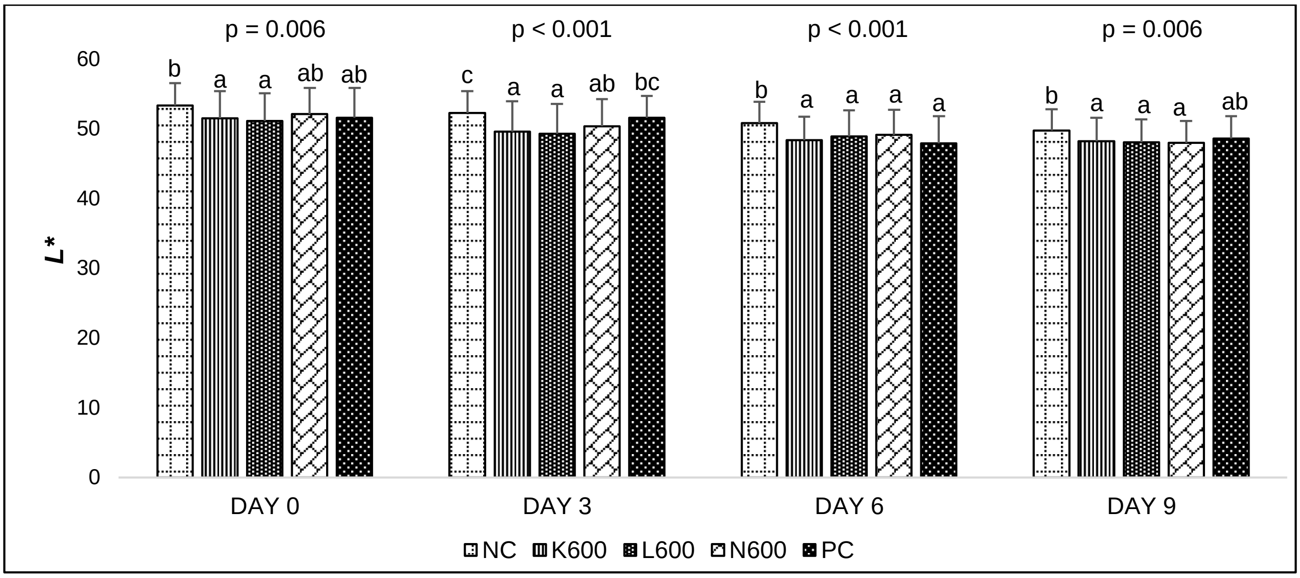

Figure 4.6. Lightness (*L**) of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

The red colour of meat plays a vital role for consumers when purchasing a meat product (Boles *et al.*, 1998). The red colour of meat comes from when the myoglobin is exposed to oxygen, which then results in the formation of red myoglobin (Mc Carthy *et al*., 2001). The gradual decrease in a*-value means a decrease in redness and can be seen from day 0 to day 9 (Figure 4.7). The NC showed the highest value throughout the 4 days and significant differences ($p < 0.001$) was found between the treatments. As NaCl levels increased, redness decreased and this could most likely be ascribed to NaCl having a denaturing effect on myoglobin proteins, which decreases their ability to bind oxygen and gave a red colour. Fresh sausages ultimately turn brown because of metmyoglobin formation during shelf-life. When sausages are packaged to include O_2, the bright red colour might only be stable up to seven days (Schivazappa *et al*., 2004). All the treatments showed a decrease in redness on each day of sampling. From the treatments that contained NaCl, the L600 treatment had the highest value and, thus, the best red colour throughout the 9 days.

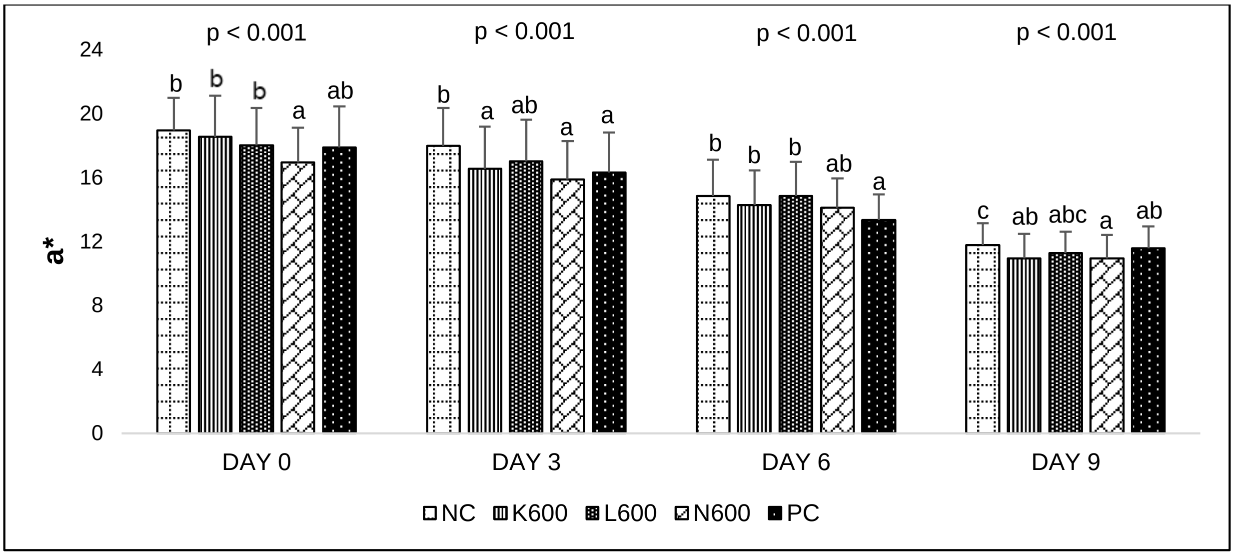

Figure 4.7. Redness (a*) value of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

There was an overall decrease in yellowness from day 0 to day 9 for all five treatment groups (Figure 4.8). Significant differences in *b**-values were observed from day 0 to day 9 ($p < 0.001$). It could be seen that the addition of NaCl did have an effect on the yellowness, since the NC had significant higher values ($p < 0.001$) than the rest of the treatments. Storage time also had an effect and it resulted in a decrease in yellowness across all the treatments.

The brightness, which is indicated by Chroma, overall decreased from day 0 to day 9 across all five treatments, signifying that there was a general decrease (Figure 4.9) in colour brightness over this period and storage time proved to have a significant effect ($p < 0.001$) on all five treatments.

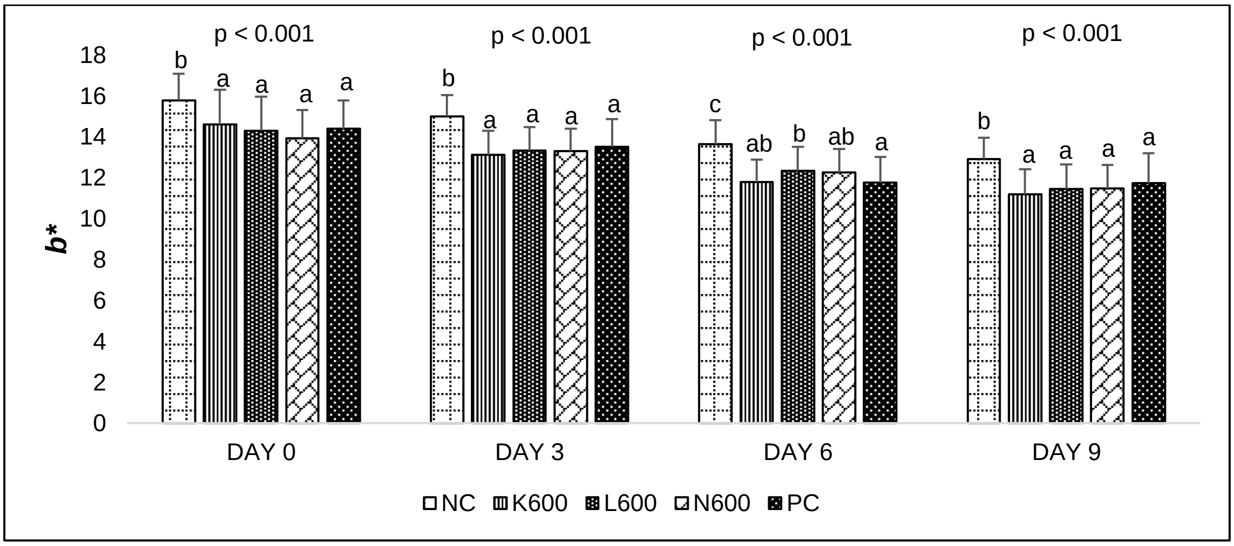

Figure 4.8. Yellowness (*b**) value of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly.

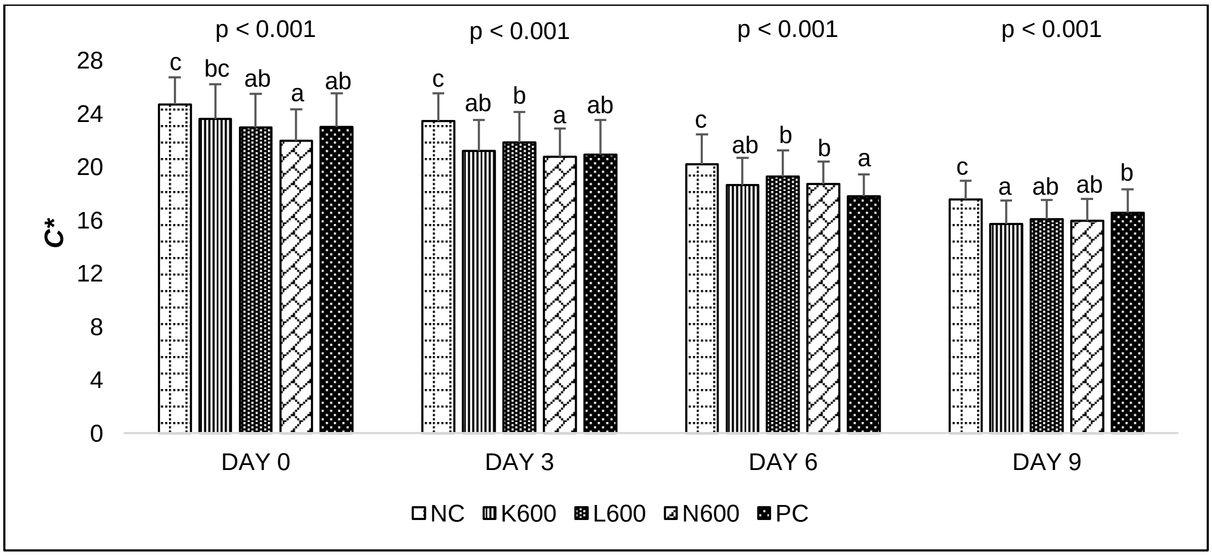

Figure 4.9. Chroma (brightness) value of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts within the same sampling day differed significantly. Error bars represent standard deviations of means.

The hue (H^*) angle also showed significant differences between treatments. The Hue angle represents the purity of colour. When H^* is nearer to 0°, it is closer to red and when H^* is nearer to 90° it is closer to yellow. The H^* of the K600 and L600 treatments were smaller and, therefor, closer to red, compared to that of the NC, N600 and PC treatments, which had higher values, which are more inclined towards yellowness. The H^* was relatively stable up to day 6, after which the values suddenly increased substantially (Figure 4.10).

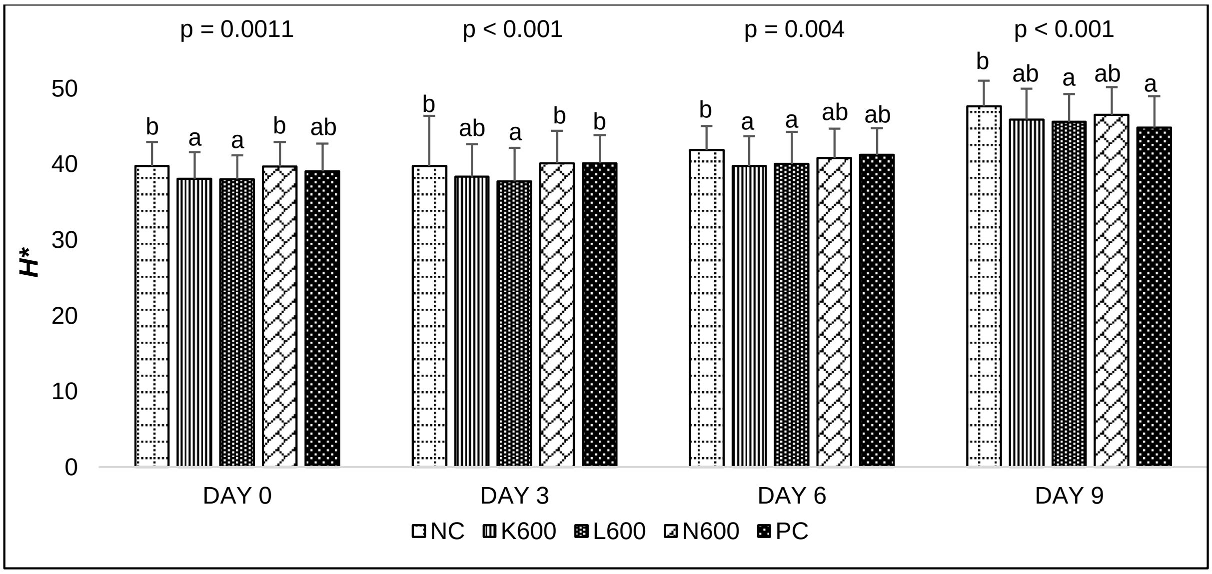

Figure 4.10. Hue (*H**) value of five Boerewors formulations based on different added NaCl and/or replacer levels over a 9-day period. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts for the same parameter differed significantly. Error bars represent standard deviations of means.

4.3.6. Thaw, cooking and total losses

Thaw and cooking losses were measured on the sausages that were frozen and the total losses were the sum of these two sets of results. The results can be seen in Figure 4.11. The thaw losses indicated that the NC had a significant ($p < 0.001$) higher value (4.81 ± 0.047%) than the rest of the treatments and in contrast with the PC that had the lowest value of 2.03 ± 0.29%. Both of the replacer formulations had higher ($p < 0.001$) thaw losses compared to the two NaCl-only treatment groups. The K600, N600 and PC treatment had the lowest cooking loss values and differed significantly from the NC, which again differed significantly ($p = 0.002$) from the L600 treatment. The PC, which contained the highest amount of NaCl, had the lowest cooking loss of 13.30 ± 4.68%, compared to the L600

Table 4.15: Effect of NaCl replacer treatment on colour parameters of Boerewors stored at 4 ^{o}C for 9 days.

Day	Treatment	*L**	Sign. level	*a**	Sign. level	*b**	Sign. level	*C**	Sign. level	*H**	Sign. level
	NC	53.29^{b} ± 3.20		18.94^{b} ± 2.06		15.78^{b} ± 1.30		24.68^{c} ± 2.04		39.77^{b} ± 3.12	
	K600	51.44^{a} ± 3.91		18.55^{b} ± 2.56		14.60^{a} ± 1.70		23.62^{bc} ± 2.60		38.05^{a} ± 3.56	
0	**L600**	51.06^{a} ± 4.03	p=0.006	18.02^{b} ± 2.32	p<0.001	14.30^{a} ± 1.66	p<0.001	22.97^{ab} ± 2.52	p<0.001	38.01^{a} ± 3.16	p=0.0011
	N600	52.05^{ab} ± 3.78		16.94^{a} ± 2.17		13.92^{a} ± 1.39		21.95^{a} ± 2.38		39.67^{b} ± 3.24	
	PC	51.54^{ab} ± 4.28		17.89^{ab} ± 2.57		14.40^{a} ± 1.39		23.01^{ab} ± 2.53		39.05^{ab} ± 3.65	
	NC	52.26^{c} ± 3.08		17.98^{b} ± 2.39		15.01^{b} ± 1.04		23.47^{c} ± 2.05		39.79^{b} ± 3.61	
	K600	49.53^{a} ± 4.36		16.54^{a} ± 2.64		13.12^{a} ± 1.18		21.21^{ab} ± 2.34		38.37^{ab} ± 4.27	
3	**L600**	49.24^{a} ± 4.33	p<0.001	17.01^{ab} ± 2.61	p<0.001	13.32^{a} ± 1.16	p<0.001	21.85^{b} ± 2.30	p<0.001	37.73^{a} ± 4.40	p<0.001
	N600	50.32^{ab} ± 3.94		15.87^{a} ± 2.41		13.31^{a} ± 1.08		20.78^{a} ± 2.10		40.13^{b} ± 4.30	
	PC	51.59^{bc} ± 3.09		16.30^{a} ± 2.53		13.50^{a} ± 1.36		20.92^{ab} ± 2.63		40.12^{b} ± 3.72	
	NC	50.78^{b} ± 3.03		14.85^{b} ± 2.27		13.65^{c} ± 1.17		20.22^{c} ± 2.23		41.90^{b}± 3.15	
	K600	48.35^{a} ± 3.39		14.29^{b} ± 2.17		11.80^{ab} ± 1.08		18.65^{ab} ± 2.02		39.74^{a} ± 3.94	
6	**L600**	48.85^{a} ± 3.77	p<0.001	14.83^{b} ± 2.14	p<0.001	12.33^{b} ± 1.19	p<0.001	19.29^{b} ± 1.95	p<0.001	40.06^{a} ± 4.20	p=0.004
	N600	49.12^{a} ± 3.59		14.12^{ab} ± 1.84		12.26^{ab} ± 1.15		18.72^{b} ± 1.68		40.82^{ab} ± 3.89	
	PC	47.87^{a} ± 3.89		13.33^{a} ± 1.63		11.77^{a} ± 1.25		17.82^{a} ± 1.64		41.22^{ab} ± 3.51	
	NC	49.72^{b} ± 3.05		11.78^{c} ± 1.36		12.92^{b} ± 1.03		17.57^{c} ± 1.40		47.60^{b} ± 3.44	
	K600	48.19^{a} ± 3.35		10.95^{ab} ± 1.54		11.19^{a} ± 1.24		15.72^{a} ± 1.75		45.86^{ab} ± 4.12	
9	**L600**	48.01^{a} ± 3.30	p=0.006	11.28^{abc} ± 1.32	p<0.001	11.46^{a} ± 1.20	p<0.001	16.09^{ab} ± 1.45	p<0.001	45.61^{a} ± 3.63	p<0.001
	N600	47.98^{a} ± 3.11		10.93^{a} ± 1.47		11.48^{a} ± 1.14		15.95^{ab} ± 1.67		46.53^{ab} ± 3.62	
	PC	48.54^{ab} ± 3.26		11.59^{bc} ± 1.36		11.73^{a} ± 1.47		16.58^{b} ± 1.74		44.80^{a} ± 4.20	

NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). *L** = lightness; *a** = redness; *b** = yellowness; *C** = Chroma; and *H** = hue angle. Means with different superscripts in the same column and within the same sampling day differed significantly.

treatment that had cooking losses of 20.36 ± 4.94%. The reason for the lower cooking loss of the PC could be ascribed to the enhanced WHC, which led to reduced cooking losses (Sofos, 1986).

The total losses indicated that there were significant ($p < 0.001$) differences between the treatments. The PC had the lowest value of 15.33 ± 4.74%. The PC, K600 and N600 treatments differed significantly from the NC and L600 treatments, which had much higher values. In terms of total losses, the lactate in the treatment with lactate as replacer (L600) had an undesired effect, since it had a value of 23.13 ± 4.16%, which differed significantly from the PC that had a value of 15.33 ± 4.74%. In terms of thaw, cooking and total losses, higher concentrations of NaCl did prove to have a positive effect in helping to reduce these losses, which in turn would also influence texture and overall acceptability.

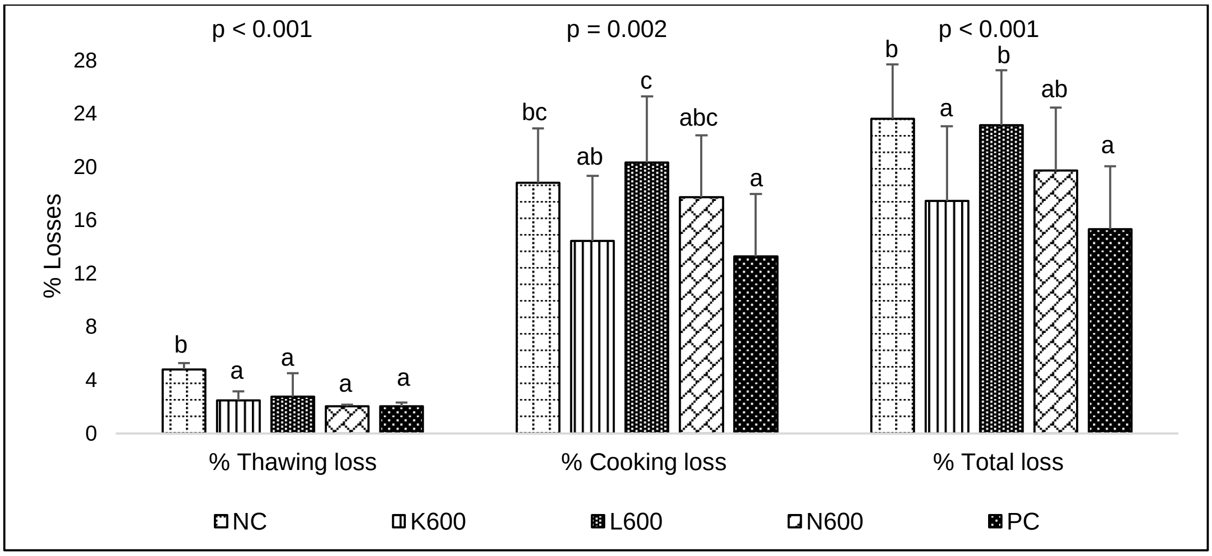

Figure 4.11. Thaw, cooking and total losses of five Boerewors formulations based on different added NaCl and/or replacer levels. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts for the same parameter differed significantly. Error bars represent standard deviations of means.

4.3.7. Sensory analysis

The 75-member consumer panel consisted mostly out of female participants and the average age was between 20 and 29 years (Table 4.16). The consumer panel continuously ranked the NC treatment lower ($p < 0.001$) than the other four treatments for all five of the chosen sensory attributes (Figures 4.12 and 4.13). For appearance, flavour, saltiness, texture and overall liking, no significant differences in rankings were found between the K600, L600, N600 and PC groups (Figures 4.12 and 4.13), indicating that consumers could not detect any differences in terms of these attributes between the different treatments. Even though there were no significant differences between the four treatments, with different levels of NaCl and added replacers, the two treatments with added replacers (K600 and L600) did have slightly higher values than the two treatments that only contained NaCl at different inclusion levels. According to the data, panel members were only able to distinguish between the NC and the rest of the treatments. They were not able to distinguish between the treatments containing different amounts of NaCl and added replacers and could, therefore, not taste a difference between these treatments.

Table 4.16: Demographic profile of the consumer panel used in this study.

Gender:	% of Total	Age:	% of Total
Female	63	< 20	9
Male	37	20-29	60
		30-39	8
		40-49	11
		50-59	8
		>60	4

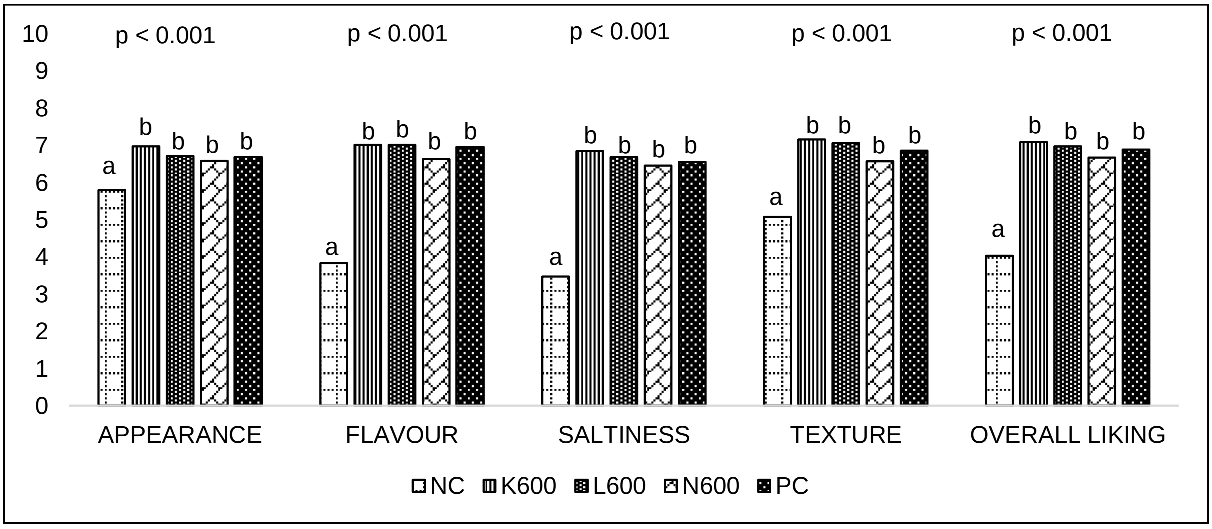

Figure 4.12. Consumer sensory rankings of five Boerewors formulations based on different added NaCl and/or replacer levels. NC = negative control (0.00% added NaCl); K600 = treatment with K as replacer (1.25% added NaCl); L600 = treatment with lactate as replacer (1.25% added NaCl); N600 = treatment containing only NaCl and no added replacer (1.25% added NaCl) and PC = positive control (1.80% added NaCl). Means with different superscripts for the same parameter differed significantly.

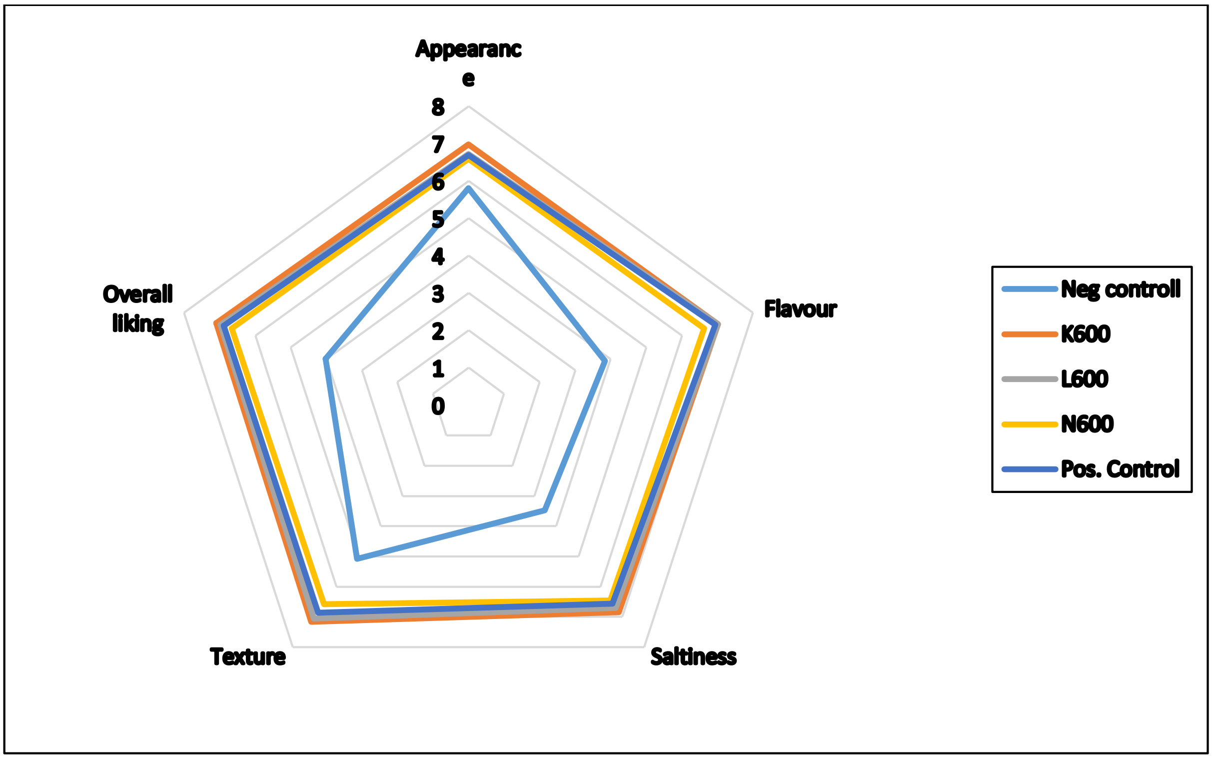

Figure 4.13: Spiderplot of consumer sensory rankings of five Boerewors formulations based on different added NaCl and/or replacer levels

4.3.8. Association of quality and stability parameters with treatment groups with different added NaCl and/or replacer combinations

In order to simplify and summarise the multitude of quality and stability parameters evaluated during this study, a multivariate, 2-dimensional PCA plot was drawn. Figure 4.14 illustrates all the parameters significantly (minimum of $p < 0.05$) affected in relation to the five different treatment groups. The two dimensions accounted for a combined 82.55% of the total variation in the data set. The first dimension (F1) explained 66.76% and the second dimension (F2) 15.79% of the variance in the data. The F1 dimension was therefore regarded as the primary dimension that divided the five treatments into two groups: PC, N600, K600 and L600 on the left and NC on the right.

The highest NaCl, Na and ash content, as well as secondary lipid oxidation values for days 6, 90 and 180, were associated with the PC and N600 treatments. Sensory attributes, such as overall liking, flavour, saltiness, appearance and texture, were strongly associated with the PC, K600, L600 and N600 treatments.

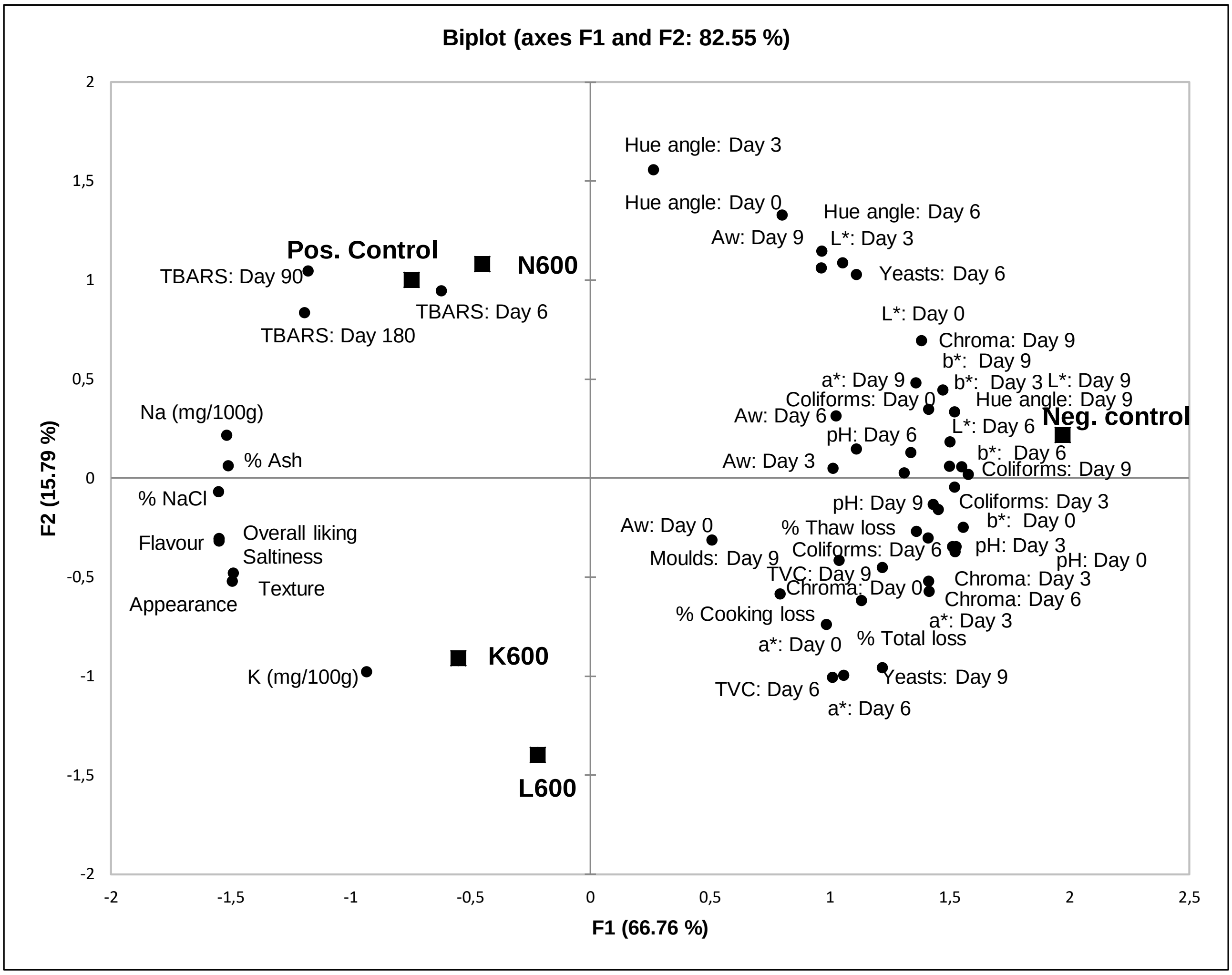

Figure 4.14: Principal component analysis of 52 quality and stability parameters of five Boerewors formulations significantly affected by different added NaCl and/or replacer combinations.

By far the highest number of significant effects on parameters was associated with the NC treatment in F1. For the NC treatment, these included: the largest Hue angles, highest yellowness and Chroma values over multiple days; the highest coliform values on days 0, 3, 6, and 9; the highest yeast count on days 6 and 9; highest mould values on day 9; the highest TVC on day 6 and 9; highest pH on days 0, 3, 6 and 9; highest a_w on days 0,3, 6 and 9; and lastly, the highest thaw, cooking and total losses percentages. As for F2, in general, the PC, K600, L600 and N600 treatments were not distributed very far from each other, showing that there is some commonality between significant effects on parameters. The proximity of the PC and N600 treatments showed great commonality, as well as that the K600 and L600 treatments. The two treatments with different added levels of NaCl and no added replacers grouped together and the two treatments with the same NaCl concentration containing different replacers grouped together. The NaCl only treatments were more closely clustered together than any other groups. In terms of both dimensions, the PC and N600 treatments were more closely clustered with the K600 and L600 treatments, showing their similar results.

4.4. Conclusions

When Na reduction needs to be implemented, the use of different NaCl or Na replacers is currently the most popular strategy to be considered. The aim of NaCl replacers is to reduce the reliance on NaCl, in order to help maintain various factors of a product, such as overall stability and quality. The primary goal is to reduce the overall Na content, without causing any adverse changes in the product.

It was found that the reduction of Na and the addition of different NaCl replacers were effective, without having a negative effect on product quality or stability. From the results obtained it was concluded that there were no significant differences between the four treatments with different NaCl levels and added NaCl replacers. None of the treatments stood out to an extent that it gave significantly better results overall in terms of parameters tested. The microbial counts were all within limits and the treatments with different Na levels and NaCl replacers overall helped inhibit microbial growth. The treatment which represents the next Na inclusion level (N600) mandatory from June 2019, was able to successfully inhibit microbial growth, which was very positive. It also showed that microbial growth could be inhibited and controlled with the reduced Na levels and also that it was not necessary to add any replacers, in order to maintain microbial stability.

When the results from all the parameters tested were considered, a shelf life of between 3 and 6 days is recommended. Within this period the results showed that the sausage was stable and all the parameters had values that indicated satisfactory overall quality and stability. Consumers were only able to distinguish the negative control from the other treatments and not between the other four treatments with different Na levels and different added replacers, which showed that the replacers did not have a negative effect on the sensory quality.

From results obtained it was clear that the next mandatory Na inclusion limit in 2019 would not have a negative effect on the chemical or microbial stability of Boerewors. As a final comment, it would be suggested that because there were no major differences between the treatments with different NaCl levels and added replacers, the best option would be only to add NaCl at the indicated legislative level, simply because it would be more cost effective and consumers might feel more at ease when there are no extra additives that they are not familiar with.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

A traditional South African sausage, better known as Boerewors, is a product that is enjoyed by many South African consumers (Nel & Steyn, 2002). Processing techniques during production include mincing, dicing or chopping and these may render the product more vulnerable to microbial, as well as oxidative spoilage. The quality of sausage is also affected by temperature, pH, a_w, surface area, as well as packaging materials (Cannon *et al.,* 1995).

In food processing, NaCl is the second most popular food additive, next to sugar (Seligsohn, 1981). Sodium chloride plays an important technological role in processed meat products, since it activates meat proteins, which increases water binding and effectively then affect the texture of the product (Hamm, 1961). This directly also helps to improve the tenderness and reduce cooking losses (Man, 2007). Other functions of NaCl include aiding in preservation and improvement of flavour (Allen, 2015).

The main source of Na in the human diet is through the intake of a salty diet (Ruusunen & Puolanne, 2005). Humans experience hedonic pleasure from the taste of NaCl (Mattes, 1997; Leshem, 2009), which results in an increase in consumption of foods that are saltier (Shultz, 1957; Tuorila *et al*., 2008). With continuous consumption of large amounts of NaCl, higher levels of the salt taste will be required to achieve the same level of hedonic response (Pangborn & Pecore, 1982). The result of the continuous need and intake of high levels of NaCl has led to rather severe implications on human health. High blood pressure is a result of continuous intake of high levels of Na (Alderman, 2000). High blood pressure is one of the main risk factors in heart disease and strokes (Sacks *et al*., 2001; Strazzullo *et al.,* 2009; Aburto *et al*., 2013).

The WHO recommends the intake of Na to not be more than 2000 mg Na/day (WHO, 2007). The meat industry will need to take on the challenge presented to produce healthier and more convenient products, in order to stimulate meat consumption (Font-i-Furnols & Guerrero, 2014). When the NaCl content is reduced in a meat product, other parameters may need to be altered, in order to keep an acceptable texture (Doyle & Glass, 2010) and good chemical and microbial stability. It is also important to maintain consumer acceptance, which is a challenge when large amounts of NaCl need to be reduced (Bobowski *et al*., 2015b).

Chapter 2 of this thesis consisted of a review on the available literature on the topic of NaCl and Na reduction. It was found that humans have a physiological need for NaCl and the effects it has on food became so likeable that it has led to NaCl being used at much higher levels than necessary, all over the world. The problem was drawn a bit closer to home, focusing on SA and the economic burden it causes, which is a direct result of increasing health problems. The functional role that NaCl plays in processed products were discussed, as well as the factors that might be affected because of partial reduction and/or replacement of Na. Different Na reduction strategies were also investigated, as well as the effects thereof, as found from previous research on this topic. It was found that there is a lack of information with regard to fresh South African sausage and that the effect of Na reduction and/or partial replacement needs to be investigated to know what the implication of the Na reduction strategies will be.

The South African Government introduced regulations that specify the maximum inclusion levels of Na across a wide range of food products, in an effort to reduce the continuous overconsumption of Na. These regulations were implemented in more than one phase. For fresh sausages the first Na level standard was made mandatory from 30 June 2016, comprising of a Na limit of 800 mg/100 g sausage. The second phase comprises of an even more strict maximum salt level of 600 mg/100 g sausage and will become effective on 30 June 2019 (DoH of South Africa, 2013).

No recent data could be found specifying the general Na content of Boerewors products currently commercially available, which presented an opportunity to build a data base, in order to establish how many of the products currently comply with this legislation and also to determine the amount of Na that still needs to be reduced for products, to comply with the current and next legislative limits. As a result, Chapter 3 took the form of a survey in order to gather information on current commercially available Boerewors. Information with regard to labelling was gathered to establish how many of the products displayed the Na content as part of the nutritional information, in relation to the labelling regulation that requires this information (DoH of South Africa, 2014) and some problems were found. The following results with regard to product labelling of NaCl or Na content were found: 60% of the labels did not indicate the addition of these additives; and 40% did indicate the addition thereof. Out of the products that indicated this addition, a mere 37.5% indicated the amount of either NaCl or Na added. It is, however, not clear whether this information is understood and useful to consumers (Scott & Worsley, 1997; Gilbey & Fifield, 2006; Marshall *et al*., 2007; FSA, 2008). The survey resulted in samples of traditional South African sausage, 20 from butcheries and 20 from supermarkets. It was found that all but one sample were within the current specified Na inclusion level of 2016, while 33% of the samples were above the limit set for 2019. For this reason, it was important to investigate the effect of further Na reduction, to know what the implication of the 2019 limit will be.

This information formed the basis of the experimental design for Chapter 4, where mandatory Na limits were implemented, without previous research or form of replacement plan. The effect of the reduction of Na and/or partial replacement was evaluated in this chapter. Until now, this is the first time that a strategy to reduce Na has been investigated in Boerewors. Salt plays an important role in controlling the texture of processed meat products (Hamm, 1961; Sofos, 1985). It also affects a_w, which in turn helps to reduce microbial growth (Mossel & Thomas, 1988; Doyle & Glass, 2010). Sodium chloride replacers have been found not to have the same inhibiting effect on microbial growth as NaCl (Dötsch *et al*., 2008). It is important to ensure that products remain safe and acceptable when NaCl is reduced, which means that other parameters need to be altered (Doyle & Glass, 2010).

It is also important to produce products that are healthier for consumers and products that can be afforded by all consumers (Temple & Steyn, 2011). It has been found that consumers can be made accustomed to lower saltiness (Bertino *et al.*, 1982; Blais *et al.,* 1986) although, it is more probable that reduced Na products will need to maintain the current level of saltiness, to satisfy the human hedonic response to NaCl (Mattes, 1997; Leshem, 2009).

When taking these factors into consideration, the need to establish whether or not NaCl replacers will be able to achieve the same results as NaCl, stands out. It also provided the opportunity to measure the shortcomings of the product in question when NaCl is added at a lower level.

The 2016 and 2019 Na limits served as the basis on which formulation was prepared for the different treatments. In addition to determining the Na content using AAS for the treatments, the negative control measured the "hidden" Na content in each of the formulations. Potassium chloride and K-lactate were used as replacers in two of the treatments. These replacers were selected, because they have been studied the most and in terms of cost are the closest to NaCl. This was the first time that the effect of these replacers on traditional South African sausage were evaluated.

The Na and replacer combinations had different effects on the losses. Overall NaCl helped to reduce thaw losses and the replacers did not have an effect. The cooking and total losses, however, showed that KCl reduced losses, while K-lactate increased losses. This is attributed to the similar protein extraction capability of KCl to that of NaCl (Munasinghe & Sakai, 2004). The K values differed according to the formulation and addition thereof. The Na contents of the treatments were successfully formulated to closely match the regulatory Na limits of 800 mg Na/100 g and 600 mg Na/100 g.

Lipid deterioration in meat products occurs due to the pro-oxidative effect of NaCl (Decker, 1998; Rhee & Ziprin, 2001). In this study, none of the NaCl or replacer formulations significantly improved the lipid oxidative stability of the sausages during refrigeration. During frozen storage however, the KCl and K-lactate helped to reduce lipid oxidation up to 90 days compared to treatments that contained only NaCl. It was expected that partial replacement of NaCl would improve lipid oxidative stability, as reported in other studies (Hernández *et al.,* 2002; Zanardi *et al.,* 2010).

None of the replacers stood out to the extent that they gave significantly better results overall in terms of chemical parameters tested, such as pH, a_w and moisture content, as well as microbial parameters. With regard to the total viable counts, there were no differences between the treatments and partial replacement of NaCl with KCl, and K-lactate did not show to have any significant effect on microbial growth. When examining results of the colour parameters tested, in general all the formulations experienced decreases in lightness, redness, yellowness and colour brightness, while colour purity increased. While the oxidative effect of high NaCl on meat product colour was confirmed, the addition of lactate as replacer did to some extent affect the meat product colour positively.

The consumer sensory analyses revealed that consumers could not distinguish between treatments, but could distinguish the negative control. No differences were found for any of the parameters tested, which included appearance, flavour, saltiness, texture and overall liking. The fact that the consumers did not favour one of the treatments more than the other was positive, because it indicated that the replacers did not have a negative effect on the taste and that Na could be reduced without adding replacers, while consumer acceptability was maintained.

From the results obtained in this study it was clear that the different treatments containing different levels of Na, as well as treatments with added replacers, were all effective in maintaining the overall quality and stability of a traditional South African sausage. Excluding any of these options from future applications would be difficult. When food safety is considered, it will, however, be necessary to further evaluate the effectiveness of these replacers against food-borne pathogens. The findings that resulted from this research may be used to offer insight into possible approaches to NaCl reduction and may also be the starting point for further research.

In SA, dried sausage, better known as "droëwors", is a very popular product. It does, however, contain large amounts of NaCl, since it is air dried at room temperature, without additional preservatives. This product is, however, currently not included in the Na reduction regulations, but in the future it may be added. The research done in this thesis may be applied as a basis in future research for Na reduction of "droëwors".

CHAPTER 6

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CHAPTER 7

SUMMARY

Due to recent regulations that were passed by the South African Government limiting the sodium content of various processed food products of which Boerewors is one, the food industry now has to abide by new specified sodium levels. These regulations were implemented in an attempt to improve public health. Sodium chloride is one of the primary additives used in Boerewors production due to different functional and preservative properties it exhibits. Sodium does however, have an adverse effect on health when consumed in large quantities.

Subsequently, a survey was done representative of the commercially available Boerewors in Bloemfontein, South Africa, in order to establish what the current sodium levels are in Boerewors, to what extent the products deviate from the current and upcoming Na limit and whether reformulation is necessary. It was found that around 60% of product labels did not include information on sodium included in the products. Most products did comply with current sodium limits and some of the products even complied with the next sodium limit for 2019.

The second part of this study looked into the implications of these sodium reductions on the overall quality and stability of Boerewors products. The treatments were formulated according to current and upcoming regulations for sodium content. The effects of current inclusion limits and also of only reduced sodium content were tested alone and also in combination with KCl and K-lactate replacers, respectively. This was done in order to establish what the implication of the regulations are, if it is sustainable, whether selected replacers can help maintain quality and stability and whether or not products will need reformulation.

From the study on the effect of reduced sodium content and addition of different replacers it was found that the a_w, pH and moisture content was not significantly affected and there are no links to deviation independent parameters such as microbial stability. The results obtained from microbial and oxidative stability evaluation as well as from sensory analyses proved to be positive. Even though the replacers did not significantly help with improving microbial and lipid oxidative stability, all the treatments were stable over the shelf-life period with all the microbial values being within limits and no detection of rancidity. The addition of replacers could not be detected sensorily by consumers and in comparison with only the addition of NaCl, they also did not prefer one product over the other. Reduced levels of NaCl and also the addition of replacers maintained overall quality and stability. The treatment that contained KCl showed to help reduce cooking and total losses. In terms of the colour parameters tested, in general, all the formulations experienced decreases in lightness, redness, yellowness, and colour brightness while colour purity increased. The oxidative effect of high NaCl on meat product colour was confirmed, the addition of lactate as replacer did to some extent affect the meat product colour positively.

The overall conclusions made after taking all the findings from results obtained into consideration, are firstly that product labels do not indicate necessary dietary information regardless of set out requirements through legislation and that there is still a large margin for improvement in providing consumers with information to help them understand legislation better and consequently to make healthier choices when buying products. Secondly, the next mandatory sodium inclusion limit in 2019 will not have a negative effect on the chemical or microbial stability of Boerewors products. And lastly, that because there were no major differences between the treatments with different NaCl levels and added replacers, the best option would be to only add NaCl at the indicated legislative level simply because it is more cost effective and consumers might feel more at ease when there are no extra added additives.

Keywords: Boerewors, sodium, sodium reduction, sodium replacement, microbial stability, chemical stability, lipid stability, colour stability, sensory quality