THE EFFECT OF SODIUM REDUCTION ON THE CHEMICAL, MICROBIAL AND SENSORY QUALITY OF PROMINENT SOUTH AFRICAN PROCESSED MEAT PRODUCTS

by

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DECLARATION

I, MacDonald Cluff, declare that the Doctoral Degree research thesis or interrelated, publishable manuscripts / published articles that I herewith submit for the Doctoral Degree qualification Ph.D. Food Science at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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

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GLOSSARY OF ABBREVIATIONS

a*	Redness/greenness colour coordinate
AAS	Atomic absorption spectroscopy
$AgNO_3$	Silver nitrate
AgCl	Silver chloride
AI	Adequate daily intake
AIDS	Acquired immune deficiency syndrome
AMP	Adenosine 5'-monophosphate
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
a_w	Water activity
B.C.E.	Before Common Era
BHI	Brain Heart Infusion
BL	Baseline treatment with 1% added NaCl (w/w)
BP	Blood pressure
BMI	Body Mass Index
BP	Baird-Parker
BPW	Buffered peptone water
°C	Degrees Celsius
*C**	Chroma
Ca	Calcium
ca.	Circa
$CaCl_2$	Calcium chloride
cfu	Colony forming units
CO_2	Carbon dioxide
conc.	Concentration
CsBr	Caesium bromide
CsCl	Caesium chloride
CsI	Caesium iodide
CVD	Cardiovascular Disease
Δ^-	Maximum overestimation
Δ^+	Maximum underestimation
DAGs	Diacylglycerols
dH_2O	Distilled water
DoH	Department of Health (South Africa)
e.g.	Exempli gratia; for example
EPEC	Enteropathogenic *Escherichia coli*
EPS	Expanded polystyrene
Eqv.	Equivalent
et al.	Et alia
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (United States)
FSA	Food Standards Agency

g	Gram
GHPs	Good hygeine practices
GMPs	Good manufacturing practices
GRAS	Generally recognized as safe
h	Hour(s)
*H**	Hue angle
HACCP	Hazard analysis and critical control points
HHP	High hydrostatic pressure
HI	High intermediate
HIV	Human immunodeficiency virus
HNO_3	Nitric acid
i.e.	Id est; that is
IFT	Institute of Food Technologists
IMP	Disodium inosinate
IOM	Institute of Medicine (United States of America)
IUPAC	International Union of Pure and Applied Chemistry
K	Potassium
KBr	Potassium bromide
KCl	Potassium chloride
kg	Kilogram
K-gluconate	Potassium gluconate
$KMnO_4$	Potassium permanganate
L^*	Lightness colour coordinate
LAB	Lactic acid bacteria
LI	Low intermediate
LiBr	Lithium bromide
LiCl	Lithium chloride
M	Molar
MA	Modified atmosphere
MDA	Malondialdehyde
mEq	Milliequivalents
Mg	Magnesium
mg	Milligram
mg/100 g	Milligrams per 100 grams
$MgCl_2$	Magnesium chloride
min	Minute
mL	Millilitre
mm	Millimetre
mM	Millimolar
mmHg	Millimetre of mercury
mmol	Millimoles
MPa	Megapascal
MSG	Monosodium glutamate
μ	Average deviation
μg	Microgram
μM	Micromolar

N	Newton
N	Normality
n	Population size
Na	Sodium
n.a.	Not applicable
NaBr	Sodium bromide
NaCl	Sodium chloride
ND	Not detected
NaI	Sodium iodide
NaK	1% NaCl (w/w) and 1% KCl (w/w) treatment
NaKGlu	1% NaCl (w/w) and 1% K-gluconate (w/w) treatment
NaKKlac	1% NaCl (w/w), 0.8% KCl (w/w), & 0.2% K-lactate (w/w) treatment
NaKYE	0.8% NaCl (w/w), 0.8% NaCl (w/w), & 1% YE (w/w) treatment
NC	Negative control
NCSS	Number Cruncher Statistical System
ND	Not detected
NH_4Cl	Ammonium chloride
NS	Not significant
NSA	Not statistically analysed
O_2	Oxygen
O/W	Oil-in-water
p	Vapour pressure of food
P	Significance level (≥ 0.05)
p_0	Vapour pressure of pure water
PC	Positive control
PCA	Principle Component Analysis
ppm	Parts per million
PSE	Pale, soft and exudative
PVC	Polyvinyl chloride
%	Percentage
R	South African Rand
RbBr	Rubidium bromide
RbCl	Rubidium chloride
RbI	Rubidium Iodide
rH	Relative humidity
rpm	Revolutions per minute
RTE	Ready-to-eat
s	Seconds
SABS	South African Bureau of Standards
SANS	South African National Standard
SLOP	Secondary lipid oxidation products
SPI	Soya protein isolate
ssp.	Subspecies
STPP	Sodium tripolyphosphate
TAGs	Triacylglycerols
TBARS	Thiobarbituric acid reactive substances
TVC	Total viable counts

UK	United Kingdom
UL	Tolerable upper intake level
US$	United States dollar
USDA	United States Department of Agriculture
vs.	Versus
VTEC	Verocytotoxigenic *Escherichia coli*
WASH	World Action on Salt and Health
WHC	Water-holding capacity
WHO	World Health Organization
W/O	Water-in-oil
WOW	Water-in-oil-in-water
w/v	Weight per volume
w/w	Weight per weight
WVTR	Water vapour transmission rate
YE	Yeast extract

THESIS OUTPUTS

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Cluff, M. (2015). Update: Sodium reduction in processed meat products. *70th SAMPA AGM* (27 May 2015, Crown National Auditorium, Crown National, 31 Nguni Drive, Longmeadow West, Modderfontein, Johannesburg, South Africa).

CHAPTER 1

INTRODUCTION

The positive correlation between sodium intake and blood pressure was first made a century ago. Through the evaluation of cross-cultural studies it was initially concluded that differences in sodium intake might be linked to, and possibly result in, changes in blood pressure. In non-industrialised societies, blood pressure were generally reported to be lower and not to increase with age, which was in stark contrast to what was happening in most industrialised societies (Alderman, 2000). Presently, there is a direct and incontrovertible link between sodium intake and high blood pressure, which in turn is a major risk factor for coronary heart disease and stroke (Sacks, Svetkey, Vollmer, Appel, Bray, Harsha, et al., 2001; Strazzullo, D'Elia, Kandala, & Cappuccio, 2009; Aburto, Ziolkovska, Hooper, Elliot, Cappuccio, & Meerpohl, 2013). Globally, this accounts for 45% of all heart disease and 51% of death due to stroke (WHO, 2008). Reducing sodium intake from food as a non-pharmacological method may delay the use of pharmacological methods in controlling blood pressure (Appel, Moore, Obarzanek, Vollmer, Svetkey, Sacks, et al., 1997).

In terms of the human diet, it is accepted that table salt, i.e. sodium chloride (NaCl), is the main source of sodium (Ruusunen & Puolanne, 2005). The hedonic response of humans to saltiness is the result of the interaction of physiological, cultural, and environmental factors that determine the need and access to high-sodium foods. In contrast to animals, there is little evidence for the existence of a 'salt appetite' in humans (Leshem, 2009; Mattes, 1997), meaning that humans are not prone to directly consume pure NaCl and find aqueous solutions of NaCl unpalatable (Cowart & Beauchamp, 1986; Moder & Hurley, 1990). Similar to many animals, humans still love salt and have a special taste organ to detect it (Leshem, 2009). Like the rat, humans have three taste transducers, of which one is dedicated just to the detection of sodium (McCaughey & Scott, 1998).

The fact that humans generally have a high level of liking of salt explains why its consumption has become problematic. How well a food is liked or preferred strongly predicts its level of intake (Schultz, 1957; Tuorila, Huotilainen, Lähteenmäki, Ollila, Tuomi-Nurmi, & Urala, 2008). In the case of salt, positive associations between salt hedonics and sodium consumption have been established (Mattes, 1997). The result of this is that consumers whom continuously consume more sodium add more salt to reach their preferred level of saltiness, as reported for tomato juice (Pangborn & Pecore, 1982) and beef broth (Stone & Pangborn, 1990). This cycle has been shown to be reversible (Bobowski, Rendahl, & Vickers, 2015a, b), although consumers are generally unwilling to change their behaviour (Grunert, 2006; Mendoza, Schram, Arcand, Henson, & L'abbe,

2014) or compromise on sensory experience for potential health benefits (Verbeke, 2006). Saltiness preference may be more closely linked to discretionary salt use (i.e., salt added to food at the dinner table) than to total sodium intake (Shepherd, Farleigh, & Land, 1984) seeing as many main contributors to total dietary sodium are not necessarily salty (Shepherd, 1988). These 'hidden' sources of sodium act as additional protagonists to the high level of sodium intake (Borghi, Meschi, Maggiore, & Prati, 2006; Zandstra, Lion, & Newson, 2016). Reportedly, the most effective way to reduce salt consumption or sodium intake is to reduce the salt content of commercially produced foods as this does not rely on consumers to change their behaviour (He & MacGregor, 2010). In response to this, 83 countries have salt reduction strategies in place or planned, 38 had established voluntary and/or mandatory sodium content targets and two countries (Argentina and South Africa) have mandatory targets in place for wide ranges of food products (Webster, Trieu, Dunford, & Hawkes, 2014).

Sodium chloride is one of the most frequently used ingredients in meat processing with functionality in terms of the flavour, texture and shelf-life of meat products (Ruusunen & Puolanne, 2005). Herein lays the greatest challenge in attempting to reduce its levels. It seems highly unlikely that a single substitute to NaCl exists, requiring that a range of functional ingredient combinations be developed. The end goal should be new products that continue to appeal to consumers in the same fashion as the replaced high-sodium products. This goal presents a seemingly infinite amount of challenges which in turn presents a legion of opportunities to re-evaluate what a meat product should embody in the 21st century. The topic of salt and/or sodium replacement has been in the spotlight for many decades, although new ingredients and technologies regularly become available for consideration. There is an almost inexhaustible number of different meat products with unique characteristics and quality parameters, requiring that each one be investigated in this regard. Many countries are eagerly stepping up to the challenge to address the issue of high sodium content. It has to be acknowledged that tried and tested solutions from one part of the world do not necessarily translate to the same successes in other parts, requiring a back-to-basics approach, at least initially.

The first aim of this study was to establish the current general sodium content of commercially produced processed meat products in South Africa.

The following hypothesis was formulated:

Recent preventative public health care developments include upcoming regulations on sodium content (Department of Health, 2013) and product labelling requirements with regard to the provision of nutritional information including sodium content (Department of Health, 2014). In light of these regulations it was suspected that the sodium content of current processed meat products would exceed these regulations, and would require

reformulation for compliance. In addition, it was suspected that with little incentive to monitor sodium content, the actual sodium content of products with voluntary labelled sodium content would vary substantially from the labelled values. The null hypothesis would be that processed meat products in their current formulations generally comply with the sodium limit regulations and that there is little variation between actual and labelled sodium content of products already providing this information.

The second aim of this study was to determine if intermediate added NaCl levels could maintain three distinct processed meat products' quality and stability.

The following hypothesis was formulated:

In response to addressing sodium reduction, the use of NaCl or sodium replacers are often the first approach taken (Terrell, 1983). The use of replacers and the resultant appearance of unknown additive names on product labels may however, be negatively received by consumers and retailer alike (Searby, 2006). Perhaps the greatest obstacle to sodium replacement is cost, with NaCl being one of the cheapest food ingredients available (Desmond, 2006). It was theorised that a "sweet spot" might exist between reducing excessive added NaCl levels to intermediate levels where the functionality (i.e., role in maintaining chemical, microbial and sensory quality) of the added NaCl would not be impaired, without having to rely on the addition of replacers. The null hypothesis would be that sodium reduction, effected through NaCl reduction at one or both intermediate levels, would have adverse effects on one or more of these parameters.

The third aim of this study was to determine if partial replacement of added NaCl could maintain a processed meat product's quality and stability.

The following hypothesis was formulated:

For consumers, maintaining the particular salty taste of a processed meat product is probably the most important factor. In general, consumers have developed an unfavourable attitude towards foods that compromise on the sensory experience in return for potential health benefits (Verbeke, 2006). When NaCl and/or sodium replacers are being considered, the original purpose of NaCl, preventing the growth of microorganisms (Dötsch, Busch, Batenburg, Liem, Tareilus, Mueller, & Meijer, 2009), has to be taken into account. Other parameters, such as flavour and texture, are also important as well as the roles of other ingredients, such as preservatives, flavouring agents and additives also become more important (Doyle, 2008). No single replacer can completely replace NaCl and no single replacer formulation can be applied to every different type of product (Desmond, 2006). This necessitates appropriate research for when replacers are to be considered for reducing

the NaCl and/or sodium content of a specific product (Sofos, 1983a). The cost of replacers may also increase the overall raw material costs of a reformulated product by 5-30% (Dötsch et al., 2009), placing emphasis on economical replacers. Potassium chloride in particular has shown great promise in a number of applications and it is the most widely and successfully used candidate (Desmond, 2006; Reddy & Marth, 1991). It would be advantageous to evaluate this "gold-standard" of replacers against other lesser applied options in combination with reduced NaCl content. The null hypothesis would be that partial replacement of added NaCl with replacers resulted in processed meat products with unacceptable changes in chemical, microbial and/or sensory quality.

The fourth and final aim of this study was to determine the effect of intermediate added NaCl levels or effect of partial replacement of added NaCl in pork banger batters on the growth and survival of *Escherichia coli* and *Staphylococcus aureus* as potential pathogens.

The following hypothesis was formulated:

Spoilage of food products is the result of microbial activities over time, usually as a result of the composition of the products (Doulgeraki, Ercolini, Villani, & Nychas, 2012). It can be defined as a situation where microorganisms are present in large enough numbers so as to cause changes in the product, making it unappealing and unsuitable for consumption (Gram, Ravn, Rasch, Bruhn, Christensen, & Givskov, 2002; Fung, 2010). An additional concern is the growth of potential pathogens that may negatively affect meat product safety such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*; Smith-Palmer, Stewart, & Fyfe, 1998). The concentration of NaCl required to limit pathogen growth vary, depending on the microbial species, pH, temperature, oxygen levels and other components in food such as moisture, fat and additives (Doyle & Glass, 2010). A change or failure in such a control system may allow the emergence or re-emergence of a pathogen (Miller, Smith, & Buchanan, 1998). Foods are very complex systems and natural or added constituents may have unexpected effects on the viability and growth of microbes (van Boekel, 2008). In this study it was suspected that other components in the formulations of pork sausage batters would exhibit strong synergistic antimicrobial effects in combination with sub-optimal storage temperatures (4 °C and 10 °C) so as to limit the growth or survival of either one of the inoculated *E. coli* or *S. aureus* strains. The null hypothesis would be that reducing or partially replacing the normally added amount of NaCl in pork banger batters would allow for the survival and growth of either one of the inoculated bacterial strains.

CHAPTER 2

LITERATURE REVIEW

ABSTRACT

The aim of this literature review was to evaluate the many factors surrounding dietary salt and sodium. There is consensus throughout the world, that too much salt is being consumed. In this literature review, the physiological role of salt in the human diet, the source and perception of saltiness are discussed. The problems caused by the chronic overconsumption and its effect on South Africans were addressed as well as the negative attitude consumers have towards reducing their sodium intake. A number of challenges facing the food industry responsible for processed meat products were identified. The various functions of sodium contributing substances were explored with a focus on the effect on meat microbiology. A number of possible substitutes, containing sodium or substitutes free of sodium were discussed in an effort to find the most suitable analogues with the broadest application in current processed meat products. The new area of modifying processing conditions were also discussed in terms of how these technologies may assist in maintaining the quality of sodium reduced products. Finally, advantages and possible opportunities for the meat industry with regard to sodium reduction were highlighted.

2.1. Introduction

Salt or sodium chloride (NaCl) as it is chemically known, is abundantly found in nature and is known to be vital for many life processes. It was even regarded as the fifth element alongside air, earth, fire and water and The Bible makes 30 references to this important chemical compound (Ball & Meneely, 1957; Dickinson, 1980). Various gustatory, spiritual and economic references have been made in historical literature and its history as a valuable food additive was traced back to around 3000 B.C.E. Ancient Roman soldiers received their payment partially in the form of salt which was called a *salarium* or allowance of salt, from which the modern English word, salary was derived (Blinkerd & Kolari, 1975).

After sugar, salt is regarded as the second most used food additive in food processing (Seligsohn, 1981). The functions of salt in food range from flavour and flavour enhancement (Gillette, 1985) to the provision of complex functional properties in various food systems. In meat processing, salt is one of the most used ingredients after the meat itself (Desmond 2006). It also acts as a preservative by lowering the water activity (a_w) which results in an inhibition of microbial growth and it

solubilizes particular muscle proteins to create stable emulsions (Forsythe & Miller, 1980; Sebranek, Olson, Whiting, Benedict, Rust, Kraft, & Woychik, 1983).

In recent decades the increased consumption of processed foods containing high levels of sodium (Na) has changed the perception of dietary salt. It is now considered a potential health threat (Doyle, 2008). There is a progressive increase in the blood pressure levels of individuals and increasing prevalence of age-associated hypertension across populations which appears to be directly correlatable to Na intake (Dickinson & Havas, 2007). To put the problem of hypertension into perspective, the number of adults worldwide whom suffer from hypertension is thought to be around 26% or 1 in 4 adults (Kearney, Whelton, Reynolds, Muntner, Whelton, & He, 2005). Initially, research suggesting reduction in Na intake was met with some controversy. Reducing Na intake from food as a non-pharmacological method was found to delay the use of pharmacological methods in controlling blood pressure (Appel et al., 1997). The conflicting opinion was raised that the benefits from Na reduction was significantly smaller than could be gained from antihypertensive drugs (Taubes, 1998). However, the long-term use of antihypertensive drugs can have adverse health effects such as arthritis, diarrhoea, tiredness (Materson, Cushman, Goldstein, Reda, Freis, Ramirez et al., 1990), diuretic-induced hyponatremia and gout (Lewis, Grandits, Flack, McDonald, & Elmer, 1996) and requires continued medical supervision (Chobanian, Bakris, Black, Cushman, Green, Izzo, Jones, Materson, Oparil, Wright, & Rocella, 2003). The link between Na intake and high blood pressure is now regarded as the best described of all dietary factors that may cause cardiovascular disease (CVD) (WHO, 2007). In 2013, the Institute of Medicine (IOM) released a new report confirming the strong relationship between Na intake and the risk for CVD. The report was controversial, in that it found insufficient evidence of benefits in limiting Na intake to between 1500 and 2000 mg/day, in contrast to the Institute's own findings in 2004 (IOM, 2013).

A number of approaches have been taken to reduce the NaCl content of processed meat products: a straight forward reduction in the amount of NaCl normally added; replacing some or all of the NaCl with other salts, or non-sodium compounds; and altered processing techniques (Terrell, 1983). With current efforts to reduce salt from processed foods the original purpose of salt to prevent the growth of potentially pathogenic and spoilage organisms should be kept in mind. This may prove to be one of the biggest hurdles to overcome. Salt-replacing ingredients and compounds, for the most part, do not have the preservative effect of salt (Dötsch et al., 2009). Other important functions such as maintaining flavour and texture and masking bitter tastes will remain a concern. It should also be kept in mind that when salt levels in food are reduced, other preservatives, flavouring agents, additives and processing techniques become more important to maintain quality and microbial safety (Doyle, 2008). No single solution exists that can be used to replace salt in meat products. A

number of functional ingredient combinations need to be developed and/or optimized to recreate products that will continue to appeal to consumers. Salt cannot be completely removed due to its various functional roles, unless other ingredients can completely fulfil those roles (Desmond, 2006). The removal or reduction of salt from processed foods should be preceded by appropriate research and should be based on the results of the research (Sofos, 1983a).

2.2. The physiological role of dietary salt

As NaCl dissolves in aqueous environments, it dissociates into cationic sodium (Na^+) and anionic chloride (Cl^-) ions (Meneely, 1973; Institute of Food Technologists, IFT, 1980). Sodium is required by humans, as by all mammals, to maintain blood volume, regulate osmotic pressure and for the transmission of electric impulses by the nervous system (IFT, 1980; Anonymous, 1981; Beauchamp, 1982). The membrane potential of cells is determined by Na and it participates in the active transport of some molecules across cell membranes (Doyle, 2008). It is the main cation responsible for regulating extracellular fluid volume and plasma volume. Other cations such as calcium and potassium interact with Na and influence its physiological effects (Adroqué & Madias, 2008). Sodium is of such importance that it makes up around 2% of the human body's mineral content (Null, 1984). Chloride in turn is essential to maintain tissue osmolarity, the acid-base balance in blood, for the activation of certain essential stomach enzymes and for the formation of hydrochloric acid in the stomach (IFT, 1980).

The Intersalt Study on blood pressure and electrolyte secretion, carried out in 32 countries, proved that humans can survive on diets within a wide range of sodium concentrations. The median urinary excretion of Na varied from 0.0046 g/day (0.2 mmol/day) for Yanomamo Indians in Brazil to over 5.60 g/day (242 mmol/day) for people in Tianjin, China (INTERSALT Cooperative Research Group, 1988). The physiological need for salt is around 0.184-0.230 g/day or 8-10 mmol/day, although Na intake in many industrialized countries is between 3.6-4.8 g/day (WHO, 2007).

The main contributions to salt in the diet are made by: drinking water; the salt naturally contained in foods; the salt added to foods during processing; and that which is added to the food during cooking and at the dinner table (Reddy & Marth, 1991). Approximately 98% of dietary Na is absorbed into the body by the intestines (Adroqué & Madias, 2008). Excessive amounts of Na and Cl from over consumption is excreted by the body to keep levels within very narrow limits (IFT, 1980). A number of hormones as well as the sympathetic nervous system in healthy humans regulate the adaptation to varying dietary salt levels needed to keep plasma levels of Na within the optimal range. In healthy adults under normal physiological conditions, urinary Na excretion through sweat

and urine is roughly equal to Na intake. Problems arise due to aging or the development of chronic diseases which negatively affects the homeostatic regulation of electrolytes. As the successful excretion of excess Na diminishes, plasma volume increases and this stresses the cardiovascular system by inducing hypertension. Hypertension in turn, is linked directly to coronary heart disease, stroke and end-stage renal failure (Doyle, 2008). The words "salt" and "sodium" are often used synonymously. It should be kept in mind that salt is only ~ 40% Na, thus 1 g of salt only contains ~ 400 mg Na (Bodyfelt, 1982). The remaining 60% of salt is Cl which is often disregarded although it is strongly linked to blood pressure. When Cl was replaced with other chemicals e.g. sodium citrate, sodium phosphate and sodium bicarbonate, positive effects on blood pressure were observed. It is suggested that with regard to blood pressure, "salt intake" is the more useful term to use (Kurtz, Al-Bander, & Morris, 1987).

2.3. The source and perception of saltiness

The salty taste of Na is regarded as the most potent stimulus for the salt taste (Beauchamp, 1982; McCaughey, 2007). The exact mechanism of salt perception and the cause of saltiness are not yet fully understood. Originally it was proposed that the Cl^- anions were responsible, recent evidence suggests that saltiness is produced by Na^+ cations (Bartoshuk, 1980). Anions are generally thought to inhibit the taste effect of cations (Beidler, 1954; Bartoshuk, 1980; Beauchamp, 1982) although the chloride anion is regarded the least inhibitory seeing as it has no taste of its own (Bartoshuk, 1980). In a human study by Murphy, Cardello & Brand (1981) the tastes of 15 halide salts: lithium chloride (LiCl); lithium bromide (LiBr); NaCl; sodium bromide (NaBr); sodium iodide (NaI); potassium chloride (KCl); potassium bromide (KBr); rubidium chloride (RbCl); rubidium bromide (RbBr); rubidium iodide (RbI); caesium chloride (CsCl); caesium bromide (CsBr) and caesium iodide (CsI) were evaluated. It was concluded that the molecular weight of the cation had no consistent effect on perceived saltiness whilst lower weight anions produced saltier-tasting salts and both heavier anions and cations produced salts that were more bitter tasting. Saltiness as one of the basic tastes perceived by humans appears to be received indifferently by new-born babies. A positive response to salt appears during the first 4 to 6 months of life and has been associated with birth weight. Lower birth weights are correlated with risk for hypertension in later life (Stein, Cowart, & Beauchamp, 2006; Beauchamp & Mennella, 2009).

The perceptibility and intensity of saltiness is also affected more indirectly through flavour perception. Flavour is an important organoleptic property of food which is regarded as a combination of odour and taste stimuli. It has been shown that taste can increase odour intensity and

vice versa (Salles, 2006). The odours of commercially available aromas of products such as anchovy, bacon, dry sausage, smoked salmon and other were shown to increase the saltiness intensity of a weak 0.02 M NaCl solution evaluated by 59 consumers (Lawrence, Salles, Septier, Busch, & Thomas-Danguin, 2009).

2.4. Saltiness as affected by temperature, moisture, viscosity, lipid, and protein content

For the salty taste to be perceived, the Na^+ ions need to be transported from the food into saliva and finally to the surface of the taste receptors. In one of the earliest reports of the effect of temperature on taste thresholds, Hahn and Günther (1933) reported that the threshold for NaCl increased with increasing temperature of a solution. Later it was reported that sensitivity to NaCl is lower at 0 °C and 55 °C with the greatest sensitivity in the range of 22-37 °C (Pangborn, Chrisp, & Bertolero, 1970). Moisture content directly affects the time-release of Na from a food matrix with Na being released faster at higher moisture content (Phan, Yven, Lawrence, Reparet, & Salles, 2008). This is explained by the fact that the salt can more easily dissolve at a higher moisture content which will normally go hand in hand with a higher water activity.

The amount of a tastant that can be released and eventually diffused is affected by food hydrocolloids that may be used to thicken the product. The rule of thumb is that flavour and taste perception decreases as the viscosity increases (Pangborn, Trabue, & Szczesniak, 1973). Ionic hydrocolloids such as xanthan and κ-carrageenan can have additional effects on saltiness perception. The positively charged Na-ions from the salt may be attracted to binding sites on the hydrocolloid. When these ions become unavailable for tasting it results in lower saltiness perception (Rosett, Shirley, Schmidt, &, Klein 1994; Rosett, Kendregan, Gao, Schmidt, & Klein, 1996). Starch can also suppress flavour and taste perception, although to a lesser extent than the non-starch thickeners at iso-viscous conditions (Ferry, Hort, Mitchell, Cook, Lagarrigue, & Pamies, 2006). The more limited effect of starch is attributed to a rapid decrease in the starch viscosity due to starch degradation by salivary α-amylase (Ferry, Hort, Mitchell, Lagagarrigue, & Pamies, 2004). Even though the effect of those hydrocolloids may have on saltiness need to be kept in mind, it should be noted that very large decreases in viscosity will have to occur before Na reduction will be realized. This makes changing viscosity an ineffective method for controlling taste perception (Malone, Appelqvist, & Norton, 2003).

Saltiness intensity is affected by fat content (Phan et al., 2008). Generally, with an increase in fat content there is an inverse decrease in the saltiness intensity. Ruusunen, Simolin, & Puolanne (2001) reported a similar result where replacement of lean pork with pork fat decreased the

perceived saltiness of sausages. They also proposed that a negative correlation exists between perceived saltiness and protein content. When water in the formulation was replaced with pork on a w/w basis, the perceived saltiness did not change. Potential exists for manipulating fat and salt content so as to maintain saltiness whilst improving the nutritional aspects of a meat product. When the salt and fat content of chicken sausages were simultaneously reduced it was possible to maintain the saltiness intensity (Chabanet, Tarrega, Septier, Siret, & Salles, 2013). Lipids in the form of oils can be both beneficial and detrimental to saltiness perception. When the lipids are present in an oil-in-water emulsion it serves as a filler which concentrates soluble components such as salt in the aqueous phase, thereby enhancing overall saltiness. It can also result in a mouth coating effect, limiting the contact between the dissolved salt and the relevant taste receptors (Busch, Yong, & Goh, 2013; Malone et al., 2003).

2.5. The sources of salt in meat products

Fresh meat is naturally low in Na and the concentration thereof is around 60-80 mg/100 g or the equivalent of 0.15-0.20 g/100 g of salt. Processed meat and meat products contain much more Na and the highest levels can be found in cured meat products and sausages (Desmond, 2006). Most processed meat products are associated with high salt content, although the actual salt content may be highly variable. Except for NaCl being the main contributor of Na in processed meat products, other ingredients can also contribute excessive amounts of Na to the final product. Examples include: acid-hydrolysed vegetable protein (HVP, 18.0% Na); monosodium glutamate (MSG, 13.6% Na) sodium ascorbate or erythorbate (11.6% Na); sodium nitrate (33.2% Na); sodium nitrite (33.2% Na) and sodium tripolyphosphate (31.2% Na) (Maurer, 1983). Typically a meat product contains around 2% salt of which the salt itself contributes around 79% of the total Na of the final meat product (Breidenstein, 1982).

2.6. The problem with too much dietary salt/sodium

The large difference between the minimum level of Na needed and the actual amount being consumed, indicates that most people consume far more salt than is needed for maintaining good health (Beauchamp & Engelman, 1991). In general, it can be deduced that the amount of salt consumed in westernized countries is driven by taste and not physiological need (Dötsch et al., 2009). Processed foods and foods served in restaurants contribute more than 75% of the dietary Na in industrialized countries. The natural Na levels in food only makes up around 10% of the total dietary intake (Mattes & Donnelly, 1991). The salt does not only come from obviously salty foods

such as snacks, but large contributions are made by processed meats and cheeses, bread and breakfast cereals, soups, sauces and ready-to-eat dinners (Doyle, 2008). Discretionary addition of salt during cooking and at the dinner table also contributes only 5-10% of the total dietary intake of salt in Europe and North America (Mattes & Donnelly, 1991). In contrast, the amount of salt added in home cooking and at the table accounts for between 72% and 76% of dietary intake in Asian countries (Andersen, Rasmussen, Larsen, & Jakobsen, 2009). Cured meats for example, have been estimated to contribute 20.5%, 20.8% and 21.0% of the Na in the Irish, British and American diets, respectively (Desmond, 2006). The latest recommendation of the WHO is that no more than 2000 mg Na should be consumed per day (WHO, 2007). South African urinary Na excretion was found to be in excess of 2.4 g Na or 6 g NaCl (Charlton, Steyn, Levitt, Zulu, Jonathan, et al., 2005).

Factors known to affect blood pressure include body mass index (BMI), activity levels, alcohol consumption and diet. Evidence gathered from various epidemiological, migration, population, intervention, treatment, genetic and animal studies have shown that higher intakes of salt or Na are directly correlated to elevated blood pressure in populations and in individuals (Charlton et al., 2005; Dahl, 1972; INTERSALT Cooperative Research Group, 1988; Lerman, Chade, Sica, & Napoli, 2005; Maseko, Majane, Milne, Norton, & Woodiwiss, 2006). Left untreated, hypertension is associated with increased incidences of diabetes, heart disease, kidney disease, and stroke and in some cases, premature death. Other conditions including gastric cancer, osteoporosis, cataracts and kidney stones have also been associated with Na intake through epidemiological studies (Cappuccio, Kalaitzidis, Duneclift, & Eastwood, 2000; Cappuccio & MacGregor 1997; Chobanian & Hill, 2000). Observations on the American population revealed that not only is the mortality rate of hypertensives higher than that of non-hypertensives, hypertensive males having a significantly higher mortality rate than hypertensive women (Ford, 2011). Globally, 62% of cerebrovascular disease and 49% of ischaemic heart disease have been attributed to elevated blood pressure (WHO, 2007).

High blood pressure or hypertension as it is also known is defined as the condition where blood pressure is chronically (over extended periods) elevated above 140 over 90 mmHg (Wardlaw & Kessel, according to Kim, Lopetcharat, Gerard, & Drake, 2012). The precise mechanism by which Na affects blood pressure is not completely understood. One theory is that in response to high salt intake, salt sensitive hypertensives do not excrete as much Na in the urine as more salt-resistant individuals. The resulting higher serum Na levels are then followed by an expansion in plasma volume, an increase in cardiac output and a prolonged increase in systematic vascular resistance (Schmidlin, Forman, Sebastian, & Morris, 2007). The concept that interventions to reduce or

prevent the development of high blood pressure would significantly improve health is universally accepted (Dickinson & Havas, 2007).

The term “salt sensitive” is used to describe individuals that experience large fluctuations in blood pressure when consuming varying amounts of salt, whilst other individuals may not experience the same significant changes. Salt sensitivity is known to prevail in individuals with hypertension, diabetes and chronic kidney disease, the elderly and people of African descent. This condition is thought to be based on genetics, or may be related to body mass index and/or other variables in the diet or lifestyle (Dickinson & Havas, 2007; He & MacGregor, 2007). Long-term animal studies on the effects of salt on hypertension have shown salt to have two distinct effects: (i) an acute increase in blood pressure in response to increased salt occurring over days or weeks and (ii) a much slower progressive response to salt that occurs over a significant portion of the lifetime of normal individuals. In some species this long-term increase in blood pressure appears to be irreversible and may correspond to the age-related increase in blood pressure observed in many human societies (Van Vliet & Montani, 2008). In 1998, a national cross-section survey completed by 13 000 South Africans showed that a quarter of South African adults suffer from hypertension. Diagnosis, management and control of hypertension are poor, particularly in the black population. An overburdened, mainly due to HIV/AIDS, public health sector cannot successfully assist the situation and therefore non-pharmacological approaches to lower blood pressure are believed to be a feasible option to curb the ever increasing rate of hypertension (Steyn, Gaziano, Bradshaw, Laubscher, & Fourie, 2001).

Normally only about 27% of dietary calcium is absorbed by the body, but intestinal uptake can change in response to suboptimal or excess serum calcium levels and the presence of other nutrients. The metabolism and intracellular transport of Na and calcium are linked and thus, high-salt diets may affect calcium retention and bone density. Several studies have shown that a higher Na intake is correlated to greater urinary losses of calcium (Carbone, Bush, Barrow, & Kang, 2003; Devine, Criddle, Dick, Kerr, & Prince, 1995; Fringes-Meuthen, Baecker, & Heer, 2008; Teucher, Dainty, Spinks, Majsak-Newman, Berry, Hoogewerff, et al., 2008).

In 2005 an international organization of experts, WASH (Word Action on Salt and Health) began to publicize the adverse effects of NaCl on health. As of 2016, WASH consisted of 527 members in over 95 countries whom engage governments and food industries in a drive to reduce salt levels in processed foods, catered foods and restaurant foods, as well as salt added during home cooking and at the dinner table (World Action on Salt and Health, 2016). The revocation of the GRAS (Generally Recognised As Safe) status of salt has been suggested. This would require food

processors to justify the amounts of salt they add to food (Dickinson & Havas, 2007). It has been found that when the salt concentration of food is radically reduced, the acceptance of these food by consumers also decrease (Mattes, 1997). This can, however, be successfully countered by repeated exposure to a low salt diet since subjects become sensitized to the salty taste (Bertino, Beauchamp, & Engelman, 1982). Thus in effect, consumers can not only adapt to high Na diets, but low Na diets as well (Blais, Pangborn, Borhani, Ferrell, Prineas, & Laing, 1986).

2.7. South Africans and high blood pressure

Charlton et al. (2005) conducted a study to: establish whether differences exist in habitual intakes of Ca, K, Mg and Na across South Africa's ethnic groups; assess the proportion of discretionary salt intake; and to identify foods that are major contributors to Na intake. Daily intake of salt were higher than recommended, with only 23% of subjects having urinary Na values below 6 g NaCl across all groups. Discretionary salt intake was found to be at 33% to 46% of the total intake and was highest in the black population. It is clear that discretionary salt intake is a much bigger problem in South Africa compared to affluent Western countries. Bread contributes the most Na to the South African diet (45.9% to 48.6%) followed by meat products and processed meat (10% and 20% for rural versus urban blacks, respectively). The effect of this high level of salt consumption is reflected in the prevalence of high blood pressure across the population found to be in the region of 45-49.9%. This is similar to that of many African and Eastern European countries (Figure 2.1). All racial groups had K intakes far below the recommended intake level with only 8% of subjects having met the recommended intake level. It is estimated that approximately 60 deaths per 100 000 (men over 30 years) and 50 deaths per 100 000 (women over 30 years) could be averted through the implementation of selected measures to reduce tobacco and salt exposure. The cost of voluntary salt reduction in South Africa would be approximately US$ 0.17 per person per year to implement (Asaria, Chisholm, Mathers, Ezzati, & Beaglehole, 2007). The South African Department of Health intend to reduce the mean population intake of salt from 8-10 g/day currently, to less than 5 g/day by 2020 (Bertram, Steyn, Wentzel-Viljoen, Tollman, & Hofman, 2012). Sodium reduction may contribute to prevent 7400 cardiovascular disease related deaths per year (Gaziano, Steyn, Cohen, Weinstein, & Opie, 2005) and the prevention of non-fatal strokes may save the overburdened South African health system R300 million annually (Bertram et al., 2012).

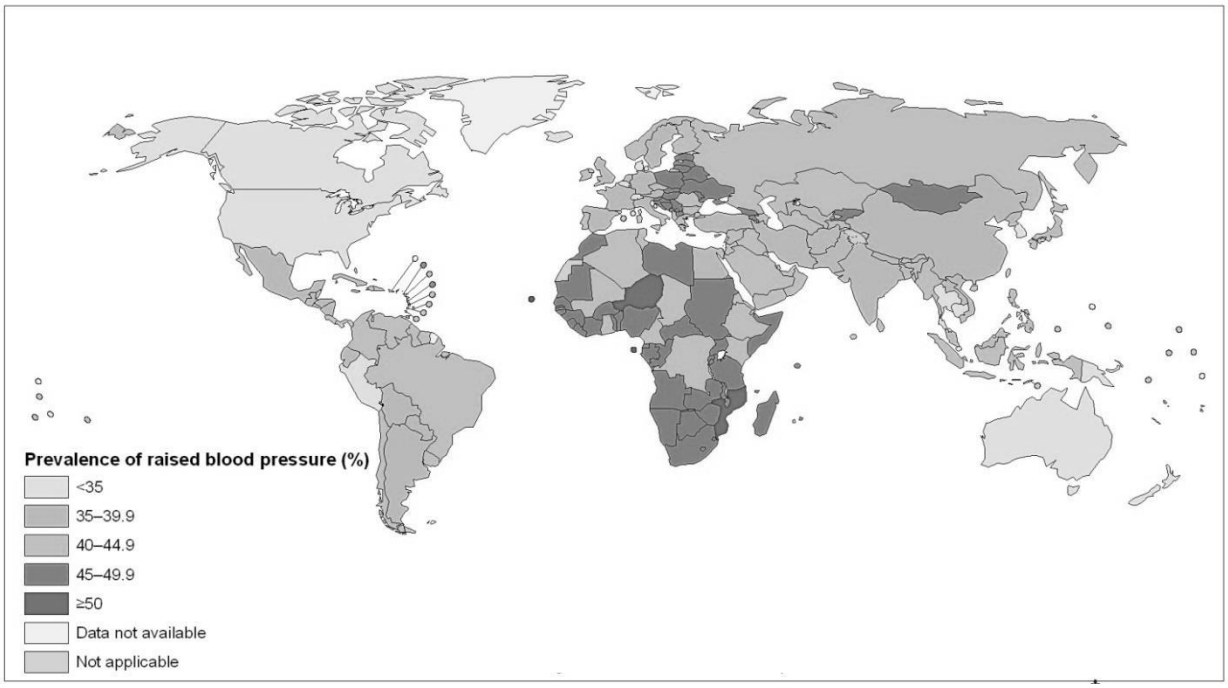

Figure 2.1. Prevalence of high blood pressure across the world for both sexes over the age of 25 in 2008. High blood pressure defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 or using medication to lower blood pressure (WHO, 2011).

Most South Africans have a poor understanding of concepts such as the difference between energy dense and nutrient dense foods, preferring less healthy food over healthier options (Temple & Steyn, 2011). As a general rule, the less healthy options contain more Na and NaCl. Another problem that exists is that healthier foods cost between 10% and 60% more on a weight basis (Rands per 100 g). It is of the utmost importance that when healthier diets are to be promoted, they remain affordable to the majority of people (Temple & Steyn, 2011). A survey carried out by an Australian group found that 60% of shoppers indicated that they would be more likely to buy reduced salt products as long as the price remained constant (Grimes, Riddell, & Nowson, 2009).

2.8. Consumers' attitude toward salt/sodium reduction

In a study by Kim et al. (2012) consumer perception of Na and Na reduction in food was quantitatively assessed. They found that most American consumers were able to identify high-sodium food as being the same as high-salt food. People from families with a history of sodium-related diseases were not more aware of the relationship between Na consumption and disease, than people with no family history of sodium-related diseases. Provision of Na content on the product label did not influence product dissatisfaction and did not necessarily increase consumer product satisfaction. Fewer consumers scan the labels of food products for salt content than for information

about the fat and sugar content (FSA, 2008; Marshall, Bower, & Schröder, 2007; Scott & Worsley, 1997).

In a more recent Canadian study, 23 dietary recommendations for reducing Na intake were evaluated in relation to the extent to which consumers engage these recommendations. The study also aimed to determine if any differences existed between gender, level of education, age, marital status and between consumers who prepare most of their food at home as opposed to those consumers who were more likely to buy ready-made food. Consumers showed the lowest level of engagement towards recommendations requiring avoidance of higher Na food items and the highest level of engagement towards recommendations requiring modification of food preparation for reducing Na intake. Consumer engagement was also highly variable in terms of the sub-groups. Older people (> 50 years) were less likely to engage recommendations relating to salt use at the table, draining and rinsing canned vegetables and beans, and with avoiding ready-to-eat dishes. Younger people (< 50 years) were in turn less likely to engage recommendations relating to the avoidance of the use of condiments, eating pizza, and limiting bread consumption. A lower level of education correlated with lower consumer engagement of recommendations related to using fresh foods in cooking and avoiding ready-to-eat meals. It is therefore important that consumer education programmes be targeted at specific sub-groups. Between men and woman as sub-groups, the following was reported. Men were less likely to engage in recommendations to use salt replacers and less likely to ask that no salt be used in preparation of their food when dining out. In comparison, women were less likely to engage recommendations related to the draining and rinsing of canned vegetables and beans and asking for sauces/dressing to be placed on the side of a plate when dining out (Mendoza et al., 2014).

England is a good example of how industry, with the participation of government, has improved consumer awareness of the adverse effects of too much Na in the diet. Over the course of 8 years, salt intake there has decreased by almost 15%. This is believed to have resulted in a decrease in mortality from stroke by 42% as well as from ischemic heart disease by 40% (He, Pombo-Rodriquez, & Macgregor, 2014). A key part to building on consumer awareness, is making sure that consumers are not confused by what they might perceive to be technical jargon. Consumers criticize the use of scientific terminology such as the use of the word sodium instead of salt on food labels (Grunert & Wills, 2007; Scott & Worsley, 1997), lacking the know-how of how to convert Na levels to salt levels which results in an inability to accurately interpret Na levels (Gilbey & Fifield, 2006; Marshall et al., 2007).

2.9. Other challenges facing the processed meat product industry

The consumption of meat and meat products remains a prominent feature of the world diet and while consumers may sometimes indicate health concerns, they generally do not seem to adopt healthier eating habits (Grunert, 2006). If the meat industry wants to stimulate meat consumption it has to face the challenges of improving environmental sustainability and animal welfare. It also needs to offer more convenient and healthier options while identifying the new and changing roles of meat and meat products in the diet (Font-i-Furnols & Guerrero, 2014). Now and for the foreseeable future microbial foodborne illnesses and outbreaks; the associated product recalls and issues relating to regulatory compliance will remain of concern. A large and ever increasing number of potential reasons for increasing food safety concerns in recent years and the future include: changes in animal production systems; increased international trade; the changing needs and expectations for minimally processed and convenient food; the projected increase in worldwide meat consumption; higher numbers of consumers at-risk for infection; emerging pathogens with increased virulence and resistance to control and treatment; advances in microbial detection; limited food handler and consumer education and training in proper food handling; and, increasing interest, awareness and scrutiny by consumers, consumer activist groups and the media (Sofos, 2008). It is regarded as an impossible feat to have complete knowledge of all factors affecting a food product, food microorganism or the production and storage conditions influencing safety and/or quality, either directly or indirectly. This means that there is always a level of uncertainty involved even though the safety of food products is a prerequisite (Smith, Imfeld, Dayan, & Roberfroid, 1999). In addition to uncertainty, there is also often a large variability. Variability can be divided into: biological variability such as raw materials or contaminating organisms; process variability such as daily variations; and variability after processing such as storage time and storage temperature. Variability requires safety factors and if variability can be better controlled, the safety factors can be decreased, thus reducing over-processing of food products (Zwietering, 2002).

Bobowski et al., (2015b) regarded maintenance of liking as one of the major challenges encountered by the industry where a sizeable salt reduction is involved. They also concluded that specific research is needed in understanding the potential response of consumers and the ability to "learn-to-like" low Na foods. A major concern of the food industry is the belief that consumers may switch to a competitor's product, if a company lowers the salt content of their product and this results in a decrease in consumer acceptability (Bobowski et al., 2015b). Consumers do seem to be increasingly unfavourable towards foods that compromise on the sensory experience of the food in return for potential health benefits (Verbeke, 2006). This sentiment can result in a large percentage of consumers rather lowering or completely avoiding the intake of a previously preferred food product

over settling for a supposedly healthier and unpalatable version (Guerrero, Claret, Bernardo, Mauri, Comaposada, & Arnau, 2011). Verbeke (2006) concluded that it is highly speculative and risky to depend on consumers' willingness to lower their expectations for taste in return for health benefits.

Consumers, especially women, generally have a favourable attitude toward specific nutritional improvements of meat and meat products as is the case with reducing fat and NaCl content (Guárdia, Guerrero, Gelabert, Gou, & Arnau, 2006). It was found that the majority of European consumers consider processed meat products unhealthy and believe them to contain considerable amounts of harmful chemicals, fat and salt (Tobin, O'Sullivan, Hamill, & Kerry, 2004). The consumer consciousness also appears to be more focused on the healthiness and nutritional properties of meat as opposed to the safety issues surrounding meat consumption (Verbeke, Pérez-Cueto, de Barcellos, Krystallis, & Grunert, 2010). With regard to the microbiological food safety and quality implications of salt reduction, little attention has been paid to the subject in both peer-reviewed literature and the media compared to that devoted to the potential beneficial cardiovascular health impacts. The perception that refrigeration has largely replaced the need for Na salts as food preservatives (Flegel & Magner, 2009) is attributed (Toarmina, 2010) to perpetuating this skewed focus.

2.10. Functions of sodium contributing substances

2.10.1. Flavour

As Na content increases there is a corresponding increase in hardness and juiciness intensity rankings associated with positive sensory quality of processed meat products such as frankfurters (Matulis, McKeith, Sutherland, & Brewer, 1995). Sodium and lithium are the only two cations with a primarily salty taste. Other minerals such as potassium and calcium are also salty, although they have other flavours that are described as "metallic" or "bitter" (McCaughey, 2007). It has been reported that the NaCl in dry fermented sausages has been partially replaced with calcium ascorbate at levels of between 28-50% with organoleptic qualities comparable to the control (García-Íñiguez de Ciriano, Berasategi, Navarro-Blasco, Astiasaŕan, & Ansorena, 2013). Salt may affect the flavour of foods in other ways than just contributing a salty taste. It can enhance the taste of some ingredients and may supress or mask the bitter flavours of others. The ability to discern bitterness is genetically controlled with about 25% of the population being nontasters (insensitive to normal levels of bitter compounds) and about 25% of the population being supertasters (very sensitive to bitter tasting compounds) (Kilcast & Den Ridder, 2007). This may present challenges in formulating foods with lower salt content. A significant decrease in the salt content of some foods

may lead to a situation where these foods become unpalatable to as much as a quarter of the population, whilst another quarter of the population may not even notice this decrease in salt content (Doyle, 2008).

Flavour perception may also suffer, through weaker flavour intensity that may be characteristic of a particular product (Chabanet et al., 2013). Other ways through which salt affects the flavour of food is through its effect on the activity of enzymes and on the growth of microorganisms. The concentration of salt significantly impacts the activities of both proteolytic and lipolytic enzymes which for example, produce important and characteristic flavour compounds during the ripening of cheese. Specific NaCl levels stimulate or depress the growth and metabolic activities of particular cheese starter cultures, yeast and sourdough starters for bread. The secondary functions of these microorganisms are to synthesize important flavour and aroma compounds (Man, 2007).

When the lean meat content in meat products increase, a reduction in perceived saltiness is found (Ruusunen et al., 2001; Ruusunen, Tirkkonen, & Puolanne, 2001) and this effect on perceived saltiness was found to be stronger than the effect of fat content on perceived saltiness (Ruusunen, Vainionpää, Lyly, Lähteenmäki, Niemistö, Ahvenainen, & Puolanne, 2005). Lower NaCl content can lead to a situation where tissue proteases can excessively act on proteins and polypeptides, thereby resulting in unnecessarily high concentrations of low molecular weight nitrogen compounds such as free amino acids and small peptides. The end result of this could be the presence of a bitter taste (Martín, Córdoba, Antequera, Timon, & Ventanas, 1998; Toldra, 1998).

2.10.2. Texture

Sodium chloride affects the texture of foods and reactions that occur during processing through its interactions with other major components in foods. In particular, salt is known to enhance the hydration of proteins and to enhance the binding of proteins to each other and to fat. These reactions cause emulsions of ground meat mixed with fat to become stabilized (Doyle, 2008). Salt added to meat activates proteins to bind more water molecules. This happens when salt moves the isoelectric point of the meat proteins towards lower pH values (Hamm, 1961). The result is increased tenderness of the meat and decreased fluid loss in heat-processed, vacuum-packaged products. The presence of salt loosens myofibrillar proteins and thus enhances their ability to bind more fat which in turn increases the viscosity to form more stable emulsions of comminuted meats (Figure 2.2). A so called meat emulsion is not a true emulsion as it does not consist of two immiscible liquids in which one is dispersed (discontinuous phase) in the other (continuous phase). It is a complex mixture of suspended particles (connective tissue as collagen fibres, myofibrils and cellular

organelles), bound and unbound water, lipid droplets and fat particles, and myofibrillar proteins in different states (hydrated, solubilised and non-solubilised). The continuous phase consists of water, dissolved salt, and myofibrillar proteins. The discontinuous phase consists of suspended non-soluble particles, and fat globules and particles (Figure 2.2) (Claus, Colby, & Flick, 1994).

Salt is also responsible for the binding of myosin proteins to each other, thereby improving the texture of processed meats (Man, 2007). The salt-solubilized myofibrillar proteins essentially form a sticky exudate on the surface of the meat pieces and then bind them together during cooking. This is due to the matrix of heat-coagulated protein that forms around the meat pieces and entraps free water and fat globules (Figure 2.2). This process enables the creation of unique meat products such as frankfurters and bologna (Monahan & Troy, 1997). The level of solubilisation is dependent on the muscle fibre type. Myofibrillar proteins from slow twitch oxidative muscles are less soluble compared to those from fast twitch glycolytic muscles under the same pH and ionic strength condition (Parsons & Knight, 1990; Xiong & Brekke, 1991; Xiong, Lou, Harman, Wang, & Moody, 2000). It can therefore be concluded that the effect of salt addition on the structure of minced beef could be different from the effect on minced pork as these two meat types also contain different proportions of slow twitch oxidative fibres, with that of pork generally lower (Astruc, Labas, Vendeuvre, Martin & Taylor, 2008).

Sodium chloride has been shown to have direct positive effects on maintaining the texture of whole muscle meat products. When NaCl was added in a smaller quantity, defects in texture such as softness were observed. This was attributed to an excess of proteolysis as a result of the intense action of tissue proteases (Martín et al., 1998; Toldrá, 1998). Reducing the NaCl content of food may require alterations in other parameters to ensure that affected foods maintain acceptable textures (Doyle & Glass, 2010). This creates technological challenges in that the formulation of low-salt meat batters requires other ionic compounds to fulfil the water-holding, protein-binding and fat-binding functions of the salt that is being replaced. Comminuted meat products become unstable and have poor texture when they contain less than 1.5% salt in their formulation (Xiong, 2007). Also, Ruusunen et al. (2001) reported higher cooking losses in hams with less than 1.4% added salt compared to hams with more than 1.7% added salt. The authors suggested that products with low salt content and high water content need extra protein or other functional ingredients to keep cooking losses low.

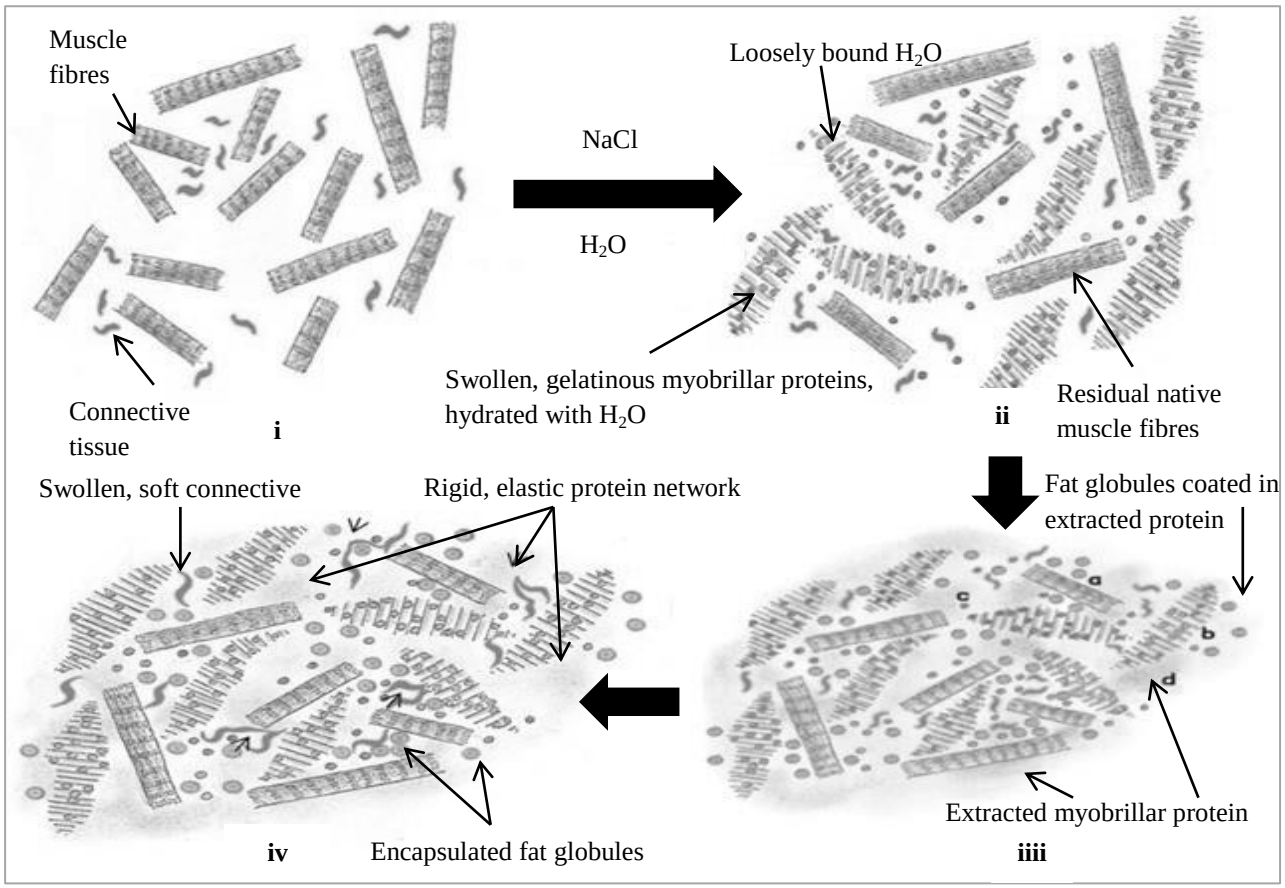

Figure 2.2. Simplified progression of the changes to components during the preparation of an emulsion meat batter. (i) Initial dry chopping, (ii) addition of water and NaCl causing the solubilisation and extraction of myofibrillar proteins, (iii) establishment of network structure of liquid and gelatinous extracted proteins and globulization of fat, (iv) heat-stabilised meat protein network and gelatinisation of connective tissue collagen (Heinz & Hautzinger, 2007).

Potassium chloride, calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) and polyphosphate compounds can be used to stabilize reduced-sodium meat emulsions. Potassium chloride has the same ionic strength than NaCl and thus interacts with meat proteins in exactly the same way as NaCl. The chlorides of calcium and magnesium are however not as effective (Gordon & Barbut, 1992; Aliño, Grau, Baigts, & Barat, 2009). Potassium phosphates can bind water and improve the stability of meat products to the same extent as the sodium phosphates, but these potassium compounds can adversely affect taste at high levels. Nonmeat proteins such as soy and milk proteins, starches from a number of plant sources and gums and alginates can act as binding agents to increase the viscosity of low-salt meat products (Desmond, 2006).

2.10.3. Preservation

Salt has been used to preserve meat for thousands of years. Weight for weight ionic substances such as NaCl decrease water activity (a_w) more than other classes of substances (Mossel & Thomas, 1988). It reduces the a_w of foods so as to hamper the growth of pathogenic or spoilage microorganisms. At the most basic level of food preservation, salt exerts a drying effect through

drawing water out of cells of both the food itself and microorganisms present in the food, through the process of osmosis (Doyle & Glass, 2010). A a_w of less than 0.92 inhibits the growth of most foodborne bacteria including *Clostridium botulinum* (*C. botulinum*), *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella* spp. and the spoilage bacteria *Pseudomonas* spp. Unfortunately, some microorganisms can tolerate lower minimum a_w: spoilage lactic acid bacteria (LAB) (0.90); *Staphylococcus aureus* (*S. aureus*; 0.83); moulds such as *Aspergillus* and *Penicillium* tolerate lower a_w than most bacteria, ranging from 0.80 to 0.83; and spoilage yeasts (0.62) (Christian, 2000; Betts, Everis, & Betts, 2007). These a_w levels mentioned are only limiting when other growth conditions are favourable. If the pH, oxygen levels and/or temperature are outside the optimum range, microorganisms may not be able to tolerate these minimum a_w levels due to cumulative stressful conditions. Gram-negative bacteria are generally more sensitive to low a_w than Gram-positive bacteria (Anonymous, 2003).

The concentration of salt needed to inhibit the growth of microorganisms varies depending on the species involved. Campylobacters are highly salt-sensitive, with optimal growth found at NaCl levels of around 0.5% (Doyle & Roman, 1982) while proteolytic *C. botulinum* can tolerate up to 10% NaCl, and under favourable growth conditions *S. aureus* can grow in the presence of > 20% NaCl (Baird-Parker, 1990). Microorganisms such as *L. monocytogenes* produce specific stress proteins in a response to high salt levels. Some toxin-producing microorganisms are only able to produce those toxins at amounts of available water much higher than the amounts at which they are capable of surviving (Duché, Trémoulet, Glaser, & Labadie, 2002). As an example, *S. aureus* can grow aerobically at 37°C at a a_w of 0.86, but is only capable of producing enterotoxin if the a_w is at least 0.90 (Baird-Parker, 1990). Growth of *S. aureus* was shown to stop at temperatures below 8°C and was only possible at 8°C if the pH and a_w was at an optimum (Valero, Pérez-Rodriguez, Carrasco, Fuentes-Alventosa, García-Gimeno, & Zurera, 2009). There is difficulty in analysing the a_w of multicomponent foods. Effective measurements of a_w may not reflect actual values in a micro-environment or at the interface between different components (Anonymous, 2003).

The use of refrigeration, heat treatment and appropriate packaging has reduced the use of salt as a preservative for many foods. These other methods of preservation are not infallible as is the case with refrigeration, especially where it is depended upon when salt as a microbial hurdle is reduced or removed. Meat products requiring refrigeration often experience abusive temperatures in retail outlets and particularly in many households where the products may be kept in refrigerators that do not maintain temperatures at or below 5°C (Juncher, Vestergaard, Søltoft-Jensen, Weber, Bertelsen, & Skibsted, 2000). For low temperatures, two conditions need to be met for microbial growth to stop: (1) the reaction rates of individual enzymes in the microorganism need to become much

slower, and (2) the low temperatures need to reduce the fluidity of the cytoplasmic membrane to the extent that it interferes with transport mechanisms (Mossel, Corry, Struijk, & Baird, 1995). Food microbiologists and food scientists can agree that for modern food production, refrigeration is of the utmost importance. However, the idea that the need to formulate food products and in effect meat products, to be safe and of quality can be replaced by only refrigeration, is erroneous (Taormina, 2010) and may even be dangerous in light of the ever present threat of meat pathogens. The haphazard removal of salt from food may not only encourage pathogen survival and growth, it may support accelerated spoilage which could negatively affect producers, distributors, retailers and consumers financially (Taormina, 2010).

Other preservatives, mainly the Na salts of organic acids (benzoate, diacetate, propionate and sorbate), sodium nitrite and various sodium phosphate compounds are often used in foods. They are applied individually or in combination with each other to prevent microbial growth (Doyle, 2008). Nitrite has been positively linked to cancer due to its role in the formation of carcinogenic N-nitrosamines (Peters, Preston-Martins, London, Buckley, & Thomas, 1994). However, its invaluable contribution in: controlling microbes (Lücke, 2003; Kabisch, Scheuer, Roedel, & Gareis, 2008); anti-oxidative effects (Arneth, 2001; Mancini, 2013) and contribution to cured meat flavour (Fischer, Bristle, Gehring, Herrman, & Gibis, 2005) cannot be overlooked and maintains its status as a necessary additive (Cassens, 1995).

2.11. Common pathogens and spoilers of meat products

Most serious meat safety issues that result in both immediate consumer health problems as well as marketplace recalls of potentially contaminated products are associated with microbial, mostly bacterial pathogens (Sofos, 2008). Public awareness about the safety of meat has increased due to the increasing number of food poisoning outbreaks with increasing severity (Maurice, 1994). The shelf-life of meat and meat products is defined as the storage time until spoilage. The point of spoilage can be defined as a certain maximum acceptable bacterial level, or an unacceptable off-odour/off-flavour or appearance. The shelf-life will depend on the numbers and types of microorganisms present, usually bacteria, initially present and their subsequent growth (Borch, Kant-Muermans, & Blixt, 1996).

For fresh meat products, *E. coli* 0157:H7 and related enteric pathogens such as *Salmonella* are of major concern and are associated with product recalls. *Listeria monocytogenes* is the major pathogen of concern in ready-to-eat meat products that allow growth of the organism during storage, or after exposure to recontamination during slicing and packaging (Sofos, 2008).

Staphylococcus aureus is commonly found in chopped meat mixes and processing plants (contaminating equipment, and carried by plant personnel). Salt and nitrite tolerant, it can also grow under a wide range of environmental conditions (Gonzáles-Fandos, Sierra, García-Lopez, García-Fernández, & Otero, 1999). The retail display of meat often involves the opening of packaging and exposure to air with subsequent storage at a higher temperature. The microbial population of pork chops prepared from loins initially stored under CO_2 at -1.5 °C, was found to be dominated by LAB during storage in air at 8 °C (Greer, Dilts, & Jeremiah, 1993). The predominant bacteria associated with the spoilage of pork include: *Bacillus thermosphacta, Carnobacterium* spp., *Enterobacteriaceae, Lactobacillus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens* (Dainty & Mackey, 1992). The *Enterobacteriaceae* reportedly develop better on pork than on beef (Boers, according to Borch et al., 1996).

Meat spoilage is usually attributed to bacteria, however, the inhibition of bacterial growth through certain processing and storage techniques may allow yeasts to become the dominant microbiota causing meat spoilage (Fleet, 1992; Osei Abunyewa, Laing, Hugo, & Viljoen, 2000). For most meat products, yeast growth is undesirable and results in spoilage of the product – e.g. gas swelling of packaged meat products; slime formation and discolouration on the surfaces of sausages; and production of off-flavours. Also of concern is the ability of some yeasts to metabolize organic acids and sodium nitrite, functioning as preservatives in some meat products (Fleet, 1992). Processed meat products have been reported to contain relatively high numbers of yeasts capable of negatively affecting quality and causing spoilage (Fleet, 1992). In a recent study by Nielsen, Jacobsen, Jespersen, Koch, and Arneborg (2008) it was concluded that yeasts did not contribute that much to meat product spoilage. The researchers were however of the opinion that due to the ability of yeasts to grow to large numbers, yeasts should not be disregarded.

2.12. Intrinsic and extrinsic factors affecting meat product microbiology

Microorganisms function at an optimum in their normal physiological environments. Extreme deviations from the optimal environmental conditions inflict stress on an organism. The extent of the deviation determines whether the organism dies, ceases to grow or experiences an increased lag time and reduced growth rate (Ray, 1986; Russell, Evans, Ter Steeg, Hellemans, Verheul, & Abee, 1995). Most bacteria can tolerate small changes in a particular environmental parameter and can adapt within minutes, hours or days (Hill, O' Driscoll, & Booth, 1995). To do this, microorganisms yield to the stress conditions and make suitable provisions for survival or attempt to resist the stress (Herbert, 1989). These changes in environmental conditions away from the optimal value cause the

induction of many elaborate stress responses which are directed at survival rather than growth (Beales, 2004). Factors that affect the microbial growth potential in food can be divided into two main groups. Intrinsic factors include physical and chemical properties of the food and extrinsic factors include temperature, packaging and storage conditions. A large number of these factors exist although the following were identified for further discussion.

2.12.1. pH

Most meats have a final ultimate pH of about 5.6 or above which makes these products susceptible to bacterial and fungal spoilage. Upon the death of a well-rested animal, the ~ 1% muscle glycogen is converted to lactic acid, causing a depression in muscle pH from ~ 7.4 to ~ 5.6, depending on the animal type. In contrast, the meat of fatigued animals is prone to faster spoilage. This is a direct consequence of a higher final pH attained after rigor mortis. Some foods resist pH change better than others due to their higher buffering capacity. Meats are one type of highly buffered food, able to resist pH changes due to the various meat proteins it contains. The ability of microorganisms to proliferate depends on their ability to bring the environmental pH to a more optimum value when it is above or below neutrality. Other environmental factors interact with pH. Substrates become more acidic as the temperature rises and salt above an optimum concentration narrows the pH growth range (Jay, Loessner, & Golden, 2005).

2.12.2. Moisture content and a_w

The preservation of food by lowering moisture content is a direct consequence of removal or binding of moisture, without which microorganisms cannot survive. It is generally accepted that the water requirements of microorganisms should be described in terms of the a_w. Water activity is defined by the ratio of the water vapour pressure of food (p) to the vapour pressure of pure water (p_0), both at the same temperature: $a_w = p / p_0$. Relative humidity (rH) is related to a_w in the following way: $rH = 100 \times a_w$ (Christian, 1963). While the salt and moisture contents of food have long been used to assess microbiological stability, a_w is regarded as being a better general indicator of microbial stability than moisture content (Sperber, 1983). Small reductions in hurdles, such as increasing a_w due to the reduction or replacement of salt, may be enough to tip the balance from microbiologically safe to unsafe (Taormina, 2010).

2.12.3. Nutrient content of the food product

Microorganisms need the following nutritional factors for growth and survival: water; a source of energy; a source of nitrogen; vitamins and related growth factors and minerals. Water is the most important nutritional factor. With regards to the other four groups of substances, moulds have the lowest requirements, followed by Gram-negative bacteria, yeasts and Gram-positive bacteria having the highest requirements. Food borne microorganisms may utilize sugars, alcohols and amino acids as sources of energy. Some microorganisms are capable of utilizing complex carbohydrates such as starches and a small number of food microbes can even attack fats for energy. Nitrogen is primarily sourced from amino acids and the B vitamins are mostly required, although in low quantities (Jay et al., 2005).

2.12.4. Temperature and the meat cold chain

An important aspect of meat distribution and consumption is the effective monitoring of the time and temperature conditions which affects both the safety and the overall meat quality. It is generally recognized that several stages of the cold chain, such as the transfer points or storage rooms, are the weakest link in the management of chilled perishable food. Meat products, unless appropriately packaged, transported and stored, will spoil in a relatively short time (Nychas et al., 2008). The meat cold chain consists of two main parts: the primary and secondary chilling. Both of these parts are important for the microbiological stability, eating quality and production yield (Koutsoumanis & Taoukis, 2005). Primary chilling takes place during the cooling of the carcass after slaughter when the temperature is reduced from body to refrigeration temperature. Rapid reduction of the carcass surface temperature can prevent microbial growth and extend product shelf-life. During subsequent handling processes such as cutting, mincing, etc., temperature increases will occur and thus secondary chilling is required to reduce the temperature of the meat below 7 °C. Secondary chilling is also of cardinal importance with relation to cooked meat products where the meat temperature should be reduced from ≈ 60 °C to 5 °C as rapidly as possible. This is done to prevent or reduce the growth of pathogens that may have survived the heat process (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

To meet market demands, producers and regulators concentrate on the development and application of structured quality and safety assurance systems to monitor products throughout their whole life cycles. Meat product safety via HACCP (Hazard Analysis and Critical Control Points) monitors raw material selection, control of conditions during processing and distribution (Koutsoumanis, Taoukis, & Nychas, 2003; Koutsoumanis & Taoukis, 2005). Distribution is the weakest link in this system. Conditions during transportation and at retail level are not under control of the

manufacturer and are prone to deviate from specifications. Furthermore, temperature control is lacking from store to domestic storage up to the time of preparation and consumption. The importance of temperature control throughout distribution becomes even more apparent in that, the risk potential, the shelf-life and final product quality of chilled products processed and packed under good manufacturing and good hygiene practices (GMPs and GHPs) are determined by the temperature conditions applied throughout the chilled distribution chain (Nychas et al., 2008).

In the meat market the dominating managing approach is related to the "First In-First Out" principle, meaning the first stock of a particular product that is taken in is also the first stock to be taken out. With regard to the vehicle used in transport, it should be provided with a good refrigeration system. A related weak point in the distribution chain is the transport period from product purchase to home refrigeration. Very little information is available on home refrigeration due to the difficulty in collecting data concerning: temperature conditions in domestic refrigerators and freezers; consumer habits; and storage periods before consumption. When the quality of chilled meat from production to final consumption is to be addressed, home refrigeration conditions should be included in the evaluation of quality losses and safety risks in the cold chain (Nychas et al., 2008).

2.11.5. Packaging

Three different packaging types can be used: air, vacuum and modified atmosphere (MA) packaging. Pork is generally stored aerobically or in MA. The shelf-life of meat increases in the following order: air, high-oxygen-MA, vacuum, no-oxygen-MA and 100% CO_2 (Borch et al., 1996). The film permeability has been shown to influence shelf-life (Newton & Rigg, 1979). A long shelf-life can be attained by using pure CO_2. The time needed to reach 10^7 bacteria/cm^2 and off-flavour, was 10 days in air, compared to 40 days in 100% CO_2 for pork stored at 4 °C (Blickstad, Eufors & Molin, 1981). Shelf-life extension by CO_2 resulted in an immediate selection of the population composition, as opposed to a gradual selection in vacuum-packaging of LAB growing at a reduced rate (Greer et al., 1993). Vacuum-packaged pork reportedly has a shorter shelf-life than beef, even in the event of both meat types being dominated by LAB. This is attributed to the faster decrease in glycogen and glucose in pork than in beef, leading to earlier initiation of amino acid degradation in pork (Boers, according to Borch et al., 1996).

2.13. Sodium requirements for microbial safety

The microbial safety, shelf life, as well as the sensory and nutritional quality of most foods is dependent on a combination of preservative factors or hurdles. Hurdles have been used for a very long time in traditional foods and remain invaluable to today's novel foods where it is purposefully selected and applied (Leistner, 1995). Usually a number of sublethal factors are combined so as to target different groups of microorganisms within a food product (Beales, 2004). Temperature, a_w (and by implication NaCl), acidity, redox potential, preservatives (NaCl) and competitive microorganisms are regarded as the most important hurdles for food preservation. A list of over 60 possible hurdles has been compiled and is regarded as being nowhere near complete (Leistner, 1999). If the concentration of a particular hurdle is too low to be effective, it should be increased and when it becomes detrimental to the food it should be decreased. Through this type of adjustment, hurdles in food can be kept in the optimal range, and a state equal to, or close to equilibrium between safety and quality can be achieved (Leistner, 1994).

Essential to public health is controlling the growth of pathogens. This is especially of importance to high-risk groups such as very young children, pregnant women, the elderly and people with immune systems weakened by chronic illnesses, immunosuppressive therapy, or chemotherapy (Doyle & Glass, 2010). Due to the great diversity of microorganisms to be found on raw meats, processing can be seen as a way to limit microorganisms to a group of well-adapted and safe organisms. However, a change or failure in the control system can allow the emergence or re-emergence of a pathogen (Miller et al., 1998). There is a possibility that the reduction in salt content of meat products may bring about such a change or failure in the control system. A large variety of meat products exist, each having various processing, packaging, storage and distribution attributes. The result of which is that each individual product has its own unique microbial environment and population (Ross & McMeekin, according to McDonald & Sun, 1999) to be taken into consideration when changes to processing or product formulation are to be made.

The concentrations of salt required to inhibit pathogens vary, depending on the microbial species, but also pH, temperature, oxygen levels and other components in food such as moisture, fat and other additives (Doyle & Glass, 2010). In a study with laboratory media by Gibson and Roberts (1986), the effect of salt concentration (1-10% w/v) in combination with sodium nitrite (0-400 µg/mL) on growth of various strains of *Salmonella* and enteropathogenic *E. coli* at three pH values (5.6, 6.2 and 6.8) were established at temperatures ranging from 10 °C to 35 °C. It was reported that these bacteria could tolerate NaCl concentration better at higher temperatures together with higher pH values (Gibson & Roberts, 1986). This may indicate problems for food products kept at higher

temperatures or experiencing temperature abuse and low acidity. Between these two groups of bacteria, *E. coli* proved to be more tolerant than *Salmonella* to salt in combination with nitrite (Gibson & Roberts, 1986). The impact of NaCl on the cold storage survival of *E. coli* O157:H7 was shown to be greater at pH 4.0 and 7.0 versus pH 5.0 and the MSG content did not enhance cold storage survival (Campbell, Bang, Isonhood, Gerard, & Drake, 2004). *Staphylococcus aureus* is regarded as the most halotolerant, non-halophilic eubacterium capable of growing at a_w values as low as 0.86 (3.5 M NaCl). It is a common cause of food poisoning in the West and growth studies have shown that exogenously supplied taurine, proline, choline and betaine are osmoprotectants of *S. aureus.* Of these, betaine is the most efficient osmoprotectants in *S. aureus* (Graham & Wilkinson, 1992). It is found in high concentrations in sugar beets and other foods of plant origin (Rhodes & Hanson, 1993) and thus will not normally be found in meat products. *Staphylococcus aureus* is recognized as an indicator of deficient hygiene in food processing and is a major cause of gastroenteritis worldwide (Soriano, Font, Moltó, & Mañes, 2002).

Salt has been shown to suppress the growth of most anaerobes when > 3.5% NaCl was used in curing whole meat pieces (Jensen, 1944). The probability of growth and toxin production by *C. botulinum* has been shown to decrease at 5% NaCl together with pH and storage temperature decrease. The use of 2% NaCl (equivalent to a_w of 0.988) in many foods had little effect on the growth from spores of nonproteolytic *C. botulinum* (Whiting & Oriente, 1997). A number of models have been created to describe the effects of combinations of environmental factors on the growth of pathogenic bacteria. The USDA (USA), Food Standards Agency (UK), Institute of Food Research (UK) and the Australian Food Safety Centre of Excellence have collaborated to create ComBase as an example. Large amounts of data on the effects of factors such as sodium chloride concentrations on the growth of pathogens and spoilage organisms have been created. ComBase as a modelling toolbox provides a quantitative method with which to predict microbial responses to changes in three or more environmental factors. Its usefulness is, however, limited due to the fact that some of the basic data is based on the growth of microbes in laboratory cultures. These models can therefore not predict exact results for particular foods, but they can provide estimates of interactions among various factors that should be tested for in actual foods (Doyle, 2008).

An understanding of the processes underlying osmotic adaptation of pathogenic microorganisms is crucial to designing new ways for controlling both food spoilage and pathogenic microorganisms. Internal osmotic pressure in bacterial cells is higher than that of the surrounding medium. This results in pressure exerted outwards on the cell wall, called turgor pressure, which is thought to provide the mechanical force necessary for cell elongation (Csonka, 1989). Despite variations in the osmotic pressure of the surrounding medium, bacterial cells need to maintain turgor pressure. Two

types of osmotic shock have been reported. The first type, hypo-osmotic shock generally results in only minor changes in cell volume. The second type, hyperosmotic shock causes major shrinkage of the cytoplasmic volume. After an extended lag phase, if the osmotic shock is not severe enough, the cytoplasmic volume will increase due to osmotic adjustments made by the cells (Csonka, 1989) and survivability will most probably be guaranteed.

A number of microorganisms are capable of adapting to elevated salt levels in foods. Compounds such as potassium, amino acids or sugars can be accumulated to prevent a large influx of sodium and the resultant outflow of water from the cells. Other strategies may include increased activity of sodium efflux systems, changes in cell morphology and membrane fatty acids (Christian, 2000; Lado & Yousef, 2007) and the production of specific stress proteins that supports survival (Cheville, Arnold, Buchrieser, Cheng, & Kaspar, 1996; Duché et al., 2002). Some microorganisms can survive very high salt concentrations even when they cannot grow or multiply. Although *Salmonella* and *E. coli* require a a_w of at least 0.95 for their growth, they can survive at a a_w of 0.85, even after 8 days (Wijnker, Koop, & Lipman, 2006).

Listeria monocytogenes, known to be salt-tolerant, has been shown to remain viable for 259 days at 4 °C in commercial cheese brine containing 23.8% NaCl with an estimated a_w of 0.80 (Larson, Johnson, & Nelson, 1999). *Listeria monocytogenes* in meat products is effectively destroyed by pasteurization, but can become a problem when product recontamination occurs during slicing and packaging. This microorganism can often be isolated from vacuum-packaged sliced meat products (Schmidt & Kaya, 1990). Exposing *L. monocytogenes* to a number of additives in low concentrations that act as hurdles is preferable over using less additives at higher concentrations (i.e. salt). Using this method, products may be secured without affecting sensory and technological properties negatively (Juncher et al., 2000).

Lactic acid bacteria is known to be a group of major spoilage bacteria of vacuum-packaged, emulsion-type sausages and other processed meats stored at refrigeration temperatures (Borch, Nerbrink, & Svensson, 1988; Von Holy, Cloete, & Holzapfel, 1991). They may be effectively destroyed during initial pasteurization with problems arising after secondary handling and packaging (Dykes, Cloete, & Von Holy, 1991), although thermotolerance has also been reported (Franz & Holy, 1996; Pérez-Chabela, Totosaus, & Guerrero, 2008). Contributing to their difficulty to being eliminated is the fact that this group of bacteria are psychrotrophic, micro-aerophilic and resistant to nitrite, salt and smoke (Egan 1983; Dodds & Collins-Thompson, 1984; Franz, Dykes, & Von Holy, 1991). In-package pasteurization is regarded as a feasible control measure to extend the shelf life of vacuum-packaged processed meats (Bell, 1983; Von Holy, Meissner, & Holzapfel,

1991) and has been shown to increase the shelf life of vacuum-packaged vienna sausages four-fold (Von Holy et al., 1991). A research group from South Africa reported that the exudate of in-package pasteurized, vacuum-packaged vienna sausages had a less severe "off" smell, even at counts of $1x10^8$ CFU/g and was less milky in appearance overall (Franz & Von Holy, 1994).

Evaluating the shelf-life of hotdog sausage, bacon, ham and salami containing either product specific typical salt contents, 20-25% reduced salt or 45-50% reduced salt, Aaslyng, Vestergaard, and Koch (2014) reported the following results. Reducing the salt content of hotdog sausage from 2.2% to 1.7% (w/w) and ham from 2.3% to 1.3% (w/w) had no effect on the shelf-life of these two products. On the other hand, bacon and salami had shelf-lives negatively affected by even mild (20-25%) salt reduction from 2.8% to 2.1% (w/w) for bacon and 3% to 2.25% (w/w) for salami. The preservation and shelf-life of processed meat should be considered as paramount when a reduction in salt content is considered. A number of studies have shown reducing NaCl levels to below those typically used, in the absence of other preservative measures, reduces the shelf-life of said meat products (Madril & Sofos, 1985; Sofos, 1983b, 1985).

2.14. The importance of sodium and the reduction thereof

The meat industry is regarded as the main user of salt in the processed foods industry (Andres, 1982; Wolf, Raper, & Rosenthal, 1983). Sodium occurs naturally in beef, pork and poultry although at levels far below that of the processed products, usually around 50 to 80 mg per 100 g of raw product (USDA, 2012). Three decades ago, in 1980 it was established that the population of a large country such as America consumed around 4.5 billion kilograms of processed meat products (Marsden, 1980). At that time most of these products contained between 1.10 and 1.30% Na, amounting to 2.75 to 3.25% NaCl (Maurer, 1983). This in turn amounted to between ~123 and ~146 million kilograms of salt. The reason for such large volumes of salt use? Salt is a key functional component in processed meat products. Its main functions include but are not limited to: together with sodium nitrite it retards the growth of *C. botulinum* (Sebranek & Bacus, 2007); is responsible for the solubilisation of muscle proteins which assists with the binding of meat, moisture and fat; acts as a seasoning or flavour agent; and acts as a preservative (Forsythe & Miller, 1980).

The physical process of extracting and solubilizing the muscle proteins which contribute to meat protein binding, fat emulsification and water holding capacity (WHC) helps to reduce cooking losses and improves the quality and texture (Sofos, 1986) by increasing tenderness and juiciness of the meat product (Desmond, 2006). The importance of WHC in meat quality is directly related to product yield (Van Laack, 1999). The addition of salt may also help to improve the technological

properties of problematic meat as in the case of pale, soft and exudative (PSE) pork. The functional properties of PSE pork are inferior to that of normal pork. Under high ionic strength conditions (through the addition of salt) in combination with the addition of polyphosphate, it was shown to improve technological properties in some regard. Cooking loss has been shown to improve although the texture was still of lower quality than that of normal pork (Torley, D'Arcy, & Trout, 2000). Salt may not be purposefully included in certain frozen meat products to primarily act as a preservative. These frozen products could, however, become microbiologically highly sensitive if allowed to thaw, and then salt levels will become a very important factor. Therefore, the reduction of salt in frozen foods should be included in future research (Taormina, 2010).

During the last three decades much research has been done on reducing the amount of Na in meat products. The taste for salt in food is specific to a defined type of food and cannot be generalized to all food types (Bertino, Beauchamp, & Engelman, 1986). One study showed that preference for higher salt use could be related to higher salt content of bread but not for potato (Shepherd et al., 1984). This means that salty taste preference is context specific. For example, if consumer acceptance of low salt meat products is to be increased, it will be necessary that all meat product manufacturers reduce the salt content of the products simultaneously and to the same levels. In the UK, cross industry Na reduction led by the Food Standards Agency (FSA) was shown to be highly effective (Dötsch et al., 2009). The Na content of processed food products can be categorized as the following example suggests: "sodium/salt free" with a maximum of 5 mg Na/100 g (or eqv. salt); "very low sodium/salt" with a maximum of 40 mg Na/100 g (or eqv. salt); "low sodium/salt" with a maximum of 120 mg Na/100 g (or eqv. salt); and, "reduced sodium/salt" with at least a 25% reduction in sodium/salt content over a similar product (European Commission, 2006).

By reducing the a_w in foods, salt acts as a critical hurdle to control the growth of both pathogens and spoilage organisms. Thus, if NaCl levels are decreased, the concentrations of other preservatives may need to be increased. Changes in ingredients and new or adapted processing techniques should also be considered. Changes in ingredients or processing techniques need to be evaluated to ensure product safety and organoleptic acceptability (Fulladosa, Serra, Gou, & Arnau, 2009). A number of strategies can be utilized to reduce the salt content of processed meat products. The use of salt substitutes is probably the most widely used strategy. Secondly, flavour enhancers which themselves have no salty taste can be used, in conjunction with salt, thereby decreasing the amount of salt needed in the product. Thirdly, the physical form of salt can be optimised to decrease the amount of salt needed (Angus, Phelps, Clegg, Narain, Den Ridder, & Kilcast, 2005). Some processed meat products such as hotdog sausage and ham have been shown to endure high salt reductions of up to 50% (w/w) without experiencing any negative effects on their sensory

properties, yield, shelf-life or safety. These products required no adaptations in their formulation or processing (Aaslyng et al., 2014). These types of results indicate that some meat products will prove to be more conducive to salt reduction than others.

The question arises whether predictions can be made on the microbiological impact of Na reduction on foods? Predictions can be made using existing published data. One possibly helpful exercise would be to correlate typical salt levels in food at different time periods with typical shelf-life expectancies and foodborne illness data (Taormina, 2010). This might, however, prove difficult to achieve in South Africa due to the lack of availability of such extensive data. The use of microbial modelling is another possible strategy, although the many possible interactions in a complex system such as a meat product may be difficult to account for.

2.15. Substitutes and replacers

Substitutes can be divided into two main groups; those containing Na that replaces NaCl as a source of Na and usually used at much lower inclusion levels. The second group consists of replacers that do not contain Na and thus does not contribute to the total Na content of the product. With regard to the substitution of NaCl with salt substitutes, it is important that these substitutes be at least in some way functional and do not compromise the safety of the product. There is great difficulty in finding an ideal substitute for NaCl, because of its unique salty taste and flavour enhancing capability (Reddy & Marth, 1991).

Consumers are accustomed to salt in processed meat products and although alternatives have become available, some consumers and even retailers may become uncomfortable with new ingredients appearing on well-known labels (Searby, 2006). Another important factor that needs to be considered by food processors is the cost of salt substitutes in relation to the cost of NaCl. Salt is very cheap, while other substitutes can incur much greater costs. It is estimated that a Na reduction of 20-30% could increase raw material costs by 5-30%, depending on the individual product (Dötsch et al., 2009). It is important for food processors to measure up the cost of a particular salt substitute against its effect on flavour, microbial safety and texture. A number of the most commonly used and researched replacers are discussed in the following section.

2.15.1 Chloride salts

Various other salts e.g. KCl, magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), ammonium chloride (NH_4Cl) and LiCl have been evaluated although each potential substitute comes with its

own drawbacks (Frodey, 1982; Bravieri, 1983). Already a number of decades ago it was concluded that the maximum levels for acceptable taste were 2.3% for NaCl, 0.75% for KCl, 0.6% for $CaCl_2$ and 0.5% for $MgCl_2$ (Wierbicki, Cahill, & Deatherage, 1957). By using a mixture of different salts, possible negative effects observed with the excessive use of a single one, may be reduced (Gimeno, Astiasarán, & Bello, 1998). The protein extractability of NaCl, KCl and LiCl has been shown to result in very similar extraction levels at 1.0 M concentration of the applied solute. With increasing concentration, KCl and LiCl both form extraction plateaus after which the amount of extractable protein gradually declines. In the case of NaCl, a continuous plateau of the same level of protein extraction is formed with increasing NaCl concentration (Munasinghe & Sakai, 2004). Lithium chloride imparts a salty taste, being only slightly sour without any bitterness. It can, however, cause nausea and becomes toxic at the inclusion level needed to act as a salt substitute (Dötsch et al., 2009). Calcium and potassium compounds also impart salty tastes, although this coincides with off-flavours described as metallic or bitter (Charlton, MacGregor, Vorster, Levitt, & Steyn, 2007; Park, Hwang, Kim, & Kim, 2009).

Potassium chloride is the most widely and successfully used salt substitute in low- or reduced salt/sodium foods (Desmond, 2006; Reddy & Marth, 1991). Frye, Hand, Calkins, and Mandigo (1986) prepared hams containing NaCl and KCl where the KCl replaced 50% of the NaCl. These hams had exceptional physical binding and acceptable sensory rankings; however, upon being fried or baked the hams had a significant off-flavour. Vacuum packaged, pasteurized pork loins were produced with NaCl or a mixture of NaCl/KCl where the amount of NaCl was replaced with enough KCl to maintain the original a_w of 0.967-0.968 of the brine. Organoleptic rankings for pork loins made with either the NaCl or the NaCl/KCl brines were similar and replacement of Na with potassium had little effect on the microbial activity, regardless of the storage temperature at 2, 5 or 10°C (Nielsen & Zeuthen, 1986). A 70% replacement of NaCl with KCl has been shown to result in dry-cured bacon with decreased hardness and saltiness and increased juiciness and bitterness (Wu, Zhang, Long, Tang, Yu, Wang, et al., 2014). The same research group reported that a final substitution level of 40% NaCl by KCl did not significantly influence the proteolysis phenomena. The sensory properties and moisture content of the final product was also not adversely affected. Except for NaCl, KCl is also widely used in protein extraction (Munasinghe & Sakai, 2004). Fresh pork sausages with very high levels (75%) of NaCl replacement with KCl had no significant negative sensory rankings (Pasin, O' Mahony, York, Weitzel, Gabriel, & Zeidler, 1989). Where KCl has partially replaced NaCl in several meat products, it yielded foods with flavour rankings compatible with those made with only NaCl using consumer taste panels (Reddy & Marth, 1991). The use of KCl in replacing NaCl is reportedly more effective in strong, hot flavoured foods

(Brandsma, 2006). Although K can functionally replace Na (Marsden, 1980; Pasin et al., 1989), it does produce a bitter and astringent taste in products made with KCl. The overall acceptance of modified KCl (containing disodium ribonucleotides) is, however, limited to a replacement level of 50% w/w of NaCl when MSG is added at any level (Pasin et al., 1989).

Replacing NaCl with KCl is regarded as having a double benefit for health as a K-rich diet further reduces the blood pressure (Abernethy, 1979; Geleijnse, Kok, & Grobbee, 2003). In South Africa, the highest contribution to K dietary intake comes from cereals, although the reported dietary intake of K is far below the recommended intake of 3330 mg per day (Charlton et al., 2005; Maseko et al., 2006; National High Blood Pressure Education Program, 1997). The WHO recommends enough dietary K should be taken to keep the Na to K ratio close to 1:1 (Joint WHO/FAO Expert Consultation, 2003).

Potassium chloride has been shown to affect microbes similarly to NaCl (Reddy & Marth, 1991; Askar, Elsamahy, Shehata, & Tawfik, 1993; Guárdia et al., 2006). Experiments with *L. monocytogenes* (Boziaris, Skandamis, Anastasiadi, & Nychas, 2007) and *S. aureus* (Bidlas & Lambert, 2008), showed that KCl can directly replace NaCl at the same molar ratio whilst maintaining the same antimicrobial effects of these foodborne pathogens. These experiments were carried out using laboratory media; the efficacy of KCl in food will have to be confirmed in future studies. When KCl is applied as a Na replacer it will most probably be most successful when used in conjunction with NaCl. The salty taste of NaCl together with taste interactions such as enhanced saltiness and supressed bitterness minimizes the negative tastes associated with KCl (Keast & Hayes, 2011).

Calcium is important in terms of metabolic functions such as blood pressure, the contraction of muscles and maintaining bone density (Aarle, Bontebal, & Potjewijd, 1980). Adequate consumption of calcium is important at all life stages and fortification of food with calcium can provide a great opportunity for increased calcium intake (Gimeno, Astiasarán, & Bello, 2001). Meat products may be well positioned for calcium supplementation as they are consumed by people of all ages (Heaney & Barger-Lux, 1991). Partial replacement of NaCl with a combination of KCl and $CaCl_2$ reduced the hardness, cohesiveness, gumminess and chewiness of dry fermented sausages, although a sensory panel still regarded these products as acceptable (Gimeno, Astiasarán, & Bello, 1999).

Frye et al. (1986) reported that pork products prepared with $MgCl_2$ gave consistently lower sensory rankings compared to similar products made with NaCl. Products that have undergone partial replacement of NaCl with $CaCl_2$ and $MgCl_2$ have been found to have lower overall pH values (Terrell, Ming, Jacobs, Smith, & Carpenter, 1981; Gimeno et al., 1998) with a decrease in pH of

0.25 units having been specifically attributed to these salts (Whiting, 1987). Lithium chloride may be the closest related Na substitute available in terms of taste, being only slightly sour without any bitterness, although it can cause nausea and is toxic at the levels needed in food (Dötsch et al., 2009).

2.15.2. Monosodium glutamate, flavour enhancers and masking agents

One of the most commonly used and recommended additives for reducing the sodium content of food is monosodium glutamate (MSG) which also has its own flavour, umami, described as the fifth taste (Kilcast & Den Ridder, 2007) and contributes directly to the perceived saltiness of a food product through its own salty taste (IOM, 2010). When the total Na content of a food product is reduced, overall saltiness is maintained by the inclusion of MSG (Ball, Woodward, Beard, Shoobridge, & Ferrier, 2002). Monosodium glutamate also has potential application as a masking agent of the perceived metallic or bitter aftertastes of specifically KCl. When 50% of the NaCl included at 2.5% in fermented cooked sausages were replaced with KCl and the formulation included 0.06% MSG, consumer acceptability was indistinguishable from the control (Dos Santos, Campagnol, Morgano, & Pollonio, 2014). Consumer-perceived health risks have put the high level use of MSG under pressure, due to the occurrence of conditions such as "Chinese Restaurant Syndrome" (Lindemann, Ogiwara, & Ninomiya, 2002) and even tachycardia (Okutucu, Evranos, Karakulak, Jam, Sabanov, Fatihoglu, et al., 2012). A relatively unknown analogue to MSG is calcium diglutamate which is approved as a food additive. It contains no Na and therefor has the potential to replace MSG as a salt replacer. It might also contribute modest amounts of Ca to the diet (Ball et al., 2002).

The discovery and formulation of substances capable of blocking bitter tastes, known as "bitter blockers", have gained a lot of attention in research done over the last two decades (McGregor, 2007). There is a number of flavour enhancing and masking agents that are commercially available. Taste enhancers are another group of compounds that activates receptors in the throat and mouth and in this fashion, compensates for salt reduction. Some common types of flavour enhancers and masking agents includes yeast extracts, lactates, MSG and nucleotides (Brandsma, 2006). Yeast autolysates are commonly used in Na reduction preparations and are capable of masking the metallic flavour of KCl. The yeast extracts are sometimes blended with KCl to offer a complete solution product (Desmond, 2006). By using a two- to three-fold higher dose of a complex commercial beef flavour, the saltiness of a 30% reduced-sodium instant beef bouillon could be partially restored. A mixture of KCl and NH_4Cl with the extra flavour was found to closely match the original flavour profile (Batenburg & van der Velden, 2011).

Pasin et al. (1989) reported that the NaCl content of pork sausage patties could be reduced by 75%, using modified KCl co-crystallised with a blend of 5'-ribonucleotides (disodium inosinate, IMP, and disodium guanylate, GMP) commercially available as Ribotide. Addition of MSG decreased the acceptable level of modified KCl to 50% w/w. The perceived saltiness was greater and consumers gave the sausages higher palatability rankings when MSG was used instead of Ribotide.

Bitter blockers such as adenosine 5'-monophosphate (AMP) works by blocking the activation of the gustducin proteins found in taste receptor cells, thereby preventing taste nerve stimulation for bitter taste (McGregor, 2004). Other examples of bitter blockers are: NeutralFres® which removes the metallic, bitter taste of KCl, but maintains a taste similar to NaCl; Mag-nifique Mimic which improves the taste of low-sodium products that contain KCl; and SaltTrim™ which simultaneously blocks the unwanted tastes of KCl without sacrificing the mouthfeel of NaCl (Desmond, 2006). Bitterness can be detected by a large group of around 25 different taste receptors collectively known as TAS2Rs which can be stimulated by a wide array of compounds. Researchers studying the structure and function of TAS2Rs have recently found probenecid (FDA approved for treating gout) capable of inhibiting a subset of these taste receptors. Probenecid does not physically block interaction between bitter molecules and the primary binding site of a taste receptor. Instead, it binds elsewhere on the receptor and modulates the receptor's ability to interact with the bitter molecule (Greene, Alarcon, Thomas, Berdougo, Doranz, Breslin, et al., 2001).

2.15.3. Phosphates

Phosphates are used in meat products to increase WHC by increasing the ionic strength of meat proteins and thus in effect, improve the cooking yield. This works in much the same way as for NaCl, where negatively charged sites on meat proteins are freed up and are then able to bind more water. Phosphates and salt work in synergy and the functionality of phosphates are therefore greatly affected by the addition of salt (Ruusunen, Niemistö, & Puolanne, 2002; Ruusunen & Puolanne, 2005). Phosphates can improve the stability of meat emulsions, product binding, texture and flavour (Bertino et al., 1982). The increased usage of phosphate in meat products may be attributed to the pressure from consumer groups to reduce dietary Na levels (Kijowski & Mast, 1988).

Research from a number of groups (Puolanne & Terrell, 1983; Sofos, 1983b; Trout & Schmidt, 1984; Barbut, Maurer, & Lindsay, 1988; Puolanne, Ruusunen, & Vainionpää, 2001) have demonstrated the usefulness of phosphates in lowering the NaCl content in various meat products including a number of pork products. Sofos (1985) evaluated the effect that sodium tripolyphosphate (STPP) had on the binding of low NaCl comminuted meat products. Meat batters

were prepared with 1.1, 1.2 or 1.3% NaCl, with or without adding 0.36% STPP. At 1.1% NaCl the product had inferior binding, however, if 0.36% STPP was included in the meat batter, binding of the low salt product (1.1%) was restored. In addition, phosphates (alkaline) exhibit antioxidant activity in meat by acting as a chelator which binds iron ions that act as catalysts for oxidation. The potential increase of the meat pH and ionic strength of phosphate is also thought to contribute to this phenomenon (Trout & Schmidt, 1984).

Phosphates can contribute over a 100 mg Na per 100 g of food, although this appears to be considerably less than NaCl itself (Table 2.1). Sodium polyphosphate contains 31.24% Na compared to the 39.34% Na in NaCl and is only used at around 0.5% compared to the 2-4% usage rate of NaCl. The usage rate of phosphates is also considerably lower than that of NaCl (Ruusunen et al., 2002; Ruusunen & Puolanne, 2005). Further reductions in the Na content of meat products can be made by replacing sodium phosphate with potassium phosphate. The extent to which Na will be reduced is dependent on the type of phosphate used and the Na content of the said phosphate, usually equal to a Na content of 0.2% NaCl or more (Desmond, 2006).

Table 2.1. Typical usage, sodium content and sodium contribution of some polyphosphate salts in a final product (Doyle & Glass, 2010).

Sodium compound	Inclusion level (%)	Sodium content (%)	Contribution of Na (mg) per 100 g food
Sodium chloride	1.5 – 2	39.34	590 - 790
Acid pyrophosphate	0.35	20.72	100
Tripolyphosphate	0.35	31.24	160
Pyrophosphate	0.35	34.57	170
Hexametaphosphate	0.35	22.55	110

2.15.4. Salt blends and changes in salt crystal structure

A number of sea salt products are available on the market as alternatives to regular refined salt. Sea salt contains a number of non-sodium compounds such as calcium, magnesium and potassium salts. Some of these non-sodium compounds make up almost 60% of some types of sea salt, resulting in a significantly lower contribution of NaCl to the diet (Kilcast & Den Ridder, 2007; Pszczola, 2007). Making use of other types of salt in formulating meat products may not be as simple as just replacing regular salt. In a study by Schöne, Mnich, Jahreis, Kinast, Greiling, Kirmse, et al., (2009), mineral salt made up of 50% NaCl, 44.5% KCl and lesser amounts of calcium and magnesium carbonates and magnesium sulphate, replaced regular salt in the formulation of a number of meat products. All these product rankings lower than the control products during sensory analysis. This was contributed to the notable negative differences in odour, taste and consistency. The Na content of these products were, however, significantly lower than that of the control products.

Currently available commercial salt replacers in the food industry are given in Table 2.2. Some salt mixtures such as Pansalt® have already been successfully patented and commercialized. Pansalt® consists of salt with half of the Na replaced with KCl, magnesium sulphate and an amino acid, ι-lysine hydrochloride (Table 2.2). According to the manufacturer, the amino acid enhances the saltiness of the salt replacer and masks bitter tastes that may originate from the potassium and magnesium, whilst increasing excretion of sodium from the body (Desmond, 2006). The flavour impact of salt replacers is reportedly unnoticeable if used in the range of 25-40% of the original salt level. Increased flavour intensity of flavours such as salty, acidic or spice may increase the amount of replacers that can be used before the distinct taste of the replacer can be picked up (Price, 1997).

Table 2.2. Commercially available salt replacers (Wallis & Chapman, 2012).

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

Not only can saltiness be manipulated chemically, but physically as well. Saltiness by NaCl only comes into play when it is in solution, thus physical changes that increase the solubility of salt crystals will increase the salty taste for a set amount of salt (Buck & Barringer, 2007). It has been shown that as much as 70-90% of Na can remain in the food matrix after swallowing (Phan et al., 2008). This results in a great loss in potential saltiness with only a contribution to total Na consumption as payoff. The size of salt crystals has the ability to influence the delivery of the salty taste. The smaller the salt particle size the faster the rate of dissolution and thus, in effect the higher the perception of salt (Kilcast & Den Ridder, 2007). Another such strategy is the use of different physical forms of salt. Highly agglomerated, flat or pyramidal crystals, with low circularity dissolved faster and to a greater extent in artificial saliva (Quilaqueo, Duizer, & Aguilera, 2015). Morton and Cargill, both companies that manufacture salt products, propose the use of fine flake and dendritic salts whose crystals dissolve more rapidly due to a larger surface area. Reportedly, these types of salt products have the potential to improve water and fat binding in meat batters and

emulsions at lower salt concentrations, although these claims need to be substantiated (Desmond, 2006). It seems that currently the manipulation of crystal morphology and size is mostly limited to application in dry foods such as potato chips, where it is applied to the surface. The possibility exists that the same form of application can be attempted for use on intermediate moisture or dry meat products such as jerky or biltong where the crystals will remain undissolved until contact with saliva is made.

2.15.5. Sodium salts of organic acids

Sorbates and benzoates are approved for use in many food applications, especially for their ability to inhibit yeasts and moulds. They have also shown to be effective against important bacteria such as *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. (Chipley, 2005; Stopforth, Sofos, & Busta, 2005). Sodium salts of organic acids are added to food to act against microbial growth and to improve texture (Doyle & Glass, 2010). Sodium lactate in conjunction with NaCl has been shown to inhibit lactic acid bacteria, *L. monocytogenes,* clostridial pathogens and spoilage bacteria (Meyer, Cerveny, & Luchansky, 2003). Lower molar concentrations of sodium lactate may inhibit the growth of various food spoilage microorganisms and pathogens better when compared to lower molar concentrations of NaCl (Houtsma, Kant-Muermans, Rombouts, & Zwietering, 1996). Benzoate, diacetate, propionate and sorbate have been shown to inhibit *L. monocytogenes* in both cured and uncured meat products (Glass, McDonnell, Rassel, & Zierke, 2007a; Glass, Preston, & Veesenmeyer, 2007b). The inherent flavours that these preservatives possess may limit their use in certain products or above certain concentrations. Due to the fact that these compounds also contain Na, their contribution to the total Na content of the final product needs to be taken into account, just as for phosphates. Especially sodium lactate with a high inclusion level (1.5% to 3%) contributes Na levels (310 to 620 mg Na/100 g food) comparable to that of NaCl (Table 2.3).

Table 2.3. Typical usage, sodium content and sodium contribution of some organic salts in the final product (Doyle & Glass, 2010).

Sodium compound	Inclusion level (%)	Sodium content (%)	Contribution of Na (mg) per 100 g food
Sodium chloride	1.5 - 2	39.34	590 - 790
Sodium benzoate	0.1	15.95	16
Sodium diacetate	0.1 – 0.4	16.18	16 – 65
Sodium lactate	1.5 – 3	20.51	310 – 620
Sodium propionate	0.3	23.93	70
Sodium sorbate	0.3	17.14	50

2.16. Stepwise reduction of sodium chloride in foods

A current technique used to gradually reduce salt in processed foods over an extended period of time without consumers being able to detect this, is known as reduction by stealth (Liem, 2011). This technique is suggested to render consumers incapable of detecting changes in the organoleptic qualities of products, whilst reducing the salt content and lowering consumers' sensitivity to the saltiness of a given product (Wallis & Chapman, 2012). The use of small stepwise reductions of between 5% and 10% of NaCl levels in foods are reportedly not noticeable by consumers if carried out over time. A reduction in the salt content of a diet over a couple of months has been shown to increase the preference for less salt in food (Bertino et al., 1982). This strategy has been applied successfully in: (1) a 33% reduction in salt content of cereals over 7 years; (2) a 33% reduction in Na content of processed cheese; (3) and reformulation of various products under the Heinz brand allowed for a 11% to 18% reduction in Na content (Kilcast & Den Ridder, 2007). Reduction of salt levels by stealth delivers positive results. However, there is a limit to the amount of salt that can be removed from a given product, without making it less appealing or unpalatable to consumers (Beauchamp, 2009).

2.17. Modified processing

Techniques used for the preservation of food are becoming milder as consumers demand higher quality, more convenient, less heavily processed, less heavily preserved (e.g., less acid, salt and sugar) and less reliant on preservatives such as sulphite and nitrite (Abee & Wouters, 1999). Monahan and Troy (1997) suggested that research should also be focused on the meat system itself and that new methods need to be investigated to enhance the functionality of the meat system with regard to formulations lower in salt content.

2.17.1. Emulsion-coating

Emulsion-coating has been demonstrated to reduce the salt level needed in chunked and formed ham. The emulsion coating consisted of uncured ham, salt, phosphate blend, glucose, sodium erythorbate, sodium nitrite and ice which were then chopped to form the emulsion. This emulsion was then mixed by weight with the chunked, formed and tumbled meat and then conventionally processed further and cooked. Three types of emulsion-coated hams containing 0.62, 1.07 or 1.97% NaCl were prepared and compared to nonemulsion-coated hams containing 0.50, 1.00 and 2.00% NaCl. After cooking, the emulsion-coated hams exhibited increases in cooking yield, breaking force and moisture retention when compared to controls without emulsion-coating. Sensory evaluation of

the products revealed that the 1.07% NaCl emulsion-coated product compared well with the 2.00% NaCl product without an emulsion-coating. This indicated a possible reduction in NaCl level using emulsion-coating (Thiel, Bechtel, McKeith, Novakofski, & Carr, 1986).

2.17.2. Application of pork fat diacylglycerols

The physical properties of the fat fraction are crucial in the formation of stable meat emulsions as fat stabilizes the solubilized protein gel and helps to prevent shrinkage of the protein matrix during cooking by acting as filler (Lurueña-Martinez, Vivar-Quintana, & Revilla, 2004). The technological quality of fat is determined by: the fatty acid composition; the position of fatty acids in the glycerol backbone; the melting point; and crystallization behaviour (Davenel, Riaublanc, Marchal, & Gandemer, 1999). The physical and chemical properties of fats and lipids can be manipulated by enzymatic modification e.g., the formations of partial acylglycerols (Rønne, Yang, Mu, Jacobsen, & Xu, 2005; Vu, Park, Lee, Kim, Nam, Akoh, & Lee, 2007). Thereby enzymatic modification of pork fat creates new ways to affect technological quality (Miklos, Xu, & Lametsch, 2011). Diacylglycerols (DAGs) have higher melting points compared to their corresponding triacylglycerols (TAGs), possibly serving as a new method to overcome problems with texture. A hydrophilic polar group in the molecular structure and surface activity enables DAGs to form emulsions and retain water (Nakajima, 2004). As WHC in meat products is normally increased by the addition of salt, a stronger water retention caused by DAGs can be speculated to reduce the amount of salt needed (Miklos et al., 2011). Cooking oils containing DAGs are already available on Japanese and American markets and have been awarded GRAS status by the FDA (Morita & Soni, 2009).

2.17.3 Pre-rigor meat

Pre-rigor meat is known to have superior functionality in terms of the extractability of myofibrillar proteins, binding and WHC. Patties made from pre-rigor ground beef had higher pH values, greater protein solubility, firmer, more cohesive, and chewier texture, and significantly lower cooking losses compared to patties made from post-rigor beef (Claus & Sørheim, 2006). The use of pre-rigor meat enables a reduction in Na content, without compromising the physical, chemical and sensory properties of emulsion-type sausages (Puolanne & Terrell, 1983). The WHC and texture of emulsion-type sausages manufactured from pre-rigor pork was also reported to be better than that made from either aged pork or pre-frozen pork. These improvements were attributed to the increased extractability of muscle proteins by the addition of phosphates working in synergy with the pre-rigor state of the meat, resulting in greater emulsion capacity (Wang, Xu, & Zhou, 2009).

2.17.4. High hydrostatic pressure processing

The food industry is interested in the use of high hydrostatic pressure (HHP) for processing food because of reported quality improvements in specific food products (Abee & Wouters, 1999). Elevated pressure can be detrimental to microbial physiology and viability. The growth of microorganisms is generally inhibited at pressures in the range of 20-130 MPa, while cell death may occur at higher pressures of between 130-800 MPa; the maximum pressure that allows growth or survival depends on the microbial species and medium composition. Membranes and ribosomes have been suggested as important determinants in pressure sensitivity (Earnshaw, Appleyard, & Hurst, 1995; Patterson, Quinn, Simpson, & Gilmour, 1995; Welch, Farewell, Neidhardt, & Bartlett, 1993). To prevent the development of barotolerant strains that can survive inside commercial pressurization equipment, a combination of pressure and another processing technology such as exposure to mild heat is suggested. This could prevent a possible threat to the safety and stability of pressure-processed foods (Abee & Wouters, 1999).

The organoleptic properties of meat products seems to be largely unaffected by HHP. A comparison of consumer hedonic rankings of ready-to-eat (RTE) meat products subjected to 600 MPa at 20°C for 180 s to those of control products revealed no difference in consumer acceptability (Hayman, Baxter, O'Riorda, & Steward, 2004). Colour changes are, however, possible in fresh meat and poultry, the colour may change due to changes in myoglobin, heme displacement or the oxidation of iron-containing compounds. These colour changes may give the meat a cooked appearance (Hugas, Garriga, & Monfirt, 2002; Ma & Ledward, 2013) without any actual heat processing, possibly creating the false impression that the product may necessarily be safe for consumption. The difficulty in upscaling HPP to processing economically viable volumes of food products and the overall high costs associated with the technology, has limited it mostly to the realm of academic interest (Ma & Ledward, 2013).

2.17.5. Water-in-oil-in-water encapsulation of salt in emulsions

Emulsions are used in many modern day foods as either water in oil (W/O) or oil in water (O/W) emulsions. The former consists of an aqueous phase dispersed as minute droplets within a continuous oil phase and the latter is the reverse of this, where there is an oil phase dispersed as minute droplets within a continuous water phase. A newer, lesser known emulsion is the water-in-oil-in-water (WOW) emulsion which is made up of three different phases. The first phase is very small water droplets surrounded by a second phase of larger oil droplets which in turn is surrounded by a third and continuous aqueous phase (Frasch-Melnik, Norton, & Spyropolous, 2010). The water

phase within the oil phase has potential to act as a carrier for flavours or active ingredients (Dieroff, 2011; Sapei, Naqvi, & Rousseau, 2011).

The potential of including an ingredient such as salt lies in the possibility that it may lead to significant reductions in salt usage. The salt will be dispersed in the water phases, thus a stronger salt perception can be created throughout the products. Another possible benefit for salt reduction lies in the ability of WOW emulsions to control the release of encapsulated ingredients, allowing for bursts of flavour in the mouth of the consumer (Frasch-Melnik et al., 2010).

The use of WOW emulsions will not be without its own set of challenges: the emulsions are difficult to prepare; thermodynamically unstable; and thus requires complex processing techniques and possibly the addition of surfactants (Dieroff, 2011). Osmotic pressure may also challenge the stability of a WOW emulsion. If the inner aqueous phase has a lower osmotic pressure than the outer continuous aqueous phase, water may migrate to the outer aqueous phase leading to the breakdown of the emulsion. This problem may be circumvented if both aqueous phases carry salt in quantities that create osmotic pressures close to one another on both sides of the oil phase (Wallis & Chapman, 2012). Frasch-Melnik (2010) reported that NaCl could be replaced successfully with KCl, with the added bonus of the oil phase having the ability to mask the bitter after taste of KCl. Using NaCl in the outer aqueous phase and KCl in the inner aqueous phase also increased the perceived saltiness, even if the NaCl inclusion level was decreased.

2.18. Positive effects of sodium reduction on meat products

In terms of other effects Na reduction may have on processed meat products, unintentional secondary effects of a positive nature may be introduced. It has been shown that at certain inclusion levels, salt exhibits prooxidative effects on various meat types. Oxidation reactions in food cause damage to lipids and proteins which negatively affects food quality (Decker, 1998). Examples of which include lipid oxidation in both raw and cooked meat, acceleration of metmyoglobin formation, and discoloration in raw meat (Rhee, 1999). The addition of NaCl at 0.5-2.5% in ground beef and ground chicken has been reported to be prooxidative after thiobarbituric acid reactive substances (TBARS) analysis was carried out (Rhee & Ziprin, 2001). Data has shown that NaCl may actually accelerate the development of rancidity in ground pork compared to when it is replaced with KCl or $MgCl_2$. The results revealed that NaCl and $MgCl_2$ increased rancidity of both raw and cooked products regardless of temperature, whereas the KCl treated products had the lowest level of rancidity (Rhee, Smith, & Terrell, 1983; Rhee, Terrell, Quintanilla, & Vanderzant,

1983). More recently, the replacement of 50% NaCl with KCl again proved to effectively lower the TBARS value of fresh pork patties (Cheng, Wang, & Ockerman, 2007).

This decrease in lipid oxidation in ground pork by replacement of NaCl with other salts has been demonstrated in a few other studies (Hernández, Park, & Rhee, 2002; Zanardi, Ghidini, Conter, & Ianieri, 2010). One group reported no effect of different salt types (NaCl, KCl and $MgCl_2$) used in different combinations on the TBARS of a fresh sausage despite the different pro-oxidative abilities of each salt type (Triki, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2013b). It is thought that non-meat additives such as salt compromise the antioxidant potential of endogenous enzymes such as catalase and glutathione peroxidase (Rhee et al., 1983; Rhee & Ziprin, 2001). Pork has distinctly higher catalase activity and stability compared to beef and chicken (Pradhan, Rhee, & Hernández, 2000; Hernández et al., 2002) and is believed to be resistant to lipid oxidation when stored raw (Rhee & Ziprin, 1987). Differences in lipid oxidation rates between products containing salt of different compositions may also disappear or become insignificant due to other additives. The presence of antioxidants in spices, the use of sodium metabisulphite and the refrigeration conditions to which all the product formulations may be simultaneously subjected to, may collectively exhibit such an effect (Ruiz-Capillas & Jiménez-Colmenero, 2009; Triki, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2013a).

During storage, meat colour is negatively affected by the presence of NaCl (Andersen, Bertelsen, & Skibsted, 1990; Trout, 1990). Heme pigment concentration has also been found to decrease with increasing NaCl concentration (Sakata & Nagata, 1992). Low salt content may also limit the formation of certain peroxides in fresh, non-heat treated products. Lipoxygenase, a non-heme containing dioxygenase inherently present in meat, is responsible for the catalysis of certain polyunsaturated fatty acids to conjugated unsaturated fatty acid hydroperoxides. The enzyme showed peak activity, at 3% w/w salt content, close to the general salt content of processed meat products. In the presence of a salt content of $\leq$ 1% w/w, the enzyme exhibited very low activity and heat-treatment of > 39 °C severely decreased activity (Jin, Zhang, Yu, Lei, & Wang, 2011).

By intentionally meeting specific food claims criteria, companies may place themselves in a position to make their products more attractive to consumers reviewing the nutritional facts panel on the product label (Appel, Angell, Cobb, Limper, Nelson, Samet, & Brownson, 2012). Low Na or reduced Na content as claims can be used as a marketing advantage although this will depend on consumer awareness and understanding of the health benefits of low or reduced Na foods. Another possibility for increasing the marketability of Na reduced processed meat products lies with realigning these products as functional meat products. It has been suggested that by reducing

unhealthy constituents, such as NaCl, and simultaneously adding bioactive ingredients, health claims can be made. These claims could in turn open up new markets for the processed meat industry (Grasso, Brunton, Lyng, Lalor, & Monahan, 2014).

When bioactives are added during processing instead of to the animal's diet, a wider range of bioactives can be delivered in the final product with better control over quantities and costs. An example of such a potential product is where 50% of the NaCl of a fresh beef sausage was replaced by a mixture of KCl, $CaCl_2$ and $MgCl_2$ salts. Overall, the Na content was reduced by 50% without any negative influence on the sensory aspects. 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 could possibly be used in nutritional claims. Sodium content decreased by 40%, K and Ca content increased 285.71% and 213.33% respectively when NaCl was replaced by a salt mixture consisting of NaCl, KCl and $CaCl_2$. These changes could be considered as a "nutritional advantage" (Gimeno et al., 1999). In an earlier study, a similar product was created using the same salts in a mixture to create a product with "nutritional benefits in relation to its mineral content". Lower sensory acceptability and decreased colour intensity was the only reported negative results of the reformulation (Gimeno et al., 1998).

South African biltong is a meat product normally produced from antelope or cattle through salting, curing and drying of the strips of meat. Salt is the main preservative and microorganisms have been shown to play a role in the development of taste, flavour and aroma (Prior, 1984). Biltong can be divided into two main groups based on consumer preference: dry or moist biltong. Dry biltong with a moisture content of 21.5-25.3 g/100 g and moist biltong with a moisture content of 35.1-42.8 g/100 g were found to contain on average 6.8 g/100 g and 4.8 g/100 g salt, respectively (Petit, Caro, Petit, Santchurn, & Collignan, 2014). Similar increases in the salt content of other heavily dried products such as salami can also be expected. An example of which is salami manufactured with a moisture content of 61.9 g/100 g and a salt content of 2.8 g/100 g (formulated as 3% w/w) and then ripened traditionally or commercially. After 90 days the moisture content and salt content of the traditionally ripened salami was 26.8 g/100 g and 5.9 g/100 g, respectively. Similarly, the moisture content of the commercially ripened salami decreased to 29.8 g/100 g and the salt content increased to 6.0 g/100 g (Moretti, Madonia, Diaferia, Mentasti, Paleari, Panseri, et al., 2004). It could therefore be advantageous to make use of replacers or alternative forms of preservation to effect a decrease in total Na content of these types of products.

2.19. Conclusions

Salt, and by association Na, has been an important additive in meat and meat products for millennia. The immense preservative power of salt has been used by ancient civilizations without any understanding of the underlying mechanics. In all probability salt will continue to be used in the foreseeable future. Humans have a specific taste for salt due to the physiological need for salt. Technologically it is one of the most effective, cheapest and most abundant additives available with which meat and its products can be manipulated. The abuse of this great usefulness, especially in recent human history, has become its greatest weakness and led to a re-evaluation of its use in food. The overuse and overconsumption of salt has had, and continue to have, serious long term detrimental effects on the health of not only individuals, but populations as a whole.

In recent decades, research has been on finding suitable analogues with which to replace salt completely, or more recently with which to offset its use. A number of possible analogues exist, although none has been found to match the functionality of salt. Most analogues have their own limits of safe use, some at even lower levels than that of salt. Some analogues are derived from complex compounds and even modified organic molecules that incur much greater costs in production than that of salt, resulting in price increases that eventually filter through to the consumer. Most probably, a few of these analogues in combination with each other, and even in combination with salt will give the best results. A less studied option gaining attraction over the last two decades involves the use of alternative processing techniques and changes to processing parameters to limit the reliance on the functions of salt. A third and more overlooked option, is the use of sizeable reductions in salt content to levels that do not compromise product safety and stability or consumer acceptability. It is safe to assume that many product formulations have remained unchanged in terms of the salt content. In recent history emphasis has been placed on making production and handling conditions more hygienic, improving the cold chain and a myriad of packaging innovations have taken place. The technological role that salt has to play, may no longer be enough to justify the large quantities of salt still being added to products.

Salt and Na reduction may also present opportunities to improve the overall image of processed meat products in terms of their nutritional value. Replacing some of the Na with compounds contributing even moderate amounts of other essential minerals such as potassium can go a long way to improve the overall nutrition of such products. The re-evaluation of processing techniques and formulations may even lead to the creation of new and exciting products to keep the attention of consumers.

CHAPTER 3

A SURVEY ON THE SODIUM CONTENT OF SOUTH AFRICAN PROCESSED MEAT PRODUCTS

ABSTRACT

In an effort to improve public health, South African regulations will come into effect to limit the amount of sodium allowed in a large group of processed foods. Processed meat products are known to contribute the second largest amount of sodium to the diet and will also be required to comply with specific limitations in total sodium content. No information is available to assess the impact that the regulations will have on these products and to what extent these products will need to be adapted. Through the use of a survey, a master list of locally available products was compiled and information on sodium and salt content were gathered. A representative subset of products were bought and analysed for sodium content. Comparisons were drawn between the actual and reported sodium content to determine the presence and extent of deviations. Actual sodium content of a further subset was then compared to class-specific regulatory limits to determine if products in their current state exceed these limits. Product labels generally overvalued and to a lesser extent, undervalued the actual sodium content of the products evaluated in this study. A few years before the regulations come into effect, a number of products were already compliant with at least the initial limits. How seamlessly processors will be able to adapt their products to the final limits, and what sodium reduction strategies will be applied, remain unclear.

Keywords: labelling; sodium legislation; sodium content; consumer education; commercial meat products

3.1. Introduction

Consumers, now more than ever before, are aware of the relationship between Na intake and hypertension (Dickinson & Havas, 2007; Doyle & Glass, 2010). In terms of the human diet it is accepted that table salt, i.e. sodium chloride (NaCl), is the main source of Na (Ruusunen & Puolanne, 2005). As many as one in four adults worldwide suffer from hypertension (Kearney et al., 2005) and the average South African urinary Na excretion was in excess of 2400 mg/day as reported by Charlton et al. (2005). In sharp contrast to these findings the physiological need for Na was approximately 90 mg/day. The latest recommendation by the WHO is that no more than 2000

mg Na/day should be consumed (WHO, 2007). Other findings by Charlton et al. (2005) revealed that: bread contributed up to 48% of all the Na in the South African diet, followed by meat and processed meat products at 10-20%, and that total salt intake ranged from 7800-9500 mg/day across racial groups. With the large amount of related deaths estimated to be preventable (Gaziano et al., 2005) and the massive amount of money estimated to be saveable (Bertram et al., 2012) it became clear that the government needed to take a proactive approach.

In what was a first for the country, the South African Government, through the Department of Health (DoH) published a draft of regulations on 11 July 2012 limiting the use of Na in certain food products (Department of Health, 2012). Initial target limits ranging from 800-850 mg/100 g Na content were proposed across all meat product categories as set out in SANS 885 (South African National Standard 885, 2011). The standard specifies the handling, preparation, processing, packaging, refrigeration, freezing, chilling and storage of processed meat products. The standard also includes microbiological and compositional requirements for these products. The proposed limits were to come into effect in two phases: an initial reduction by 30 June 2016 and a final reduction by 30 June 2018 (Table 3.1). A revised draft of the regulations was released on 20 March 2013. It contains three categories of meat products: processed meat products such as viennas, polonies and reformed hams (classes 6, 12 or 14), processed meat products such as bangers, blanched pork sausages and schnitzels (classes 7, 10, or 11) and raw-processed meat sausages. There are increased Na limits for category 8 (consisting of classes 7, 10, and 11) and a timeline that was extended to 30 June 2019 (Department of Health, 2013). Under the regulations relating to the labelling and advertising of foodstuffs, a tolerance for no more than 20% in excess of the target Na value will be allowed and values should be verified once every ten years unless reformulations occur which necessitates re-analysis (Department of Health, 2014).

Near the end of 2015, an official amendment was proposed by the meat industry to be called, “The Regulations Relating to the Reduction of Sodium in Certain Foodstuffs and Related matters, R.214 of 20 March 2013: Amendment.” This document proposed that Categories 7 and 8 be listed correctly as cured and uncured respectively. Changes to the Na limits of Categories 7 and 8 were also proposed with that of Category 7 to increase substantially from 850 mg and 650 mg to 1300 mg and 1150 mg, while the Na limits of Category 8 were proposed to receive further reductions from 950 mg and 850 mg down to 850 mg and 650 mg. The initial limit for Category 9 was proposed to remain the same (800 mg/100 g) while that of the second limit was proposed to increase by a 100 mg to 700 mg/100 g. This amendment has not appeared for comment on the Department of Health’s website and there were no formal notices of its existence at the time of finalizing this thesis. In light of this, the regulations in the draft of 2013 were followed and used as

reference throughout this thesis. In order to anticipate how great an effect the Na limit regulations would have on current formulations of commonly available processed meat products, it was necessary to gather information on product labelling with regard to NaCl and Na content. It was also important that a number of products be sampled and analysed for their actual Na content. With this information at hand it would then be possible to establish how much the actual Na content of products deviated from their labelling. It would also be possible to establish how much current product formulations deviated from the future Na limits and to what extent these formulations would need to be adapted. In literature, Na is commonly decreased in formulations with a number of replacers added to make up the difference, although it is not clear if the need for replacement is great enough in general applications.

Table 3.1. Development of the proposed sodium regulations related to processed meat products from 2012 to 2016. (Adapted from: Anonymous, 2015; Department of Health, 2012, 2013).

Category	Foodstuff class	Maximum Total Sodium per 100 g foodstuff	Date on which the total Sodium reduction becomes effective
First draft: Regulation R533			*11 July 2012*
8.	Processed meats (as defined)	850 mg Na	30 June 2016
		600 mg Na	30 June 2018
9.	Raw-processed meat sausages (all types)	800 mg Na	30 June 2016
		600 mg Na	30 June 2018
Second draft: Regulation R214			*20 March 2013*
7.	Processed meat (classes 6, 12 or 14 of the SANS 885:2011) - uncured	850 mg Na	30 June 2016
		650 mg Na	30 June 2019
8.	Processed meat (classes 7, 10, or 11 of the SANS 885:2011) - cured	950 mg Na	30 June 2016
		850 mg Na	30 June 2019
9.	Raw-processed meat sausages (all types) and similar products	800 mg Na	30 June 2016
		600 mg	30 June 2019
Proposed further amendments			*November 2015*
7.	Processed meat (classes 6, 12 or 14 of the SANS 885:2011) - cured	1300 mg Na	30 June 2016
		1150 mg Na	30 June 2019
8.	Processed meat (classes 7, 10, or 11 of the SANS 885:2011) - uncured	850 mg Na	30 June 2016
		650 mg Na	30 June 2019
9.	Raw-processed meat sausages (all types) and similar products	800 mg Na	30 June 2016
		700 mg Na	30 June 2019

3.2. Materials and methods

3.2.1. Product selection methodology

Seven retailers in Bloemfontein city (South Africa) were identified and selected on the basis of being representatives of all the national retailers in South Africa. These retailers were visited at random and a master list of as many possible unique processed meat products were compiled. The whole process of identifying and adding products to the master list took place over the course of one month. The list was in no way a complete representation of all the processed meat products

available as it was compiled in one geographical location and products were excluded that were indicated on shelves but found to be unavailable at any of the retailers over the surveying period. The list of products included canned, ambient storage, fresh (shelf-life of less than 9 days), chilled (shelf-life of weeks or months) and frozen products. On the master list the following data for each product was recorded: brand name; product name; size; salt and or sodium content if available.

Product labels that provided no salt or Na content information were recorded with "not indicated". The resulting master list consisted of 497 products containing beef, chicken, pork and turkey meat and excluded any fish and seafood products. Chicken and turkey were included as many of the comminuted-type products contain variable amounts of this meat used in conjunction with mostly pork, except for Halaal certified products. The list was then organized by arranging each product into the most fitting product category from 1.1 to 15.1 (Table 3.2) according to the South African National Standard 885 (SANS, 2011) which describes regulations on the handling, preparation, processing and composition of processed meat products. Categorizing products into specific subclasses were problematic in some cases. A number of subclasses appeared to be very closely related and their existence could not be justified. It would have been helpful if emphasis on the uniqueness of each subclass and differentiators from other subclasses were indicated. The existence of Class 14 as "Unspecified class" with "Unspecified" as an example of a product was found to be especially puzzling. This class will be directly affected by the proposed regulations (Department of Health, 2013), although anyone perusing the regulations or SANS 885 Standard would be unable to determine which products, if any, falls within this class.

The products within a specific category, and where relevant, subcategory, were listed and assigned consecutive numbers from one onwards. If a category/subcategory contained more than 15 products, a total of 15 were randomly selected using the random number generator add-in for Microsoft Excel 2010. If there were 15 or less products, all the products were included in the final list. This amounted to 238 products across all categories that were then regarded as being random representatives across all the processed meat product types. The products were bought in batches from any retailer where it was available, except for products that were exclusively sold at certain retailers, such as in-house brands and small volume manufacturers that only supply specific retailers.

The bought products were placed in cooler boxes packed with refreezable ice packs and transported back to the meat processing facility at the University of the Free State where they were individually minced through a small household electric mincer (LOGIK, Model RSH-011765-018) fitted with a

3 mm mincing plate. The resulting mince was thoroughly mixed and filled into pre-numbered, tight-lidded plastic cuvettes (30 mL volume) and stored frozen at -18 °C until analyses were carried out.

Table 3.2. Classification of South African processed meat products according to SANS 885 (2011).

Class	Subclass	Description	Examples*
1.	1.1	Whole muscle, cured, heat treated	Country ham, pastrami, cooked silverside, gammon
	1.2	Whole muscle, dry cured, heat treated	
2.	2.1	Whole muscle, uncured, heat treated or partial heat treated	Carpaccio, roast beef (uncured)
3.	3.1	Whole muscle, uncured, no or partial heat treated and air dried products	Uncured biltong
	3.2	Whole muscle uncured, no or partial heat treated and air dried products undergoing lengthy maturation period (min 21 days)	"Nitrite-free" dried ham
4.	4.1	Whole muscle, dry cured, no, or partial heat treatment	Jerky
	4.2	Whole muscle, cured, no, or partial heat treated	Bacon
5.	5.1	Whole muscle, cured, no or partial heat treated and air dried products	Cured biltong
	5.2	Whole muscle, dry cured, no or partial heat treated and dried products	Prosciutto crudo
6.	6.1	Comminuted, cured, heat treated	Polony
	6.2	Liver spreads, pate and terrines	Liver paste
	6.3	Products in aspic	Brawn
	6.4	Products in aspic: Suelze, other products containing cured meat pieces in aspic	Sülze
	6.5	Products made from blood	Blood sausage
7.	7.1	Comminuted, uncured, no or partial heat treated products	Bangers
8.	8.1	Comminuted, uncured, no or partial heat treated and dried products	Droë wors
9.	9.1	Comminuted, cured, no or partial heat treated, dried and fermented products	Salami
10.	10.1	Comminuted, uncured and heat treated products	Uncured Chicken viennas
11.	11.1	Reformed, uncured, no or partial heat treated products	Schnitzels
12.	12.1	Reformed, cured, heat treated from single species	Reformed hams
	12.2	Reformed, cured, heat treated from mixed species	Luncheon loaves
13.	13.1	Reformed, cured, no or partial heat treated products	Reformed bacon
14.	14.1	Unspecified class	Unspecified
15.	15.1	Coated products – Maximum coating of 50% allowed, meat component in accordance with Class 1 to 14	Chicken nuggets

* Examples of products limited in SANS 885, adapted to include examples for each subclass.

3.2.2. Sodium content determination

Sodium content was determined by atomic absorption spectroscopy (AAS) using a Varian SpectrAA-300 Atomic Absorption Spectrometer (SMM Instruments, Johannesburg, South Africa) with GTA-96 graphite tube atomizer furnace fitted with a 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. A stock NaCl solution was prepared by weighing of 0.501 g (accurate to 0.001 g) NaCl, transferring it to a 200 mL volumetric flask, dissolving it in distilled H_2O, dilution to the mark and thorough mixing. Two hundred and fifty millilitres distilled water was added to six dry 500 mL beakers and then 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mL of the stock solution was added and mixed. These further dilutions served as standards in calibrating the AAS

before sample measurements were taken. Sample absorbance values were captured and processed using the Varian SpectrAA-300/400 Plus software package. For AAS analysis the samples were reduced to a mineralized form free from any organic compounds by dry ashing according to a modified method by Nielsen (2010). A sample was first thawed over night at 4°C and 10 g was weighed off into a porcelain crucible (ø = 30 mm). Up to 10 samples at a time 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 crucible was removed, cooled to room temperature and enough 55% nitric acid (HNO_3) was added to wet the sample. The crucible was then returned to the furnace for 1 h 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. Ten millilitres of a 1:2 HNO_3:H_2O solution was added to the crucible and this was placed on a heated sand bath for 5 min until bubbles started to form and all crystals were dissolved. The sample was removed from the sand bath, cooled to room temperature before being filtered through a Whatman® nr. 1 filter paper (Sigma-Aldrich, Johannesburg, South Africa) and into a 100 mL volumetric flask to prevent any possible contamination of the AAS with foreign bodies. The crucible and filter paper was rinsed three more times with 10 mL distilled H_2O, each time allowing the filter paper time to run dry. The volumetric flask was then made up to volume with more distilled H_2O. After carefully inverting the volumetric flask ten times, the solution was poured into a clean pop-top container, three quarters of the way. The container was closed and kept frozen at -18 °C until a day before AAS analysis when it was thawed overnight at 4 °C.

3.2.3. Calculating Na content from converted NaCl content data

For various products the labels reported NaCl content and not Na content. For these products, additional data was generated by converting NaCl values from the labels to Na values. The Na content was calculated with the formula:

Calculated Na (mg) = [(mg NaCl / 100) x 39.33]

Where 39.33 represents the rounded down percentage that Na makes up of the molar mass of NaCl taken as 58.44 g/mol, where Na = 22.99 g/mol and Cl = 35.45 g/mol. All molar masses were taken from the official IUPAC Periodic Table of the Elements (Wieser, Holden, Coplen, Böhlke, Bergland, Brand, et al., 2013).

3.3. Results and discussion

3.3.1. Na and NaCl content on product labelling

The number of product labels describing the Na and/or NaCl content in the nutritional information table on the packaging were determined (Figure 3.1). Forty three per cent of the product labels identified in the survey provided no information with regard to Na or salt content. This result may be regarded as a major omission in providing consumers with nutritional information in a form that they will be able to understand. Only 3% of the labels provided the salt content while 47% described the Na content. Although such a large group of products disseminate the relevant information, it can be assumed that very few consumers are able to understand the real implications (Gilbey & Fifield, 2006; Marshall et al., 2007) and there is a risk of some consumers regarding the term "sodium" or "sodium chloride" as unnatural chemical additives added to the products (Grunert & Wills, 2007; Scott & Worsley, 1997). In a study by Van der Merwe, Bosman, and Ellis (2014) the opinions and usefulness of food labels to urban and rural consumers were evaluated. At least 82% of consumers felt that a food label is a good source of information, although only 65% felt that it was easy to understand the information. It might therefore, be wise to include a clarification of the previously mentioned terms in future consumer education programs, and why the use of the word "sodium" rather than "sodium chloride" or "salt" is the more accurate terminology in terms of a particular food product's contribution to total Na consumption and the effect thereof on overall BP. Greater consumer education with regard to why knowing the Na content of food products is important is also needed. Consumers showed dissatisfaction when information on calories, trans fat, cholesterol, and protein were omitted from labels, although they were indifferent to the presence or absence of sodium content information (Cannoosamy, Pugo-Gunsam, & Jeewon, 2014).

The fact that such a large number of processors voluntarily provide this information may be encouraging and a good example of how an industry may self-conform to provide consumers with important nutritional information. Currently it is not yet required by law to include salt or preferably, Na values, in the nutritional information table which, in itself is also not currently mandatory on a food product label (Department of Health, 2010). An amendment to the regulations was released in 2014 for comment, which will by law require this information on all food product labels with only a few exemptions (e.g., 100% tea, coffee, and herbs) in a minimum mandatory nutritional information table (Department of Health, 2014). At the time of writing, the amendment has not yet superseded the previous regulations which came into effect in 2012.

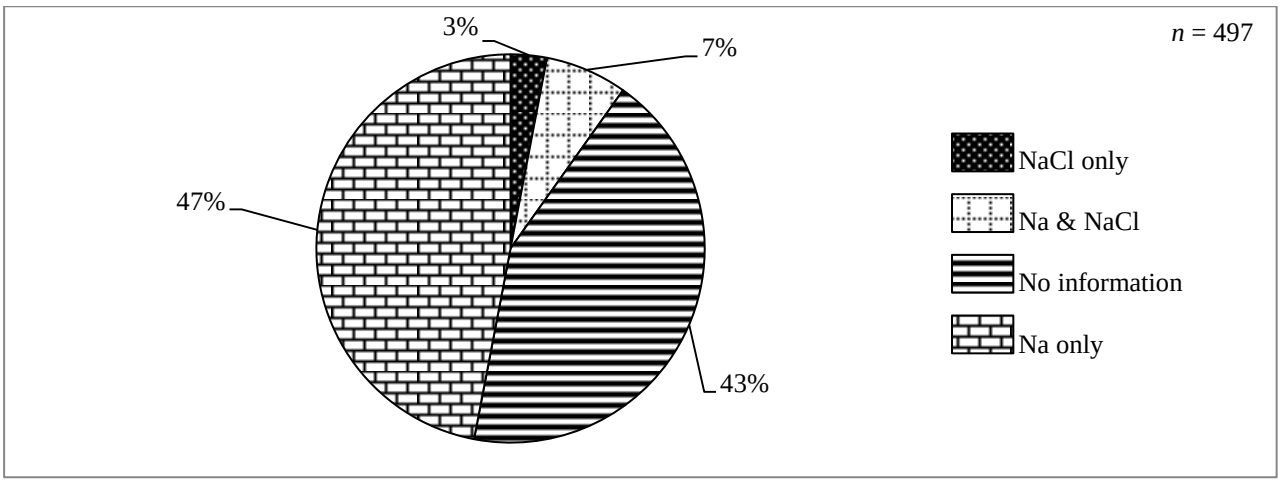

Figure 3.1. Processed meat products grouped according to the availability of Na and/or NaCl content information on the product labelling.

3.3.2. Distribution of products according to product subclasses

After all the products on the master list totalling 497 individual products were classified into their respective classes and subclasses according to SANS 885 (2011) as described in Table 3.2, large differences in representation were found (Figure 3.2). The greatest representation was found for subclasses: 1.1 (9.26%), 4.2 (10.87%), 6.1 (38.43%), 7.1 (21.33%), and 15.1 (3.62%). These five classes represented products such as: whole muscle hams, bacons, polonies, sausages, and nuggets as respective examples. These types of product are the high volume products often presented in a high variety of variants, brands and price points. The more specialized and normally more expensive and/or less favoured products were represented by subclasses with much lower percentages. For some subclasses no representative products could be found and this included subclasses: 3.2, 4.1, 5.2, 6.3, 6.4, 6.5, and 13.1. Products in these subclasses such as “nitrite-free” ham, jerky, prosciutto crudo, brawn, Sülze, and blood sausage are specialized products not commonly available in most supermarkets although they can be found through speciality shops, delicatessens and butcheries. Determining the main subclasses of processed meat products by representation across a large variety of products also allowed for the identification of subclasses on which to further focus the research project.

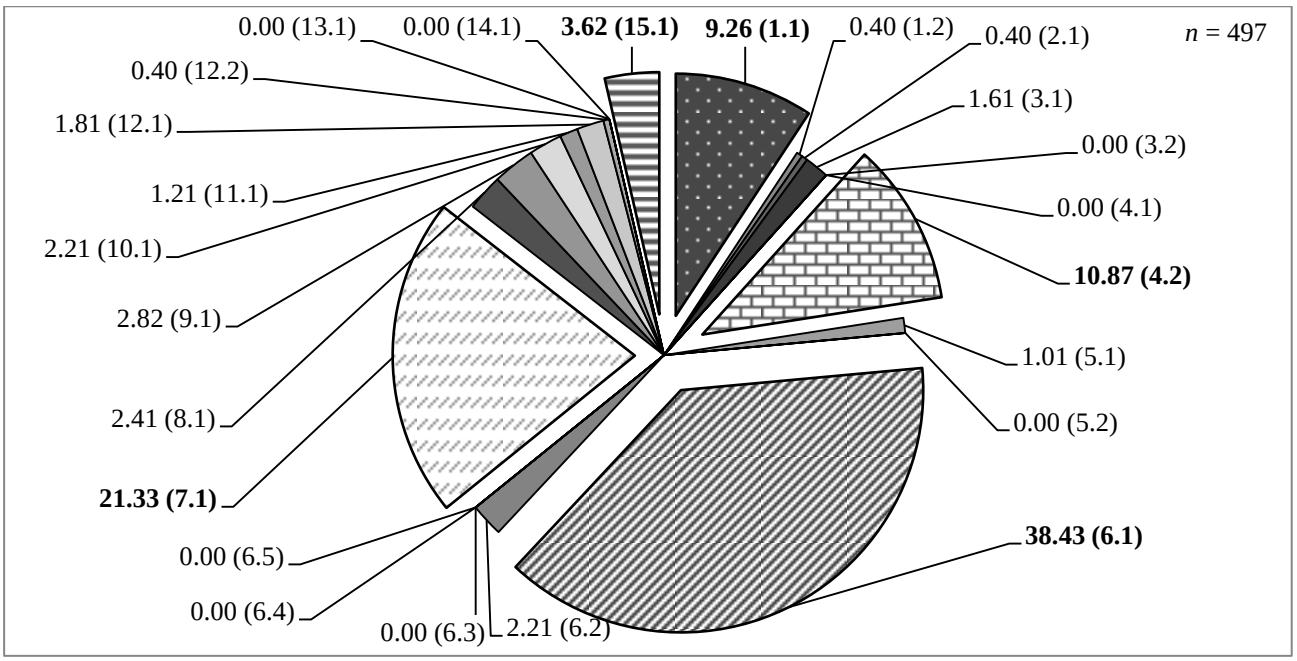

Figure 3.2. Percentage representation (first value) of 497 processed meat products surveyed in local supermarkets across all meat product subcategories (in brackets). Subcategories as described in Table 3.2.

3.3.3. Maximum, minimum and average Na content of the bought products

The highest maximum Na content was found in class 3.1 representing meat products such as biltong, which are already associated with having high Na content (Table 3.3.). Incidentally, this class also had the highest minimum Na content of any class. This class is currently excluded from the Na regulations probably due to concerns as the effective processing of this product without the salting-out effect of NaCl. It is clear however, that at some stage this situation will require some attention so as to significantly decrease the amount of Na this class contributes to overall Na intake. The lowest maximum Na content was found in class 6.3, represented by products in aspic such as brawn. This was attributed to the fact that this type of product is sold as a RTE product, having undergone heat-treatment and it often contains vinegar which provides an additional preservative effect. The lowest minimum Na content was found in class 11.1, represented by reformed products such as chicken nuggets. These products are normally heat-treated so that the crumb adheres to the meat product and developes an appealing golden colour before flash-freezing and frozen storage. These products therefore do not require NaCl as main preservative. The average Na content of the regulation-affected classes reflected that most of these classes already fall within the two regulatory limits for a given class. Off concern is that classes with high Na content such as class 3.1, 8.1, and 9.1 were excluded from the regulations at the time of writing.

Table 3.3. Classification of the 238 bought processed meat products according to SANS 885 with maximum, minimum and average Na content per class and the applicable regulatory limits.

Class (SANS 885)	Number of products	Max Na (mg/100 g)	Min Na (mg/ 100 g)	Avg. Na (mg/100 g)	Applicable Na limits (mg/100 g)
1.1	17	839.57	427.28	686.72 ± 118.46	n.a.
1.2	-	-	-	-	
2.1	-	-	-	-	n.a.
3.1	4	2250.00	1229.30	1831.60 ± 439.20	n.a.
3.2	-	-	-	-	
4.1	11	1675.88	434.35	971.33 ± 444.29	n.a.
4.2	14	1228.11	330.50	715.99 ± 227.46	
5.1	1	750.00	750.00	NSA	n.a.
5.2	-	-	-	-	
6.1	87	1344.31	272.98	755.13 ± 199.02	850 mg (2016) 650 mg (2019)
6.2	8	845.00	399.76	676.72 ± 154.80	
6.3	3	609.69	569.71	589.70 ± 28.27	
6.4	-	-	-	-	
6.5	-	-	-	-	
7.1	32	807.95	356.96	585.21 ± 130.42	950 mg (2016) 850 mg (2019)
8.1	13	2600.00	874.13	1605.61 ± 486.68	n.a.
9.1	12	1706.59	914.09	1313.39 ± 244.61	n.a.
10.1	6	624.68	354.83	510.94 ± 110.57	950 mg (2016) 850 mg (2019)
11.1	11	824.61	259.87	551.14 ± 177.39	950 mg (2016) 850 mg (2019)
12.1	4	799.60	616.88	719.16 ± 75.61	850 mg (2016)
12.2	2	854.58	590.00	722.29 ± 187.09	650 mg (2019)
13.1	-	-	-	-	n.a.
14.1	-	-	-	-	850 mg (2016) 650 mg (2019)
15.1	13	647.15	296.85	440.11 ± 131.44	n.a.

An absence of any representative products indicated by (-)
n.a. = not applicable
NSA = Not statistically analysed

3.3.4. Actual Na content versus labelled Na content of the five main subclasses

All the products that had label values for Na were compared to the actual values determined by AAS. The labels that described NaCl content were first converted to Na values with the previously discussed formula. The purpose of this part of the survey was to establish the differences between label values and actual values and if processors that report Na or NaCl content voluntarily, actually produce products with Na levels within a close range of the reported values. In Figures 3.3 to 3.8 the products were grouped together and then according to class on separate figures. Labelled Na or converted NaCl content as displayed on the labels were plotted as 0 values on the x-axis and positive and negative deviations of the actual values were plotted against the y-axis. Label values higher than the actual values resulted in negative values, indicating an overvaluation of Na content while label values lower than the actual values resulted in positive values, indicating an undervaluation of Na content. The maximum overvaluation was indicated by Δ^-, maximum

undervaluation as Δ^+ and average deviation from the label values by μ. Sodium content as displayed on the product label was indicated by values in brackets for the Δ^- and Δ^+ products only.

The ~57% of product labels with information on Na and/or NaCl content were represented by 135 individual products (Figure 3.3). The greatest number of product labels overvalued the Na content of the products with a margin of μ = -179.50 mg/100 g. This was attributed to processors rather erring on the safe side with products containing less Na than what is suggested on the labels. The Δ^- = 1064.50 mg/100 g was a gross overvaluation. The specific product was a cook-from-frozen, crumbed pork patty product with a low risk to microbial shelf-life. Such a large difference might, however, present a microbial risk in meat products not kept in a frozen state. It is possible that the processor has issues with controlling the formulation or simply that the product was reformulated to a lower Na content and packaged in surplus packaging stating old values. A much smaller group of product labels undervalued the Na content and a Δ^+ = 695.00 mg/100 g was found. This was substantially lower than the maximum overvalued Na content, although this situation may contribute a substantial amount of extra Na to the diet without consumers being aware. This product was a "droëwors", or dried sausage product made from ostrich. It is possible that the product was dried to a level much lower than normally allowed before packaging, or the processor undervalued the concentrating effect that drying normally has on the salt content.

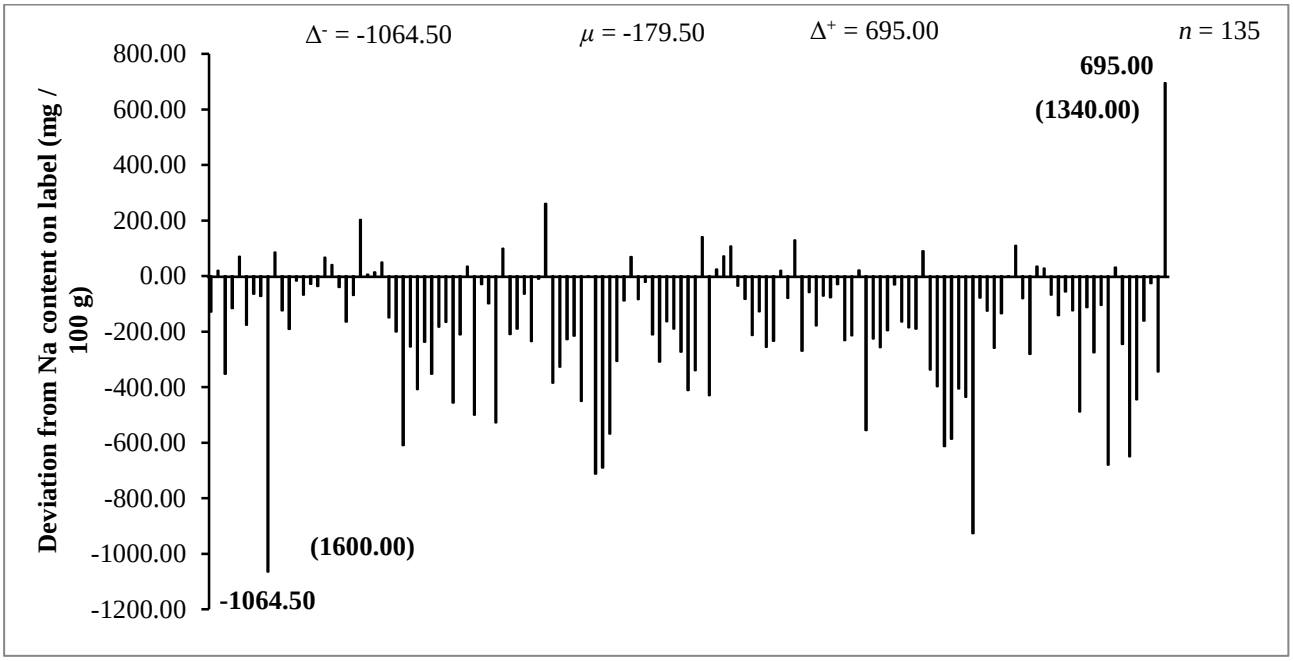

Figure 3.3. Deviations of actual sodium content from label values of all the processed meat products analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

Products in Classes 1.1 and 1.2 (as described in Table 3.2) resulted in a small group of only 12 individual products (Figure 3.4). For the most part, the Na content of these products were overvalued with a μ = -157.72 mg/100 g similar to that of the entire group of products in Figure 3.3. The Δ^- = -489.20 was again greater than the μ. The product was a cooked beef tongue which is a refrigerated RTE product. In contrast to the Δ^- product of the main group which was frozen, such a major deviation in a product which is sold for immediate consumption might be problematic with regard to overall shelf-life and microbial safety. This type of product does, however, also rely on the overall low temperature, vacuum packaging and added nitrites in its preservation. The Δ^+ of 107.29 was much closer to the label value compared to that of the overall group. This product was a pre-sliced and packaged RTE chicken product which is usually also consumed in small quantities as part of a sandwich and probably posed little risk to consumers unknowingly increasing their daily Na intake.

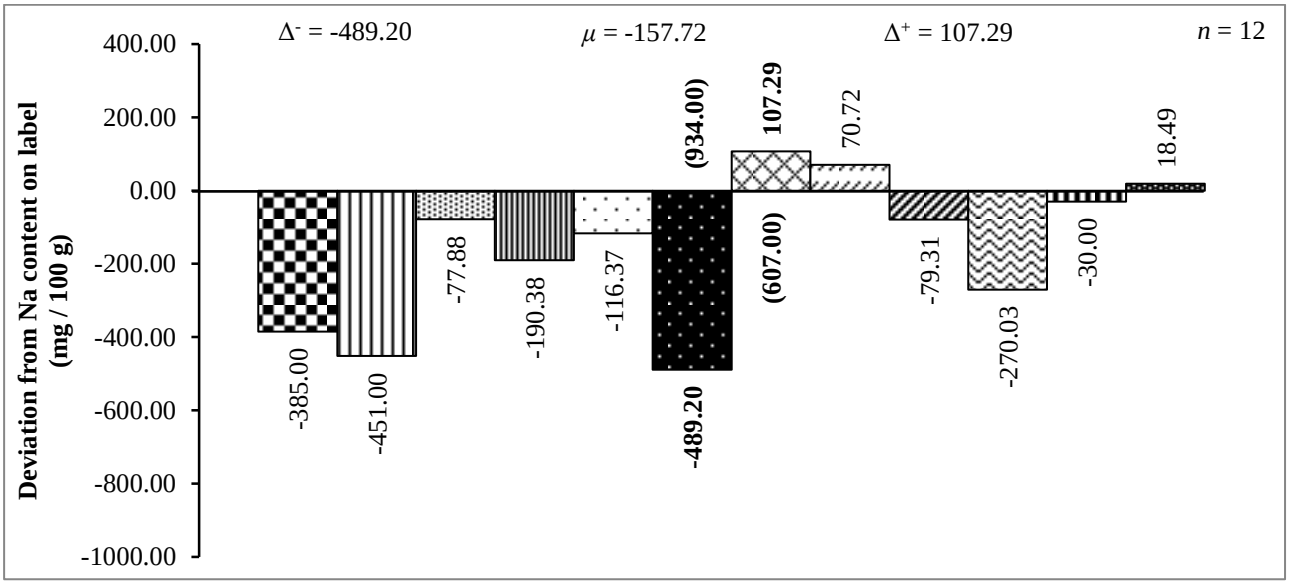

Figure 3.4. Deviations of actual sodium content from label values of processed meat products in Class 1 analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

Products in Class 4.1 and 4.2 (as described in Table 3.2) resulted in another small group of products with n = 11 (Figure 3.5). The Δ^- of -712.35 mg/100 g of this class was lower than that of the overall group of products. This product as well as the second highest Δ^- product (-690.85 mg/100 g) were both reduced fat bacon products. The fat tissue of bacon does not absorb the brine solution as well as muscle tissue and the specific batches that were analysed might have contained substantially higher proportions of fat than what was formulated for. This, or possibly deviations in the brine formulations, may have contributed to the deviations from the label values. Class 4 had the highest μ at -324.01 mg/100 g indicating that these products' labels mostly overvalued the Na content more

than that of the overall group of meat products. The Δ^+ of 202.25 mg/100 g was much lower than that of the overall group of products and was from a diced bacon product. It was also the only overvaluation of Na content for Class 4. This deviation may be attributed to an error in formulating the overall Na content of the final product.

Products in Class 6.1-6.5 (as described in Table 3.2) resulted in the largest group of products sampled with n = 42 (Figure 3.6). The Δ^- of -684.25 mg/100 g of this class was lower than that of the overall group of products as well as that of Class 4, but higher than that of Class 1. This product was a RTE chorizo sausage which might have had a formulation error or incorrect labelling with regard to Na content. The μ = of –152.36 mg/100 g of this class was the second lowest of any of the five classes after that of Class 15, indicating that processors of this class of products also rather added too little Na rather than too much. The Δ^+ of 260.04 mg/100 g was much lower than the overall Δ^+, the highest of all five classes and was for a local Drakensberger sausage.

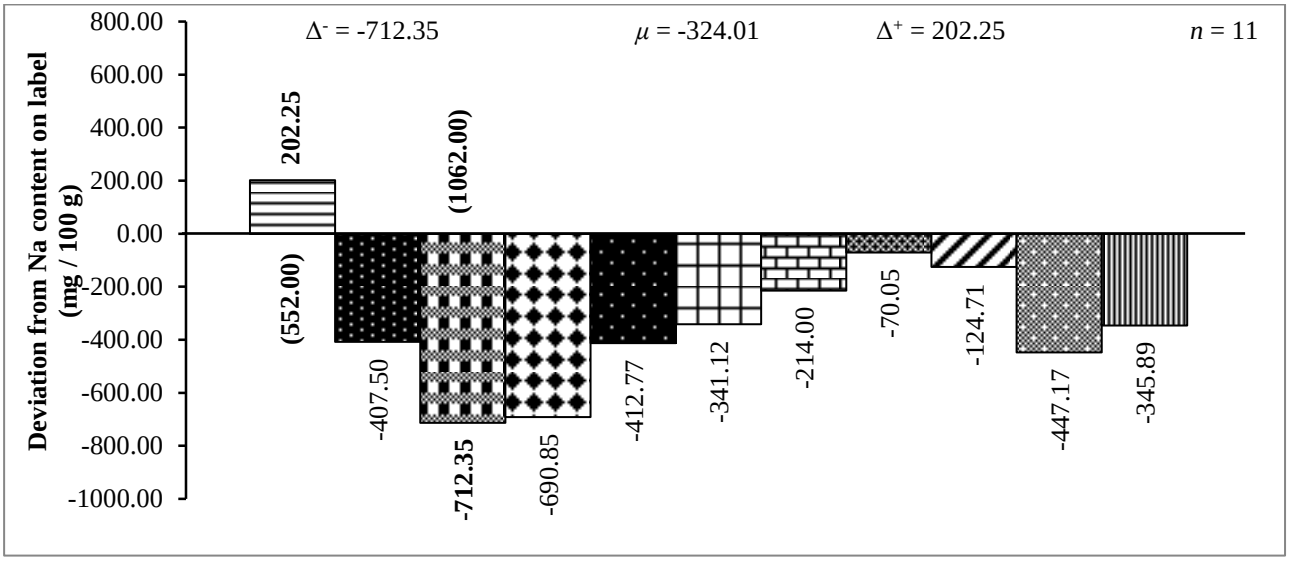

Figure 3.5. Deviations of actual sodium content from label values of processed meat products in Class 4 analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

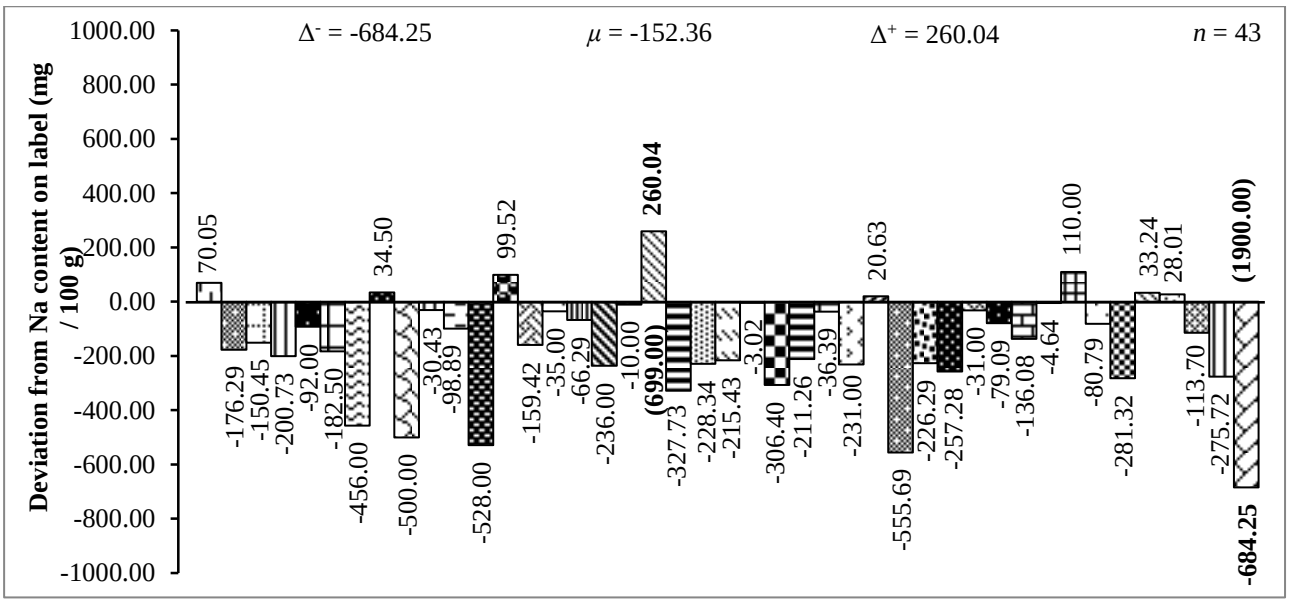

Figure 3.6. Deviations of actual sodium content from label values of processed meat products in Class 6 analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

Class 7.1 consisted of n = 13 individual products (Figure 3.7). This class had the highest Δ^- = -1064.50 compared to the whole group of products and compared to the other four classes. The specific product was previously discussed under the observations on the entire group of products (cook-from-frozen, crumbed pork patty). The μ = -202.13 mg/100 g was the second highest after that of Class 4 and higher than the general μ of all the products. The Δ^+ of 23.40 mg/100 g was the lowest of any of the five classes and the only undervaluation for this class. The product was a fresh, raw pork sausage manufactured for a supermarket chain with very high manufacturing standards although it cannot be concluded that this low level of deviation was due to the precise formulation of the Na content.

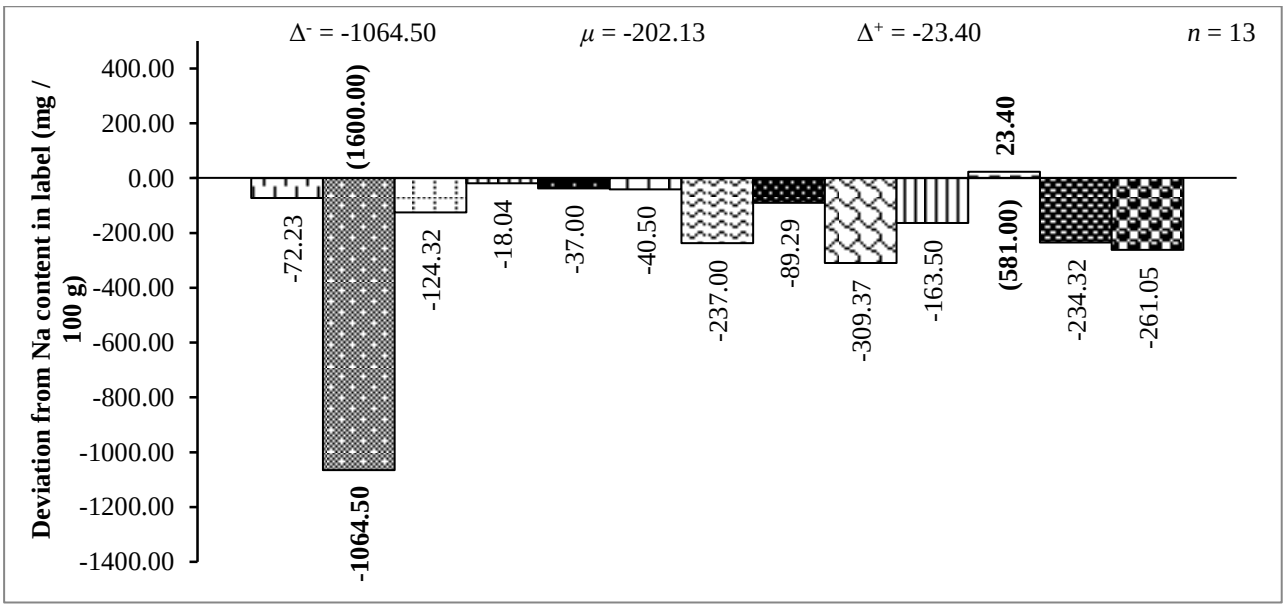

Figure 3.7. Deviations of actual sodium content from label values of processed meat products in Class 7 analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

The final class, Class 15, consisted of n = 12 individual meat products (Figure 3.8). This group had a Δ^- = -256.42 mg/100 g and a Δ^+ = -28.00 mg/100 g, showing the lowest overall deviation from label values across the five classes. This was also the only class where the Na content was overvalued and not undervalued, with an average of μ = -113.79.

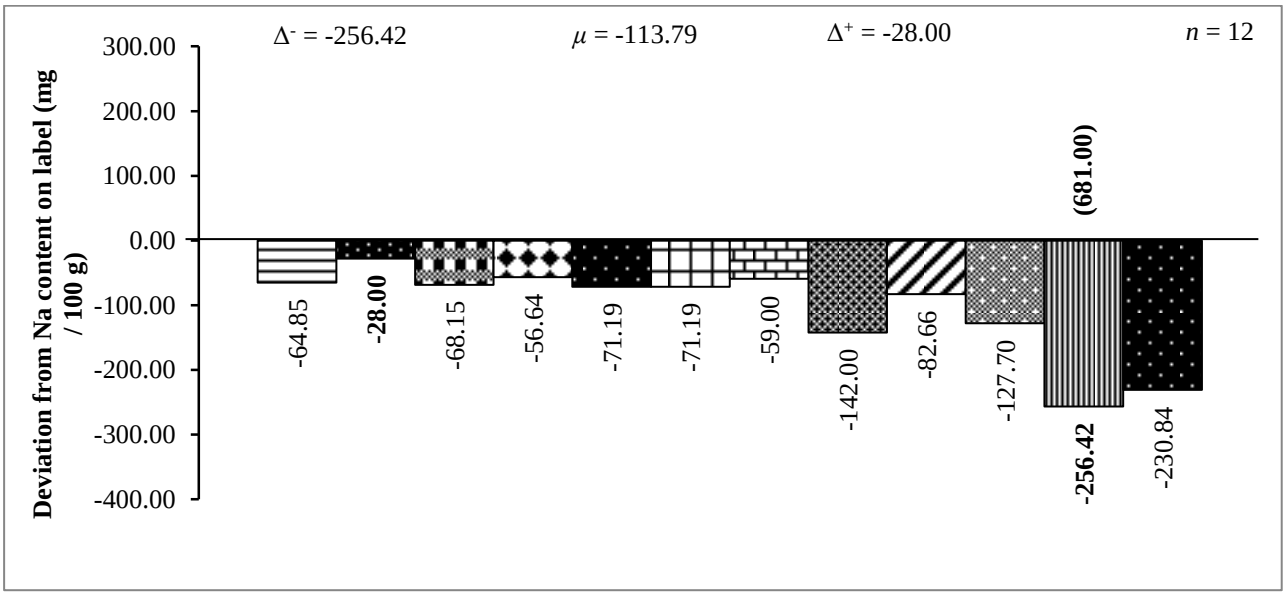

Figure 3.8. Deviations of actual sodium content from label values of processed meat products in Class 15 analysed in this study. Δ^- = maximum overvaluation; μ = average deviation from labelled sodium content; Δ^+ = maximum undervaluation; and values in brackets indicate labelled sodium content for Δ^- and Δ^+ only.

3.3.4. Sodium content distribution and current level of compliance of the three largest classes of meat products

Classes 4, 6 and 7 were the three largest classes with regards to product numbers (Figure 3.2). The actual Na content of the products within these three classes where then compared to the Na content limits (Department of Health, 2013) in an effort to establish how many products would fall outside the limits and to what extent. The actual Na content values of each individual product represented an average of duplicate values for each product. All the products within a specific class were included regardless of labelling information. Class 4 represents the third largest class of products (10.87%), although this class was excluded from the sodium regulations. Products such as bacon form part of this class (Table 3.2) and it is a high volume product with reported sales of 9705.32 tonnes in the twelve months up to August 2015 (Andrew Cocks, South African Meat Processors' Association president, personal communication, October 2015). Bacon is generally criticized for its high Na content and for containing nitrates and nitrites linked to gastrointestinal cancers by the formation of *N*-nitrosamines during high temperature cooking (Ford, 2011). There was no indication as why this class was excluded in the regulations, although it is anticipated that it may be included in future Na content limit related regulations.

Class 4 products exhibited a wide range of Na content values (Figure 3.9) with a Δ = 1345.38 mg/100 g between the maximum and minimum Na content values. The lowest Na content was 330.50 mg/100 g, the highest was 1675.88 mg/100 g and the population mean was 801.81 mg/100 g. Processors of this class of products had varied ideas of what they regarded as suitable Na levels for their own product(s). Twenty of the 25 products would have complied with a theoretical initial limit of no more than 950 mg/100 g and 17 products would have complied with a theoretical final limit of no more than 850 mg/100 g. These two theoretical values would therefore represent realistic and appropriate limits. Taking tolerances of no more than 20% (1140 mg/100 g and 1020 mg/100 g for 2016 and 2019, respectively) into consideration, only four products needed some form of reformulation with reduced Na content to fall within the tolerance of the initial theoretical Na content limit for 30 June 2016 and only five products needed reformulation to fall within the tolerance of the theoretical Na content limit for 30 June 2019. There would clearly not have been a large difference in adapting products from the first theoretical Na limit and tolerance level to that of the second theoretical Na limit and tolerance level. It is advisable that a singular change to the final limit and tolerance level be made so as to limit reformulation costs. With a difference in Na of only 100 mg/100 g it's also conceivable that consumers would not have been able to detect a significant change in saltiness.

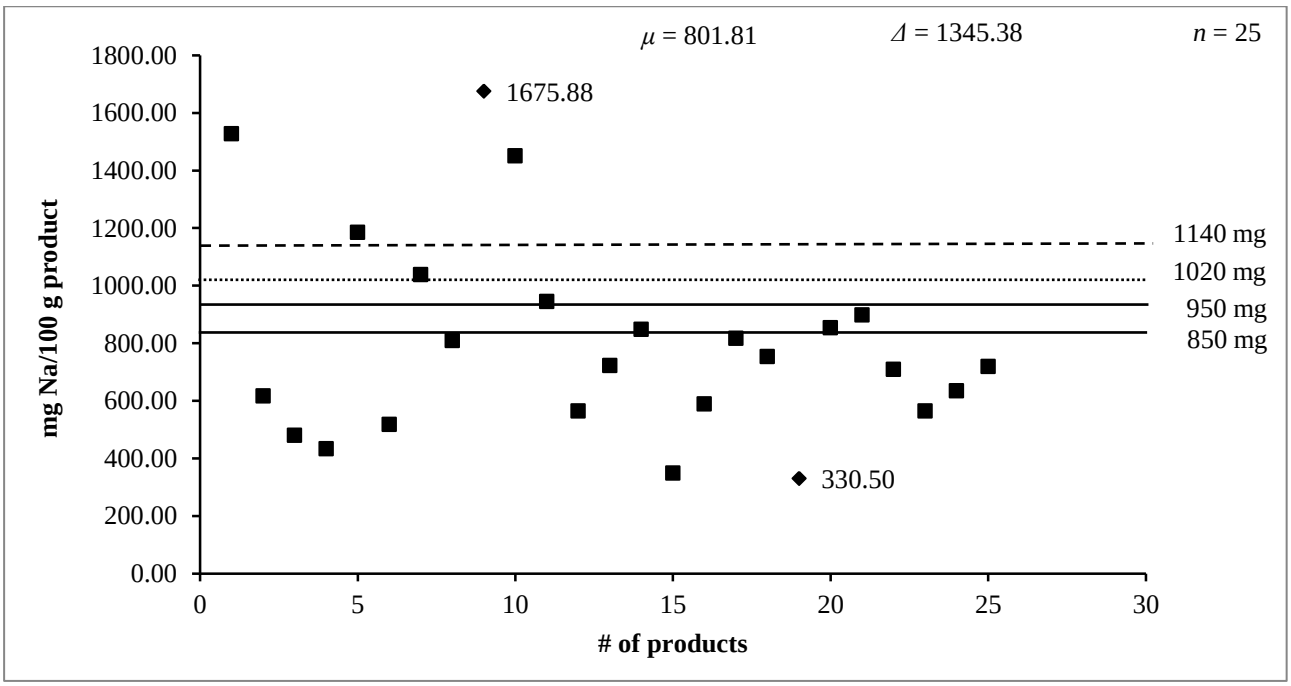

Figure 3.9. Distribution of Class 4 meat products based on actual Na content. Theoretical Na target limits for 2016 and 2019 in solid lines (─), the 2016 20% tolerance in a dashed line (---), and the 2019 20% tolerance in a dotted line (**…**).

Class 6 products were distributed over a narrower range of Na content values than that of Class 4 with a Δ = 1054.60 mg/100 g between the maximum and minimum Na content values (Figure 3.10). This range still represented a considerable variation equal to more than 2000 mg of NaCl if it was accepted that table salt was the main contributor of Na. The lowest Na content was 289.71 mg/100 g, the highest was 1344.31 mg/100 g and the population mean was 749.77 mg/100 g. This large variation in determined Na levels can mainly be ascribed to the wide variety of different products in this class that required different amounts of NaCl and Na-contributing components in their formulations. Sixty nine of the 97 products already complied with the initial Na limit of no more than 850 mg/100 g and 23 products complied with the final Na limit of not more than 650 mg/100 g. In light of the proposed amendment to the 2013 draft of regulations that would allow an increase in the initial limit from 850 mg/100 g to 1300 mg/100 g and an increase in the final limit from 650 mg/100 g to 1150 mg/100 g, there seems to be no validity for these increases as most products would easily conform or be reformulated to conform to the existing drafted limits.

Only 6 products needed reformulation to meet the 20% tolerance limit (1020 mg/100 g) for 2016 and 40 products needed reformulation to meet the 20% tolerance limit (780 mg/100 g) for 2019. At the time of analysis the 2019 Na limit and limit tolerance were a number of years away, it can therefore be expected that processors will reformulate their products in due time.

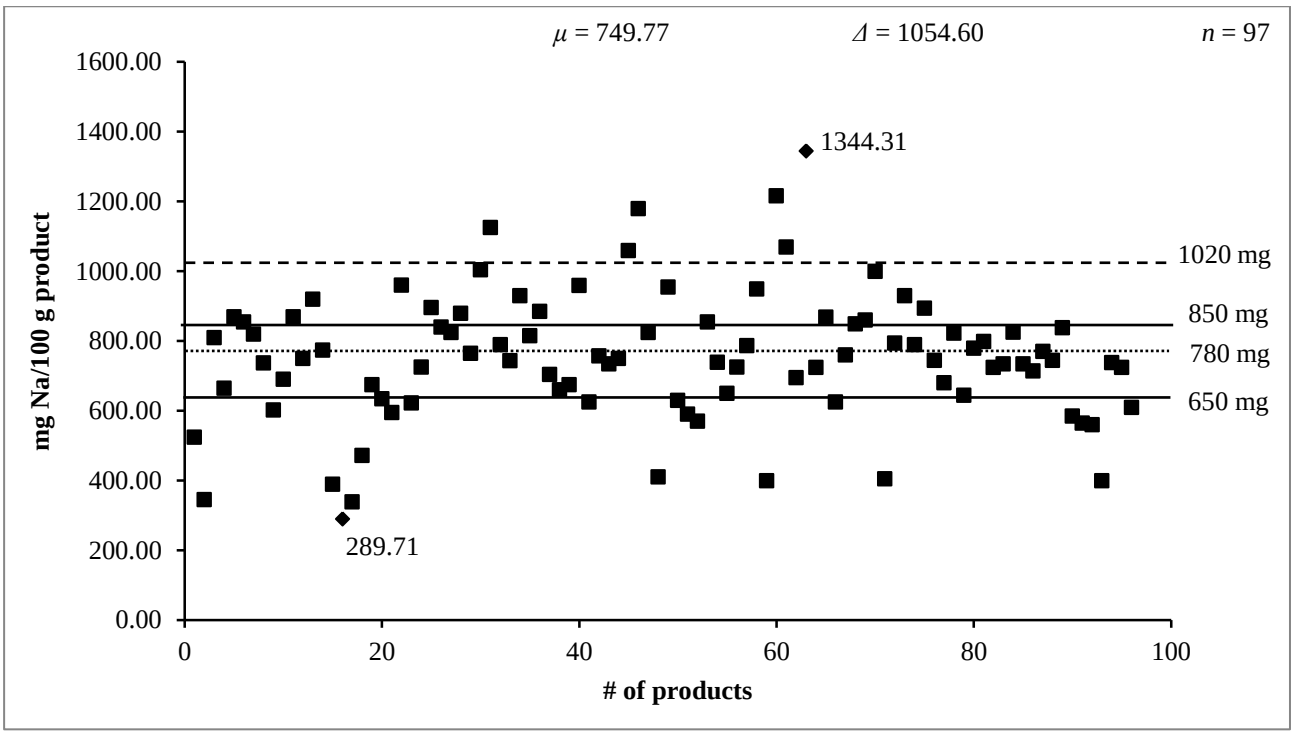

Figure 3.10. Distribution of Class 6 meat products based on actual Na content. Regulation Na target limits for 2016 and 2019 in solid lines (─), the 2016 20% tolerance in a dashed line (---), and the 2019 20% tolerance in a dotted line (**…**).

Class 7 products were distributed over the narrowest range of Na content values between the three classes of processed meat products. The maximum Na content was 869.57 mg/100 g, the minimum Na content was 356.96 mg/100 g and the Δ = 589.12 mg/100 g. Thirty of the 32 products already complied with the initial Na limit of not more than 800 mg/100 g and 16 products complied with the final Na limit of not more than 600 mg/100 g. No products required reformulation to meet the 20% tolerance limit (890 mg/100 g) for 2016 and only seven products required reformulation to meet the 20% tolerance limit (720 mg/100 g) for 2019. The very low Na content of some of these products were unexpected, considering that a large number of these products were fresh sausages products which generally have a short shelf-life of not more than 6 to 9 days.

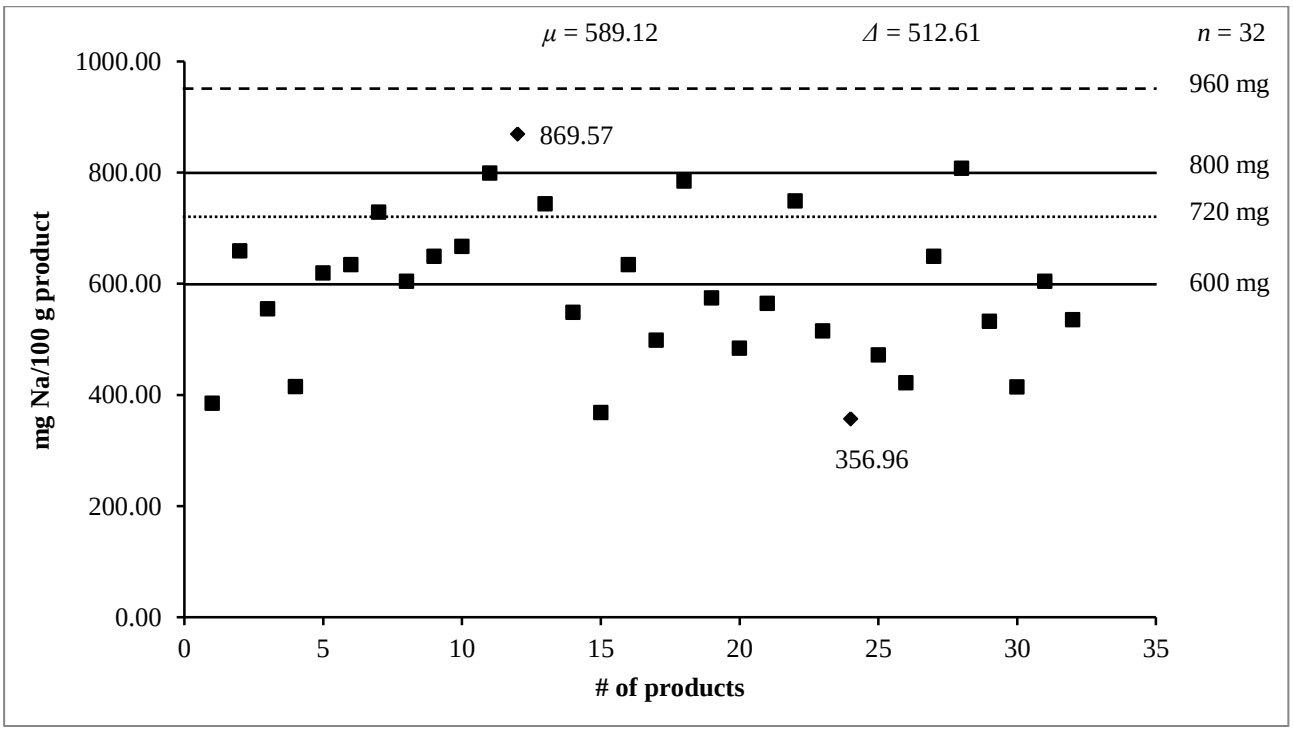

Figure 3.11. Distribution of Class 7 meat products based on actual Na content. Regulation Na target limits for 2016 and 2019 in solid lines (—), the 2016 20% tolerance in a dashed line (---), and the 2019 20% tolerance in a dotted line (…).

3.4. Conclusions

The number of drafts of the South African regulations aimed at limiting the Na content of certain foodstuffs was encouraging. It indicated a commitment by the government to improve public health, and the willingness of industry to participate in the drafting process. Only through partnership a realistic set of regulations can be created that not only takes public health into consideration, but also the challenges and limitations facing processors in reducing the Na content of their products. A number of issues between the regulations and processed meat product standards were identified with little time for correction before the first implementation date comes into effect. Many subclasses seemed too similarly defined calling into question their existence; Class 14 (undefined) exists without a useful description of its scope, and the increases proposed for the latest draft were unnecessarily high.

By conducting a survey on commercially available processed meat products displayed in local retailers, less than 60% of the product labels described Na or NaCl content. Processors were, however, not under obligation to provide this information, a situation expected to change in the near future. Not only could this information help to educate consumers as to how much they are consuming, but it could also help to make savvy consumers more assured of the types of products they choose to buy. Surveying all the available products also made it possible to establish the five largest classes of processed meat products. The smaller classes represented more exclusive and

sometimes more expensive products while the largest classes represented the higher volume, lower cost products available to most consumers. Comparing the products with Na content labelling to their actual Na content, most product labels overvalued Na content which was ascribed to processors erring on the safe side. Overestimation was greatest for frozen products which, under normal circumstances, would pose no increased microbial risks. Underestimation was greatest for dried meat products where processors probably failed to anticipate the concentrating effect of drying. This may lead to consumers underestimating Na intake from consuming the affected products.

The three largest classes were then compared to their proposed Na limits with one class currently excluded from the regulations and theoretical limits used instead. Taking what would seem a rather generous tolerance for exceeding the limits into consideration, it was found that across all three classes only a small number of products truly exceeded the regulatory limits. It was theorised that straightforward reformulation by reducing the Na content could be attempted. This would bypass the often difficult to execute and costly use of Na replacers. It would, however, still be of great importance to evaluate the microbial safety and maintained product quality of these straightforward reformulations.

CHAPTER 4

THE CHEMICAL, MICROBIAL, SENSORY AND TECHNOLOGICAL EFFECTS OF REDUCED SALT LEVELS AS A SODIUM REDUCTION STRATEGY FOR BACON, BANGERS AND POLONY

ABSTRACT

The reduction of sodium in processed meat products is synonymous with the use of various kinds of replacers. There has been little assessment of the use of intermediate added salt levels as a sodium reduction strategy in itself and no examples of using regulatory limits as the final reduction goals. In this study, reduction in added NaCl content of 68% and 34% for bacon, 47% and 26% for polony, and 50% and 26% for bangers were evaluated against no added NaCl negative controls and normal added NaCl level positive controls. Higher intermediate NaCl levels compared favourably with the positive control levels while minor defects were noticeable at lower intermediate NaCl levels. These findings provide a starting point for further investigation of sodium reduction in processed meat products.

Keywords: sodium reduction; processed meat products; microbial shelf-life; oxidative stability; technological stability; sensory acceptability

4.1. Introduction

Salt is the second most used food additive besides sugar (Seligsohn, 1981) and in meat processing it is the most used component besides the meat itself (Forsythe & Miller, 1980; Sebranek et al., 1983). Technologically, salt is critical in manipulating and controlling the texture of processed meat products by activating meat proteins which leads to greater water binding. This process occurs when the dissolved salt moves the isoelectric point of the meat protein towards lower pH values (Hamm, 1961). Normally, at lower pH values not close to the isoelectric point of meat, the meat proteins are less soluble and this may result in less adequate binding properties (Sofos, 1985). Other consequences of addition of salt to meat products include: increased tenderness of the meat; decreased fluid loss in heat-processed, vacuum-packaged products; increased viscosity due to increased fat binding; and the binding of myosin proteins to each other, which improves texture (Man, 2007). Microbiologically, NaCl has been used for thousands of years to preserve meat against the growth and resultant activities of microbes. This is primarily through the effect that

NaCl has on decreasing a_w, w/w more than any other ionic substance (Mossel & Thomas, 1988). Salt also exerts a drying effect through the action of osmosis, thereby withdrawing moisture directly from the foodstuff and any microbes it might contain (Doyle & Glass, 2010).

Humans have an innate taste for salt and derive hedonic sensations from its consumption (Mattes, 1997). The consumption of meat and meat products remain an important component of the world diet and while consumers may sometimes indicate health concerns, they generally do not seem to adopt healthier eating habits (Grunert, 2006). If the meat industry wants to stimulate meat consumption, it has to face the challenges of offering more convenient and healthier options whilst identifying the new and changing roles of meat and meat products in the diet (Font-i-Furnols & Guerrero, 2014). Nowhere is this more relevant than with the efforts being made to reduce the Na content of processed meat products. Reducing the NaCl content may require alterations in other parameters to ensure that affected foods maintain acceptable texture (Doyle & Glass, 2010) and a robust chemical and microbial shelf-life. Maintaining consumer acceptance and preference is also two of the major challenges encountered by the industry when sizeable NaCl reduction is involved (Bowboski et al., 2015). Increased production costs that can affect the affordability of these altered products need to be avoided to protect the most price sensitive consumers.

From the results of the survey on the actual Na content of processed meat products in South Africa compared to the imminent Na limiting regulations (Department of Health, 2013) it was found that most products already comply with the earliest 2016 Na limit and a substantial number of products comply with the final 2019 Na limit. It was then theorized that straightforward reductions to the limits may be applied without requiring the addition of Na and or NaCl replacers (Chapter 3).

The aim of this chapter was to investigate the potential of using the Na limit regulations as adjusted added NaCl levels in a straightforward reduction of total Na content without replacers. This was applied to three distinct processed meat products each representing one of the established three highest retail volume classes: bacon from Class 4, polony from Class 6 and banger sausage from Class 7. Effects were investigated on chemical and microbial shelf-life, sensory and textural quality and yields and 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 project was sourced from an owner-operator meat processing plant in Bloemfontein, South Africa. For the manufacturing of the bacon, fresh pork loins were

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 loins were deboned by removing the whole *longissimus lumborum* muscle from the back rib followed by the removal of the skin, subcutaneous excessive fat tissue and any excessive meat trimmings. The resulting deboned loins weighed ~ 1.60 kg and these weights were recorded as the green weight before brine injection.

Fresh lean pork consisting of a minimum of 90% lean meat and 10% fat (90/10) and good quality firm pork backfat was sourced less than 24 h before being used in the manufacturing of the banger sausages and stored at 4 °C until needed. For the polony, fresh lean beef (90/10) and good quality pork backfat was used. It was also sourced less than 24 h before required and kept at 4 °C.

All the chemical additives were sourced from Merck (Johannesburg, South Africa); casings (natural and synthetic), spices, starch, phosphates and preservatives were sourced from Crown National (Johannesburg, South Africa) and monosodium glutamate (MSG) was sourced from Freddy Hirsch (Johannesburg, South Africa). Individual components came from single batches with the same batch codes where more than one container was used.

4.2.2. Formulation of bacon, polony and bangers

The basis of formulation for the polony was the two Na limits as set out in the South African Regulations (Department of Health, 2013) (Table 3.1 on p. 49). For the bangers the first initial draft of the regulations (Department of Health, 2012) was used as this coinsided with the period during which the bangers were formulated and manufactured. As a result the applied Na limits were 800 mg Na by 30 June 2016 and 600 mg Na by 30 June 2018 and not the more recent 950 mg Na by 30 June 2016 and 850 mg Na byJune 2019 as set out in a new foodstuff category in the 2013 draft. An initial Na limit due by 30 June 2016 and lower Na limit due by 30 June 2019 was used for polony and bangers, with the Na limits for the bacon being theoretical limits as Class 4 products were not included in the most current draft of the regulations. This resulted in two treatment groups per product type which was increased to four treatment groups with the inclusion of negative and positive control groups. The contribution to total Na content per 100 g of product made by every formulation component except for added NaCl, was estimated and was subtracted from the final Na content. The difference between these two values was then designated as the amount of Na that was included through the addition of NaCl. For the three negative controls, the final products only contained Na inherent to all the other components in the formulations. For the positive controls, set amounts of NaCl were added regardless of the Na contributions made by other components. The

amounts of NaCl added were based on levels currently associated with each product type. These levels were decided upon after consultation with numerous South African spice companies as to the current usage levels in the three respective products.The treatment group batch sizes were as follows: ~ 1.60 kg deboned pork loin plus 20% pump for each bacon formulation, 3 kg emulsions for each polony formulation, and 2.5 kg sausage batters for each sausage formulation.

4.2.2.1. Bacon

Brine was applied by means of intramuscular injection. The brines were calculated to give a 20% final pump and differed from each other only on the basis of added NaCl content (Table 4.1). This class of products designated as Class 4 or "*Whole muscle, cured, no, or partial heat treated"* products (SANS 885, 2011) was excluded from the regulations, although included in the study as stated in Chapter 3 on p. 60. Therefore, two theoretical Na content limits were selected on which two intermediate added NaCl treatment groups were based. The limits consisted of an initial limit of no more than 800 mg/100 g product and a final limit of no more than 600 mg/100 g product. The initial limit group or higher intermediate (HI) group was formulated to contain two-thirds of the NaCl content of the 2.50% added NaCl positive control (PC) and the final limit or lower intermediate (LI) group was formulated to contain a third of the NaCl content of the 2.50% added NaCl PC group.

Table 4.1. The composition of the four 2 kg brines formulated with different added NaCl levels used for the production of back bacon.

Composition (%)	**NC**	**LI**	**HI**	**PC**
Formulated Na content of the final product (mg Na/100 g)	~ 210.15	~ 536.59	~ 863.03	~ 1193.40
Theoretical limits of the bacon (mg Na/100 g)	n.a.	600.00	800.00	n.a.
Water	93.604	88.624	83.644	78.604
NaCl	0.00	4.98	9.96	15.00
Dextrose	3.00	3.00	3.00	3.00
Sodium tripolyphosphate	3.00	3.00	3.00	3.00
Sodium ascorbate	0.30	0.30	0.30	0.30
Sodium nitrite[†]	0.096	0.096	0.096	0.096
Total	100.00	100.00	100.00	100.00

NC = negative control (0.00% added NaCl); LI = lower intermediate (0.83% added NaCl); HI = higher intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl)
[†]Sodium nitrite formulated not to exceed 160 mg/kg product
n.a. = not applicable

The negative control group contained 0.00% added NaCl with only the other brine components as sources of Na. This resulted in: a NC formulated to 210.15 mg Na/100 g (0.00% added NaCl plus

inherent Na content); a LI formulated to 536.59 mg Na/100 g (0.83% added NaCl plus inherent Na content); a HI formulated to 863.03 mg Na/100 g (1.66% added NaCl plus inherent Na): and a PC formulated to 1193.40 mg Na/100 g (2.50% added NaCl plus inherent Na) (Table 4.1). A brine weight equal to 20% of each individual loin's green weight was injected for a 20% pump on each loin. The total formulated Na content of the LI group at 536.59 mg/100 g was lower than the limit set for 600 mg/100 g and the total formulated Na content of the HI group at 863.03 mg/100 g was higher than the limit set for 800 mg/100 g although this was still lower than a 20% tolerance of 960 mg/100 g. In order to maintain a 2 kg brine batch size across all four treatments, the difference in added NaCl was compensated for by adjusting the amount of water used from 93.604% for the 0.00% added NaCl group to 78.604 for the 2.50% added NaCl group.

4.2.2.2. Polony

The four treatment groups were formulated on the basis of added NaCl content (Table 4.2). The Na content limits of Category 7 (Department of Health, 2013) for this class of product designated as Class 6 or "*Comminuted, cured, cooked and chilled products*" (SANS 885, 2011) were used as the basis for the two intermediate added Na treatment groups. The limits consist of an initial limit of no more than 850 mg/100 g product by the 30th of June 2016 and a final limit of no more than 650 mg/100 g product by the 30th of June 2019. The 2016 Na limit treatment group or higher intermediate (HI) group was formulated with 723.80 mg/100 g added Na (1.84% added NaCl) and the 2019 Na limit treatment group or lower intermediate (LI) group was formulated with 523.18 mg/100 g added Na (1.33% added NaCl). This resulted in a 2016 Na limit treatment group with ~ 850.32 mg/100 g total Na and a 2019 Na target group with ~ 649.69 mg/100 g total Na (Table 4.2), respectively, named HI and LI henceforth. The two control treatments consisted of the positive control (PC) representing the current general Na content for this type of processed meat product and a negative control (NC) with no added NaCl. The PC group was formulated with 983.43 mg/100 g added Na (2.50% added NaCl) and the negative control group was formulated with 0.00 mg/100 g added Na (0.00% added NaCl). This resulted in a PC group with ~ 1109.94 mg/100 g total Na and a NC group with ~ 126.52 mg/100 g inherent Na, respectively. In order to maintain a 3 kg batch size across all four treatments, the difference in added NaCl was compensated for by adjusting the amount of lean beef used from 52.50% for the 0.00% added NaCl group to 50.00% for the 2.50% added NaCl group. Adjustment of the added water content from 14.75% to 16.75% depending on the added salt level has been reported (O'Flynn, Cryz-Romero, Troy, Mullen, & Kerry, 2014). In contrast it was decided that the slight adjustment in meat content would introduce the least amount of variation between treatments.

The additive pack contained the following: tapioca starch (61.07%), sucrose (25.64%), dextrose (7.26%), sodium ascorbate (3.05%), MSG (1.92%) and citric acid (1.06%). Monosodium glutamate was included in these formulations as it is consistently used in these types of processed meat products in the industry. The spice pack was formulated to contain the following ground spices in the following quantities: white pepper (26.44%), yellow mustard (20.22%), coriander seed (15.56%), mace (14.89%), cayenne pepper (10.89%), pimento (6.22%), paprika (3.89%) and garlic (1.89%). Both packs of dry components were mixed together just before being added to the emulsion.

Table 4.2. The composition of the four polony emulsions formulated with different added NaCl levels.

Composition (%)	**NC**	**LI**	**HI**	**PC**
Formulated Na content (mg Na/100 g)	~ 126.52	~ 649.69	~ 850.32	~ 1109.94
Regulatory limits (mg Na/100 g)	n.a.	650.00	850.00	n.a.
Lean beef 90/10	52.50	51.17	50.66	50.00
Ice water	19.97	19.97	19.97	19.97
Pork backfat*	*11.36*	*11.36*	*11.36*	*11.36*
Ice water*	*11.36*	*11.36*	*11.36*	*11.36*
Soya protein isolate*	*2.28*	*2.28*	*2.28*	*2.28*
NaCl**	0.00	1.33	1.84	2.50
Additive pack	1.637	1.637	1.637	1.637
Spice pack	0.45	0.45	0.45	0.45
Sodium tripolyphosphate**	0.40	0.40	0.40	0.40
Butchers Red (E127)††	0.03	0.03	0.03	0.03
Sodium nitrite**†	0.013	0.013	0.013	0.013
Total	100.00	100.00	100.00	100.00

NC = negative control (0.00% added NaCl); LI = lower intermediate (1.33% added NaCl); HI = higher intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
*Ingredients and quantities for cold fat pre-emulsion consistent for all treatments shown in italics
**Salt pack specific to each treatment
†Resulting in a final concentration of 130 ppm nitrite in product
†† Commercial product giving a final concentration of 30 ppm erythrosine in product
n.a. = not applicable

4.2.2.3 Bangers

Four treatments of pork bangers were formulated to each contain one of four final Na levels (Table 4.3). The Na content limits of Category 9 (Department of Health, 2013) for this class of products designated as Class 7 or "*Comminuted, uncured, no or partial heat treated products*" (SANS 885, 2011) were used as the basis for the two intermediate added Na treatment groups. The limits consisted of an initial limit of no more than 800 mg/100 g product by the 30th of June 2016 and a final limit of no more than 600 mg/100g product by the 30th of June 2019. The 2016 Na limit treatment group or higher intermediate (HI) group was formulated with 590.05 mg/100 g added Na (1.50% added NaCl) and the 2019 Na limit treatment group or lower intermediate (LI) group was formulated with 393.37 mg/100 g added Na (1.00% added NaCl). This resulted in a 2016 Na limit

treatment group with ~ 786.78 mg/100 g total Na and a 2019 Na target group with ~ 590.41 mg/100 g total Na (Table 4.3), respectively, named HI and LI henceforth. The two control treatments consisted of the positive control (PC) representing the current general Na content for this type of processed meat product and a negative control (NC) with no added NaCl. The PC group was formulated with 780.74 mg/100 g added Na (2.00% added NaCl) and the negative control group was formulated with 0.00 mg/100 g added Na (0.00% added NaCl). This resulted in a PC group with 983.15 mg/100 g total Na and a NC group with 197.67 mg/100 g total Na.

In order to maintain a 2.50 kg batch size across all four treatments, the difference in added NaCl was compensated for by adjusting the amount of lean pork used from 56.81% for the 0.00% added NaCl group to 54.81% for the 2.00% added NaCl group as was done during the polony formulation. The spice and additive pack contained the following: sodium tripolyphosphate (21.58%), coriander (21.58%), MSG (14.39%), dextrose (7.19%), white pepper (7.19%), nutmeg (5.40%), sodium metabisulfite (5.04%), black pepper (5.04%), cardamom (4.46%), sodium ascorbate (3.60%), sage (2.16%), ginger (2.01%), and mace (0.36%).

Table 4.3. The composition of the four banger batters formulated with different added NaCl levels.

Composition (%)	**NC**	**LI**	**HI**	**PC**
Formulated Na content mg Na/100 g	~ 197.67	~ 590.41	~ 786.78	~ 983.15
Regulatory limit mg Na/100 g	n.a.	600.00	800.00	n.a.
Lean pork 90/10	56.81	55.81	55.31	54.81
Pork backfat	22.50	22.50	22.50	22.50
Ice water	15.00	15.00	15.00	15.00
Rusk	3.50	3.50	3.50	3.50
Spices and additives	1.39	1.39	1.39	1.39
Starch	0.80	0.80	0.80	0.80
NaCl	0.00	1.00	1.50	2.00
Total	100.00	100.00	100.00	100.00

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl)
n.a. = not applicable

4.2.3. Manufacturing of the processed meat products

Three separate replicates were manufactured for each product type at least one month apart. This was done to negate variations in raw materials and processing and environmental conditions. A single replicate consisted of all four treatment groups (described below). For each of the three products a fourth replicate was manufactured specifically for sensory analysis and for the bacon and bangers, fifth replicates were manufactured specifically for cooking losses and yield.

4.2.3.1. Bacon

Twenty percent brine was added to each individual loin weight to reach the final weight up to which brine had to be injected to reach a pump of 20%. The brine was injected by hand using a single needle brine stitch pump and injection sites were 40 mm apart and all around the surface of the loin. The loins were placed in individual stainless steel containers, wrapped with a plastic bag and stored overnight at 4 °C to allow for even distribution of the brine. The loin surfaces were then dried in a smoker (Crown Mills, Johannesburg, South Africa) until the air temperature inside the smoker reached 60-65 °C, without a significant rise in the internal temperature of the bacon. Smoking followed using oak chips, pre-wetted and kept overnight in a sealed bag, at 65.5-68.5 °C for 25 minutes until the surfaces obtained a golden brown colour. The smoked loins were removed, wrapped in plastic bags and frozen overnight at -18 °C. Tempering at 4 °C for 3 h was followed by slicing the loins into 3 mm diameter slices, packaging of 250 g quantities in vacuum bags and vacuum sealing of the packages (SAFARIVAC Model TM 400 Series, Pretoria, South Africa). The packages were then kept under retail refrigeration conditions (fluorescent lighting and 4 °C storage temperature) throughout the shelf-life study. For each of the four treatment groups per replicate there were 12 packets of bacon after packaging, resulting in a total of 48 packets per replicate.

4.2.3.2. Polony

For the manufacturing of the polony models, cold fat emulsions were needed to stabilize the fat component in the final emulsions during subsequent processing and to minimize encapsulation of the spices that may mask the associated flavours. A constant ratio of 1:5:5 of soya protein isolate (SPI; BIDFood Solutions, Johannesburg, South Africa) to ice water to pork backfat was used, typical of cold fat emulsions (Table 4.2). Some of the water and all of the pork backfat and SPI was first emulsified as a separate pre-emulsion. For this the ice water was placed in a bowl cutter (OKTO 20L Bowl Cutter, Crown National, Johannesburg, South Africa) and chopped at slow speed while all the SPI was added. This mixture was then chopped at high speed until a homogenous gel-like consistency was reached. The pre-minced pork backfat was added at low chopping speed. Chopping at high speed followed after all the pork backfat was added and continued until a creamy, shiny and homogenous texture was formed. The pre-emulsion was divided into four 750 g quantities for each of the four treatment groups and kept at 4 °C until needed.

The beef and pork backfat was separately reduced in size by mincing through a 3 mm mincing plate (OKTO No. 32 mincer, Crown National, Johannesburg, South Africa) and each minced component was thoroughly mixed by hand. The pre-minced beef and salt pack was chopped together at low speed in the bowl cutter for three rounds (Table 4.2). Half of the ice water containing the pre-

dissolved Butchers Red (10% of erythrosine BS, E127) as colourant was added and chopping continued at high speed until a strong binding was achieved and a temperature of 8 °C was reached. Half of the remaining ice water, the spice pack and the cold fat pre-emulsion were added and chopping continued to a temperature of 8.5 °C. The rest of the ice water was added followed by chopping until a temperature of 12.5 °C was reached. The final polony emulsion was filled into nylon casings (65 mm diameter by 500 mm length), the ends of which was twisted and crimped closed with 5 mm flat width stainless steel poly clips, producing three individual polonies each with a final length of 150 mm per casing. Twelve polonies (four samples and 3 sampling intervals) of ~ 180 g each were manufactured per treatment group one month apart. The polonies were loaded into a steam cooker (Crown National, Johannesburg, South Africa) and cooked at 72.2-75.5 °C to a final internal temperature of 68.5 °C. This was followed by immediate cooling in ice water and storage at 4 °C.

4.2.3.3. Bangers

The lean pork and backfat were minced separately through a 13 mm mincing plate fitted to the Okto mincer. The two separate batches of pork and backfat were then thoroughly mixed to obtain homogenous raw materials. For each treatment, specific amounts of pork, backfat, ice water, rusk, spices and additives, and NaCl were weighed out (Table 4.3). The ice water, rusk, spices, additives, and NaCl were mixed continuously for 1 min and left standing for 5 min to allow for proper hydration of the rusk and starch and for proper dissolving of the water-soluble additives. The pre-weighed pork and backfat were added to the mixture and the batter was mixed thoroughly before being minced through a 3 mm mincing plate. Natural hog casings with a diameter of 28-32 mm were then filled with the sausage batter using a manual sausage filler (Trespade, Crown National, Johannesburg, South Africa). This resulted in a single, continues roll of sausage with a weight of 2.50 kg per treatment. Banger links were formed in the roll and 24 individual pieces of sausage were then cut from each roll, each weighing ~ 80 g. This resulted in a total of 96 sausages per replicate. Each individual sausage was then placed in an expanded polystyrene (EPS) tray containing an absorbent pad, over-wrapped with polyvinyl chloride (PVC) film and stored either at 4 °C under retail refrigeration-type conditions, including fluorescent lighting, for fresh product shelf-life determination (16 sausages per treatment per replicate) or at -18 °C for frozen product lipid stability determination (eight sausages per treatment per replicate). For the fourth replicate the 24 sausages per treatment group were weighed after manufacturing, placed in individual EPS trays containing absorbent pads, over-wrapped with PVC film and stored at 4 °C for up to 9 days.

4.2.4. Sampling

4.2.4.1. Bacon

Sampling intervals were based on time and selected as: day 0, after final packaging of the bacon; day 15 and day 30 of refrigerated storage at 4 °C. Four packets of bacon per treatment group were randomly selected for quadruplicate chemical, microbial and textural analysis for each sampling interval.

4.2.4.2. Polony

Sampling intervals were based on time and selected as: day 0, after the model reached a final internal temperature of 4 °C; day 90 and day 180 of refrigerated storage at 4 °C. For each sampling interval, four polonies per treatment group per replicate were randomly selected for quadruplicate chemical, microbial and textural analysis.

4.2.4.3. Bangers

In South Africa, bangers are typically sold as both a fresh sausage (mostly at butcheries) and a frozen sausage (mostly at the frozen food section of major retailers and supermarkets). It is also common practice for fresh sausage to be bought and frozen at home. To this end, both a fresh product shelf-life of up to 9 days as well as a frozen product shelf-life of up to 180 days were used during which sampling took place. The fresh product shelf-life sampling took place on days 0, 3, 6 and 9 and the frozen product shelf-life 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 refrigeration losses, a group of 12 sausages and for thaw, cooking and total losses another group of 12 sausages per treatment group per replicate were used with individual sausages weighing ~ 80 g. For the sensory analysis, each continuous roll of sausage was cut into 500 g portions for easier handling during cooking.

4.2.5. Yield, refrigeration, thaw, and cooking losses

Yield and various losses were determined for the bacon while various losses were determined for the bangers as follows:

4.2.5.1. Bacon

The weights of the pork loins just before injection were recorded as the green weight of the loins. Percentages of pump, shrinkage after smoking, processing yield, drip loss, cooking loss and total loss were calculated with the following formulas:

% Pump = (weight after pump – green weight) / green weight x 100

% Shrink after smoking (%) = [(weight after injection – weight after smoking & freezing) / weight after injection] x 100

% Processing yield (%) = [(weight after smoking & freezing – green weight) / green weight)] x 100

For drip loss the weight of the bacon was recorded before vacuum-packaging and storage at 4 °C for 7 days as the pre-packaged bacon weight and after the storage period the bacon was removed from the packaging before it was blotted dry with paper towels and weighed again as the blotted-dry bacon weight. Drip loss was then calculated as follows:

Drip loss (%) = [(pre-packaged bacon weight – blotted-dry bacon weight) / pre-packaged bacon weight] x 100

The blotted dry bacon used to determine the drip loss was then cooked according to treatment groups in a convection oven at 160 °C for 5 min before being flipped over and cooked for a further 5 min. The cooked bacon of each treatment group was then removed from the oven, placed on paper towels and allowed to cool to room temperature before being weighed again to establish the cooked bacon weight. Cooking losses were then calculated as follows:

Cooking loss (%) = [(uncooked bacon weight – cooked bacon weight) / uncooked bacon weight] x 100

Total loss (%) = [(pre-packaged bacon weight – cooked bacon weight) / pre-packaged bacon weight] x 100

4.2.5.2. Bangers

Twelve of the sausages were refrigerated under the same conditions as for the fresh product self-life determination. After the 9 days the sausages were removed from their packaging and weighed. No blotting was required as no pooling of exudate was found in the packaging nor did any condensation form on the sausages. Refrigeration loss was then calculated with the formula:

Refrigeration loss (%) = [(initial weight – weight after refrigeration) / initial weight] x 100

The other 12 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 for gradual thaw, 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

And 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.

4.2.6. Chemical analyses

Various chemical analysis techniques were used across all three product types. All the bacon of one of four packets per treatment group per replicate was minced using a hand mincer and mixed to obtain a homogenous sample. Three plastic cuvettes with tight fitting lids were filled with the sample and 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 of the polony and the banger samples.

4.2.6.1. NaCl and Na content

Salt and Na content was determined on day 0 samples as representative of finished products. For NaCl, the Volhard method was used to volumetrically and quantitatively determine the amount of chloride present in the sample which was then used to calculate the amount of NaCl present in the sample (AOAC, 2005). Sodium content was determined by atomic absorption spectroscopy (AAS) using a Varian SpectrAA-300 spectrometer (SMM Instruments, Johannesburg, South Africa). The converted Na content was calculated as described in section 3.2.3 in Chapter 3 on p. 52. For AAS analysis, the samples were reduced to a mineralized form free from any organic compounds by means of ashing (Nielsen, 2010).

4.2.6.2. pH measurements

pH measurements were done directly by using a suitable 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, South Africa) to record quadruple pH measurements per treatment group per replicate at room temperature. Each day before use, the pH meter was calibrated with standardized buffers (Merck, Johannesburg, South Africa) with pH values of 4.01 and 7.00 respectively.

4.2.6.3. Water activity

A homogenously mixed sample was filled into a water activity container (height of 5 mm and diameter of 39 mm) to the appropriate level and covered with a container lid. The water activity was determined using a Novasina Thermoconstanter TH 200 (Labotec, Midrand, South Africa) water activity meter. After equilibrium was reached with deionized distilled water, quadruplicate measurements per treatment group per replicate were made at a temperature of 22 °C. The results were reported as % relative humidity (% rH) and converted to a_w values by dividing each value by a factor of a 100.

4.2.6.4. 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, Sofos, and Schmidt (1993) to determine the effect of the two intermediate added NaCl levels on lipid oxidation. Frozen samples were defrosted overnight at 4 °C. The TBARS results were quantified in terms of milliequivalents (mEq) malondialdehyde (MDA) per kg of sample as other TBARS may also be present (Beltran, Pla, Yuste, & Mor-Mur, 2003). Analysis took place after day 0 and after 90 and 180 days of storage at 4 °C. Moisture content (%) was a second parameter established (AOAC, 2005) as it is required in the calculation of TBARS.

4.2.7. Microbial analyses

The microbial analysis techniques applied across all three product types were consistent with differences only in the collection times and how samples were taken for further analysis.

4.2.7.1. Bacon

A 10 g sample was aseptically weighed off into a sterile 207 mL WhirlPak™ bag (Lasec, Bloemfontein, South Africa) before 90 mL of a sterile 0.1 M phosphate buffer was added and the sample was homogenized for 1 minute in a Stomacher® 400 Circulator (Seward, Lasec, Bloemfontein, South Africa). Further serial dilutions (10^{-2} to 10^{-5}) were prepared using 1 mL of the 100 mL sample (10^{-1} dilution) into 9 mL sterile 0.1 M phosphate buffer. Further 1 mL volumes of each dilution was then pour-plated using Standard Plate Count Agar (SPCA; CM0463, Oxoid; all Oxoid products from Quantum Biotechnologies, Johannesburg, South Africa) for determination of total viable counts (TVC); Violet Red Bile Agar (VRBA; CM0978, Oxoid) with 4-methulumbelliferyl-β-D-glucuronide (MUG) for coliforms and *Escherichia coli* (*E. coli*); and Rose-Bengal Chloramphenicol Agar (CM0549, Oxoid) with Chloramphenicol Supplement (SR0078, Oxoid) for yeasts and moulds. A further 1 mL was spread-plated on pre-poured Baird-Parker Agar (BP; CM025, Oxoid) plates containing Egg Yolk Tellurite Emulsion (SR054C, Oxoid) for *Staphylococcus aureus* (*S. aureus*) determination. Plates for TVC enumeration were then incubated at 32 °C for 48 h; plates for coliform, *E. coli* and *S. aureus* enumeration were incubated at 37 °C for 24 h; and plates for yeast and mould enumeration were incubated at 25 °C for 4 days. Enumeration of visible colonies was performed by means of a manual colony counter (Harrigan, 1998). Visualization of *E. coli* colonies on VRBA with MUG was by fluorescence light.

4.2.7.2. Polony

Initially the entire surface of the nylon casing around the polony was sprayed with a 70% ethanol-in-water solution and wiped dry with a sterile paper towel. A pre-sterilized pair of scissors (dipped in 98% ethanol and held to Bunsen burner) was used to cut-away half of the nylon casing before the casing was pulled back to reveal the polony surface. A 10 g sample was collected aseptically from each ~ 180 g polony through the use of a pre-sterilized (as for the scissors) cheese trier. The cheese trier was inserted perpendicular to the length of the polony and slowly pulled out for a representative sample across the whole diameter of a polony. Further processing of the samples proceeded as described for the bacon in 4.2.7.1.

4.2.7.3. Bangers

For the microbial analysis of the bangers, 10 g samples were also used. First the natural casing around the sausage batter was removed aseptically with a pair of flame-sterilised tweezers. The batter was transferred to a sterile petri dish and thoroughly mixed using a flame-sterilised spatula to

homogenize the entire sample. Further processing of samples then proceeded as described for the bacon in 4.2.7.1.

4.2.8. Physical analyses

Physical analyses included colour measurements performed on the bacon and bangers and texture measurements on the polony. No colour measurements were performed on the polony due to the addition of the colourant erythrosine at a consistent level of 30 ppm (Table 4.2) to all four formulations. The intense artificial pink colour masked any natural red colour development and was expected to mask any possible differences in natural colour.

4.2.8.1. Bacon – colour

Colour measurements were performed on days 0, 15 and 30 on the uncooked, vacuum-packaged bacon. Four packets of each of the four treatments per time interval per replicate were used and measurements per package were done in sextuplicate through the clear plastic of the vacuum bag. Colour measurements were made using a Minolta CR 400 Chroma Meter (Konica Minolta, Cape Town, South Africa) with 8 mm measuring area at a 0° viewing angle. The supplied white calibration tile was first placed inside a clean vacuum bag identical to those used for packaging the bacon and the instrument was calibrated. The CIELAB colour space (CIE, 1986) was used for measurements where L^* represents lightness, a^* represents redness, and b^* represents yellowness. Metric Chroma (C^*) as well as Metric Hue angle (H^*) was calculated by using the a^* and b^* values in the respective formulas: $C^* = \sqrt{a^{*2} + b^{*2}}$ and $H^* = tan^{-1}\left(\frac{b^*}{a^*}\right)$ (Ripoll, Joy, & Muñoz, 2011; Tapp, Yancey, & Apple, 2011).

4.2.8.2. Bangers – colour

Colour measurements were performed only on the refrigerated bangers on days 0, 3, 6 and 9 before the PVC film was removed and samples for microbial analysis were taken. Four sausage samples of each of the four treatment groups were used and measurements per sausage were done in sextuplicate. An individual sausage was gently rubbed along the length to obtain a flatter surface before measurements were taken through the PVC film. The white calibration plate was covered in the same film, any air bubbles between the film and plate was rubbed-out and the plate was used to calibrate the colorimeter. Colour measurements were made using a Minolta CR 400 Chroma Meter (Konica Minolta, Cape Town, South Africa) with 8 mm measuring area at a 90° angle perpendicular to the sample surface. The same colour parameters were measured as for the bacon in 4.2.8.1.

4.2.8.3. Polony – texture

Texture was evaluated through the use of Warner-Bratzler shear force determinations in order to establish the "first-bite" hardness of the four treatment groups. Objectively, the term hardness can describe the measurable resistance to deformation or the "first-bite" of a product as perceived through actual sensory evaluation (Bourne, 1982). The term hardness can be expanded to describe a scale ranging from soft to firm to hard (Szczesniak, 1963). Shearing of a sample is actually the result of a cutting action rather than a true shear, and consists of a combination of tension, compression and shear forces (Bourne, 1982). Four polonies per treatment group per time interval per replicate were collected after day 0 and 3 and 6 months to investigate the effect that the two intermediate added NaCl levels would have on the texture just after manufacturing and over the course of 6 months. A 12.5 mm diameter core borer was used to take three samples per polony perpendicular to width, resulting in 65 mm (length) x 12.5 mm (diameter) samples. If a core sample had a visible void on its surface, as a result of an air pocket caught in the heat-coagulated batter, it was rejected and a new core sample was taken. Two sampling points per sample were selected, each halfway from the centre of the sample to either end. An Instron Universal Testing Machine (UTS, Model 334; Advanced Laboratory Solutions, Randburg, South Africa) was fitted with a static load cell (2519 Series S-Beam) with a 2000N load cell capacity. A Warner-Bratzler meat shear force fixture (Model S17002) with 1 mm thick V-notch blade with 0.5 mm cutting radius was fixed to the load cell. The total compression extension of 40 mm at 200 mm/min crosshead speed was used to determine the shear force, expressed in Newton (N), needed to slice completely through each sample. Data was digitally captured using the Instron Bluehill 2 (V.2.26) software.

4.2.9. Consumer sensory evaluation

Sensory analysis by way of consumer panels were performed on all three products using the fourth replicates of each product type specifically manufactured for this purpose. Sensory analysis of the bacon and polony was carried out within 14 days from the date of manufacture so as to ensure a fresh batch of products fit for human consumption. The banger replicate for sensory analysis was frozen directly after manufacturing at -18 °C and defrosted overnight at 4 °C before the sensory analysis.

4.2.9.1. Bacon

Packets of bacon (250 g) from each of the four treatment groups were individually cooked as described under section 4.2.5.1. and kept warm (50 °C) in aluminium covered glass bowls in a convection oven. The oblong oval pieces of bacon were cut into pieces measuring 40 mm by 40 mm

before being placed in small glass bowls and covered with squares of aluminium foil that were then tucked in around the edges of the bowls. The bowls were kept warm (50 °C) on a hot plate with special care taken not to allow the samples to dry out or cook any further. Each container was marked with a randomized, three-digit code unique to each sample. Four glass bowls representing one of the four 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 consisting of staff and students from the Agricultural Building of the University of the Free State was used. The panel consisted of 59 females and 16 males, ranging from 19 to 61 years of age with an average age of 29 years. 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 so as to mask any possible colour variations between different samples.

Respondents were first expected to taste a paper strip 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, 4-page questionnaire consisting of four nine-point hedonic rankings per page, ranging from 1 = dislike extremely to 9 = like extremely (Table 4.4). The respondents were then expected to ranking each sample individually for the following attributes: taste, saltiness, texture and overall liking. Apple juice diluted with bottled water in a ratio of 1:3 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 (Guárdia 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 prior to sensory evaluation.

4.2.9.2. Polony

The nylon casings were removed and the polony was sliced into 5 mm thick disks. Individual disks were placed in odour-free, lidded plastic containers. The samples were kept at a room temperature of 22 °C shortly before presentation to panel members. 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 55 females and 20 males; ranging from 19 to 61 years of age with an average age of 31 years. All other procedures were carried out as for the sensory analysis of the bacon.

Table 4.4. Simplified example of the hedonic sranking 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

4.2.9.3. Bangers

Samples of each treatment group were defrosted overnight at 4 °C. The portions of different treatment groups were cooked as described under section 4.2.5.2. 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. 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 57 females and 18 males; ranging from 21 to 58 years of age with an average age of 34 years. All other procedures were carried out as for the sensory analysis of the bacon in 4.2.9.1.

4.2.10. Statistical analyses

All results were captured in multiple spread sheets in Microsoft Excel 2010. The experimental design consisted of four treatments and three replicates thereof for all three product types for most methods of analysis. Exclusions to this included: sensory analysis; refrigeration, shrinkage, thaw, and cooking losses; and yield where only one replicate per product type was used. An analysis of variance (ANOVA) procedure (NCSS, 2007) was used to determine the effect of salt replacement level, storage time and replicate on chemical composition, microbial quality, chemical stability and technological properties of the model meat products. Significant interactions between salt replacement level and storage time were analysed by two way analysis of variance (NCSS, 2007). Differences were considered statistically significant at the $P < 0.05$ level or lower. Treatment means were then compared by means of the Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

4.3. Results and discussion

The results of the three products were grouped and discussed in three separate sections. This was done to better organize the data as the results of the different product types shared no interactions.

4.3.1. Bacon – Main effects and interactions

Salt level, storage time and replicate were the main effects and salt level with storage time was identified as the main interaction between main effects (Table 4.5). Differences in added salt levels had the largest number of significant effects on various parameters of the bacon. Effects on changes in chemical parameters, colour, losses and sensory properties were the most numerous, while those on microbial parameters were limited. Storage time resulted in significant changes in chemical, microbial and colour parameters and were not applicable to chemical composition and sensory parameters nor refrigeration, thaw and cooking losses. Replicate had significant effects on more parameters than that of storage time. This emphasized the importance of using multiple replicates since variations in raw materials and environmental conditions are then accounted for. The interaction of salt level with storage time mainly affected colour parameters and to a lesser extent chemical stability and microbiological parameters.

4.3.2. Bacon – Shrinkage, processing yield, drip, and cooking losses

The NC group experienced a higher ($P < 0.05$) percentage of shrinkage after smoking compared to the LI group (Figure 4.1). No significant differences in shrinkage were found between the LI, HI and PC groups. With the addition of both NaCl and polyphosphate (LI, HI and PC) the myofibrils swell and take up more water (Offer, Knight, Jeacocke, Almond, Cousins, Elsey, et al., 1989) due to improved WHC which is directly related to yield (Van Laack, 1999). At higher NaCl levels the reverse may occur leading to shrinkage of the myofibrils that first leads to the internal accumulation of moisture and then to its eventual expulsion from the meat (Offer et al., 1989). Myofibrils from muscles with a high proportion of white fibres, such as pork, respond to lower concentrations of NaCl (Knight & Parsons, 1988).

Significant differences existed between the four treatment groups for the processing yield. The NC group had a lower ($P < 0.05$) yield compared to the LI group. The HI and PC groups had marginally but not significantly, lower yields respectively, compared to that of the LI group. It was expected that NaCl addition would improve the yield, and that an increase in yield would be directly correlatable with an increase in salt content. From these results it was concluded that a lower added NaCl level of only 0.80% (LI group) resulted in the highest overall processing yield compared to NaCl levels of 1.20-1.80% that are generally used in bacon (Pegg, 2004). These results may be explained by the fine balance that exists between salting-in and salting-out of a meat product as a result of brining.

Table 4.5. ANOVA of the main effects and interactions on various parameters of bacon formulated with different added NaCl levels.

Parameter	NaCl level	Storage time	Replicate	NaCl level X storage time
Chemical properties and composition of bacon immediately after manufacturing				
% Ash	$P < 0.001$	NSA	NS	NSA
% NaCl	$P < 0.001$	NSA	NS	NSA
mg NaCl/100 g	$P < 0.001$	NSA	NS	NSA
mg Na/100 g	$P < 0.001$	NSA	NS	NSA
% Moisture	NS	NSA	$P < 0.001$	NSA
a_w	NS	NSA	$P < 0.001$	NSA
pH	NS	NSA	$P < 0.001$	NSA
Changes in chemical parameters of bacon during storage at 4 ^oC up to 30 days				
% Moisture	NS	$P < 0.05$	$P < 0.001$	NS
pH	$P < 0.01$	$P < 0.001$	NS	$P < 0.001$
a_w	$P < 0.05$	$P < 0.001$	$P < 0.01$	NS
TBARS	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$
Changes in the microbiological parameters of bacon during storage at 4 ^oC up to 30 days				
TVC	$P < 0.05$	$P < 0.001$	$P < 0.01$	$P < 0.05$
Coliforms	NS	NS	$P < 0.001$	NS
E. coli	NS	NS	$P < 0.001$	NS
S. aureus	NS	NSA	$P < 0.05$	NS
Yeasts	NS	$P < 0.001$	NS	NS
Moulds	NS	NS	$P < 0.001$	NS
Changes in the colour parameters of bacon during storage at 4 ^oC up to 30 days				
L*	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$
a*	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.001$
b*	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Chroma	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Hue angle	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.05$
Refrigeration, thaw and cooking losses				
Yield on green (%)	$P < 0.05$	NSA	NSA	NSA
Shrink after smoking (%)	$P < 0.05$	NSA	NSA	NSA
Drip loss (%)	$P < 0.001$	NSA	NSA	NSA
Cooking loss (%)	$P < 0.001$	NSA	NSA	NSA
Total loss (%)	$P < 0.001$	NSA	NSA	NSA
Sensory properties of pork bacon with different NaCl levels				
Taste	$P < 0.001$	NSA	NSA	NSA
Texture	$P < 0.001$	NSA	NSA	NSA
Saltiness	$P < 0.001$	NSA	NSA	NSA
Overall liking	$P < 0.001$	NSA	NSA	NSA

NS = Not significant
NSA= Not statistically analysed

The amount of drip loss experienced by the NC group and to a limited extent, the LI group, was higher ($P < 0.001$) than the very limited drip losses of the HI and PC groups. In terms of cooking losses, as much as 40% of the weight of the meat can be lost as water (Offer et al., 1989). Significant differences ($P < 0.001$) were observed between multiple treatment groups: the NC group (42.24 ± 0.78%) had the highest cooking loss and the two intermediate groups of LI and HI had the expected amount of cooking losses at 40.16 ± 2.58% and 39.71 ± 4.50%, respectively. At the highest added NaCl level (2.50%) the PC group had a lower cooking loss of 32.92 ± 1.53% as a result of improved WHC leading to reduced cooking losses (Sofos, 1986). From the total losses as the sum of all the losses it was concluded that phosphate alone (NC group) could not sufficiently control losses to any significant extent. Surprisingly, an added NaCl level of 0.80% (LI group)

compared favourable to that of a 1.66% added NaCl level (HI group), although only the control level of 2.50% added NaCl (PC group) could greatly limit losses during processing and cooking.

Table 4.6. Shrinkage, processing yield, drip, cooking, and total losses of four bacon groups based on added NaCl content.

Treatment	Shrinkage after smoking (%)	Processing yield (%)	Drip loss (%)	Cooking loss (%)	Total loss (%)
	n = 12	*n* = 12	*n* = 12	*n* = 12	*n* = 12
NC	6.29 ± 2.19^{b}	11.03 ± 2.06^{a}	11.18 ± 2.37^{c}	42.24 ± 0.78^{b}	48.70 ± 1.53^{c}
LI	3.63 ± 1.56^{a}	13.51 ± 1.41^{b}	3.63 ± 0.55^{b}	40.16 ± 2.58^{b}	42.33 ± 2.42^{b}
HI	4.71 ± 0.57^{ab}	12.55 ± 0.53^{ab}	0.75 ± 0.27^{a}	39.71 ± 4.50^{b}	40.16 ± 4.57^{b}
PC	4.15 ± 1.68^{ab}	13.04 ± 1.51^{ab}	0.66 ± 0.49^{a}	32.92 ± 1.53^{a}	33.37 ± 1.64^{a}
Sign. level	$P < 0.05$	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl).
Means with different superscripts in the same row significantly.

4.3.3. Bacon − Chemical analyses

4.3.3.1. Bacon − Ash, NaCl and Na content

The mineral content of the four treatment groups were determined as the percentage ash present after preparing the samples for AAS (Table 4.7). The different added NaCl contents of each formulation resulted in different ($P < 0.001$) percentages of ash content. Higher percentages of ash correlated directly with higher percentages of added NaCl as the inorganic matter left behind mainly consisted of metal oxides, enhanced by the amount of NaCl added to the product (Honikel, 2008). The percentage of ash present also served as a crude method of confirming the identity of each bacon sample according to added NaCl content.

Salt was present at a base level of 109.34 ± 23.54 mg/100 g in the NC group with all contributions made by formulation components other than added NaCl. The NaCl content of each subsequent NaCl added treatment group increased ($P < 0.001$) with increasing amounts of added NaCl. One of the main factors limiting the accuracy of the Volhard method of analysis is that the amount of NaCl in a sample was indirectly calculated from the amount of chloride present in the sample. This totally disregarded the Na contributed to the final product originating from other sources that were not necessarily associated with chloride such as: sodium tripolyphosphate, monosodium glutamate, and sodium nitrite. The NaCl content was then converted to Na content values for the Na content per 100 g of product, using the percentage Na normally contributing to the molecular weight of pure NaCl (39.33%). Comparisons were drawn between the calculated Na content (converted from the

Volhard results) and the actual Na content (determined by AAS analysis). At a very low Na content (NC) this value grossly underestimated the actual Na content at 43.00 ± 9.26 mg/100 g compared to 190.83 ± 92.93 mg/100 g. This level represented a baseline Na content equal to the sum of all the contributed Na excluding NaCl, in all four formulations. At intermediate Na content (LI and HI); the calculated Na values were surprisingly close to the actual values. At high added NaCl content (PC) the Na content was overestimated (1062.69 ± 83.66 mg/100 g) compared to the actual value (980.83 ± 143.94 mg/100 g).

Table 4.7. Salt and Na content of four bacon formulations containing different added NaCl levels.

Treatment	**NC**	**LI**	**HI**	**PC**	**Sign. Level**
% Ash *n* = 12	$1.38^{a} \pm 0.12$	$2.18^{b} \pm 0.15$	$2.96^{c} \pm 0.43$	$4.15^{d} \pm 0.69$	$P < 0.001$
mg NaCl/100 g *n* =12	$109.34^{a} \pm 23.54$	$1008.75^{b} \pm 97.43$	$1641.39^{c} \pm 215.99$	$2702.00^{d} \pm 211.18$	$P < 0.001$
mg Na/100 g (converted) *n* = 12	$43.00^{a} \pm 9.26$	$396.74^{b} \pm 38.32$	$645.56^{c} \pm 84.95$	$1062.69^{d} \pm 83.06$	$P < 0.001$
mg Na/100 g (actual) *n* = 12	$190.83^{a} \pm 62.93$	$399.50^{b} \pm 81.79$	$662.83^{c} \pm 116.25$	$980.83^{d} \pm 143.94$	$P < 0.001$
mg Na/100 g (formulated)	~ 210.15	~ 536.59	~ 863.03	~ 1193.40	n.a.
Theoretical limits mg Na/100 g	n.a.	600	800	n.a.	n.a.

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same row differ significantly
n.a. = Not applicable

Formulated total Na content values gathered from estimating the contribution made by each component in the formulation safely overestimated the amount of Na present in each formulation of the three NaCl added treatment groups. The actual Na content of the two intermediate groups which were formulated not to exceed the precise regulatory limits, were found to be far below their respective limits. In a more practical situation, similar product formulations would possibly lend themselves to being fine-tuned to levels closer to the limits with the safety of a 20% tolerance for exceeding the regulatory limits.

4.3.3.2. Bacon − pH, a_w and moisture content

The pH values of the four treatment groups were closely grouped together after day 0 with no significant differences between the groups (Table 4.8). All the values were slightly higher after 15 days with no significant differences between treatment groups. Significant differences were only found after 30 days with that of the HI and PC groups being higher ($P < 0.001$) than that of the LI

and NC groups. If it was accepted that lower pH would improve microbial shelf-life stability due to the selection for LAB bacteria that would out compete possibly more problematic microorganisms (Gibson & Roberts, 1986), then the LI and NC groups had the more preferable pH values of the four groups. Another scenario that may explain the higher pH values of the HI and PC groups involves the synergistic relationship between NaCl and alkaline phosphates. The functionality of phosphates can be enhanced with greater amounts of NaCl being present (Ruusunen et al., 2002; Ruusunen & Puolanne, 2005).

Significant differences in the pH values of the NC and LI groups as a function of storage time (Table 4.9) supported the results of the treatment effect on pH. The pH values of both the NC and LI groups on day 0 and day 15 were higher ($P < 0.001$) than on day 30. The interaction between added NaCl level and storage time confirmed that pH values were less stable ($P < 0.001$) after 30 days at no (NC) and very low (LI) added NaCl content (Figure 4.1). It can be deduced that for the same phosphate levels, but differing added NaCl levels, the phosphate in the HI and PC groups provided greater buffering capacity against declines in pH.

Table 4.8. Changes in the basic chemical parameters of four bacon formulations containing different added NaCl levels over a 30 day shelf-life.

Day	Treatment	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level
		n = 12		*n* = 12		*n* = 12	
0	**NC**	5.77 ± 0.20	NS	0.9401 ± 0.0145	NS	74.84 ± 1.79	NS
	LI	5.72 ± 0.15		0.9400 ± 0.0138		73.61 ± 1.81	
	HI	5.74 ± 0.09		0.9391 ± 0.0772		75.14 ± 1.06	
	PC	5.76 ± 0.12		0.9322 ± 0.0075		73.40 ± 1.57	
15	**NC**	5.81 ± 0.14	NS	0.9448^{ab} ± 0.0116	P < 0.001	75.68 ± 0.88	NS
	LI	5.79 ± 0.09		0.9538^{b} ± 0.0054		74.39 ± 2.48	
	HI	5.85 ± 0.09		0.9491^{b} ± 0.0076		75.82 ± 1.07	
	PC	5.84 ± 0.15		0.9380^{a} ± 0.0085		74.40 ± 1.76	
30	**NC**	5.48^{a} ± 0.17	*P* < 0.001	0.9424 ± 0.0152	NS	74.84 ± 1.15	NS
	LI	5.53^{a} ± 0.15		0.9468 ± 0.0720		74.53 ± 1.83	
	HI	5.79^{b} ± 0.16		0.9454 ± 0.0058		74.31 ± 1.24	
	PC	5.73^{b} ± 0.13		0.9476 ± 0.0046		74.57 ± 1.32	

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
NS = Not significant

No significant differences in a_w were found between the four treatment groups after day 0 (Table 4.7). The a_w values of the NC and the LI groups were slightly higher than that of the HI and PC groups. This was attributed to the a_w-lowering effect of NaCl (Forsythe & Miller, 1980; Sebranek et al., 1983). After 15 days, the a_w of the LI (0.9538 ± 0.0054) and the HI (0.9491 ± 0.0076) groups

Table 4.9. The storage time effect over a 30 day shelf-life at 4 °C on the basic chemical parameters of four bacon formulations containing different added NaCl levels.

Treat.	Storage time	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level	TBARS (mEq MDA/kg)	Sign. level
		n = 12		*n* = 12		*n* = 12		*n* = 12	
NC	**Day 0**	$5.77^{b} \pm 0.20$	*P* < 0.001	0.9401 ± 0.0145	NS	74.84 ± 1.79	NS	$0.14^{c} \pm 0.02$	*P* < 0.001
	Day 15	$5.81^{b} \pm 0.14$		0.9448 ± 0.0116		75.68 ± 0.88		$0.11^{b} \pm 0.02$	
	Day 30	$5.48^{a} \pm 0.17$		0.9428 ± 0.0152		74.84 ± 1.15		$0.09^{a} \pm 0.01$	
LI	**Day 0**	$5.72^{b} \pm 0.15$	*P* < 0.001	$0.9400^{a} \pm 0.0138$	*P* = 0.005	73.61 ± 1.81	NS	$0.17^{c} \pm 0.02$	*P* < 0.001
	Day 15	$5.79^{b} \pm 0.09$		$0.9538^{b} \pm 0.0054$		74.39 ± 2.48		$0.14^{b} \pm 0.03$	
	Day 30	$5.53^{a} \pm 0.15$		$0.9468^{ab} \pm 0.072$		74.53 ± 1.83		$0.11^{a} \pm 0.02$	
HI	**Day 0**	5.74 ± 0.09	NS	$0.9391^{a} \pm 0.0072$	*P* < 0.005	$75.14^{ab} \pm 1.06$	*P* < 0.01	$0.19^{b} \pm 0.03$	*P* < 0.001
	Day 15	5.85 ± 0.09		$0.9491^{b} \pm 0.0076$		$75.82^{b} \pm 1.07$		$0.17^{b} \pm 0.03$	
	Day 30	5.79 ± 0.16		$0.9454^{ab} \pm 0.0058$		$74.31^{a} \pm 1.24$		$0.11^{a} \pm 0.02$	
PC	**Day 0**	5.76 ± 0.12	NS	$0.9322^{a} \pm 0.0075$	*P* < 0.001	73.40 ± 1.57	NS	$0.18^{b} \pm 0.04$	*P* < 0.001
	Day 15	5.84 ± 0.15		$0.9380^{a} \pm 0.0085$		74.40 ± 1.76		$0.17^{b} \pm 0.03$	
	Day 30	5.73 ± 0.13		$0.9476^{b} \pm 0.0046$		74.57 ± 1.32		$0.12^{a} \pm 0.02$	

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
NS = Not significant

were higher ($P < 0.001$) than that of the PC group (0.9380 ± 0.0085) which had the highest NaCl content (Table 4.8). After 30 days, there were no significant differences in a_w between any of the four groups. The a_w of the three NaCl containing groups, LI, HI, and PC were significantly affected by the storage time effect (Table 4.9). The a_w of the LI group was higher ($P = 0.05$) on day 15 than on day 0. This was also true for the HI group, where the a_w was higher ($P < 0.005$) on day 15 compared to on day 0. For the PC group this effect was delayed with a higher ($P < 0.001$) a_w on day 30 over that of both day 0 and day 15.

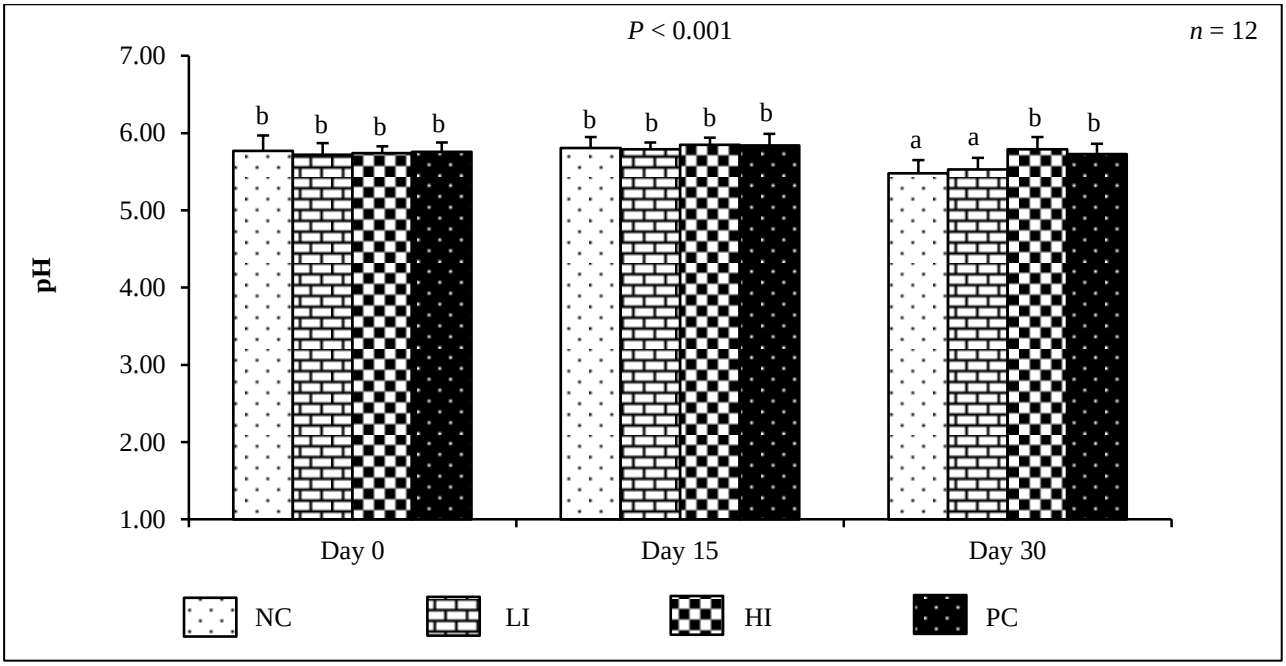

Figure 4.1. The effect of the interaction between added NaCl level and storage time on the pH of bacon during storage at 4 °C for up to 30 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

Moisture content remained constant throughout the 30-day shelf-life with no significant differences between any of the four treatments (Table 4.8). In terms of the storage time effect, HI had a higher ($P < 0.01$) moisture content on day 15 compared to day 30 (Table 4.9). The fact that the moisture content generally remained constant regardless of treatment or storage time was a direct result of the portions of bacon being packaged in vacuum bags with very low moisture permeability and that no drying effect was possible which could have affected the a_w of any of the treatment groups.

4.3.3.3. Bacon − Lipid oxidative stability

Lipid oxidative stability of the bacon was determined in terms of the quantification of TBARS over time with MDA as indicator of the level of secondary lipid oxidation products (SLOP) in the four

treatment groups. Salt acts as pro-oxidant working in synergy with the heme and non-heme iron in meat products to contribute to accelerated lipid oxidation through catalytic reactions (Rhee & Ziprin, 2001; Kiliç, Şimşek, Claus, & Atilgan, 2014). It reportedly also acts as pro-oxidant on its own at inclusion levels between 0.5 and 5% (Rhee & Ziprin, 2001), causing damage to lipids which negatively affects food quality (Decker, 1998). On day 0, significant differences were already found between treatment groups with the three treatments containing additional salt being more oxidised (Figure 4.2). The TBARS of the NC group (0.14 ± 0.02 mg MDA/kg) was lower ($P < 0.05$) than that of the three added NaCl groups of LI (0.17 ± 0.03 mg MDA/kg), HI (0.19 ± 0.03 mg MDA/kg) and PC (0.18 ± 0.04 mg MDA/kg) with all TBARS values far below that of a organoleptic threshold of 0.5 mg MDA/kg (Wood, Enser, Fisher, Nute, Sheard, Richardson, Hughes, & Whittington, 2008)

This result could be explained by the fact that day 0 of the bacon shelf-life evaluation was actually ~ 48 h after the loins were originally injected with brine either containing added NaCl or not. Therefore, NaCl where present, already had substantial time to contribute to lipid oxidation reactions. After day 15, the level of TBARS decreased regardless of the added NaCl content. This resulted in the NC (0.11 ± 0.02 mg MDA/kg) and LI (0.14 ± 0.03 mg MDA/kg) groups having lower ($P < 0.002$) TBARS than that of the HI (0.17 ± 0.03 mg MDA/kg) and PC (0.17 ± 0.03 mg MDA/kg) groups. After day 30 the TBARS of all four treatment groups were at their lowest levels with only that of the NC group (0.09 ± 0.01 mg MDA/kg) lower ($P < 0.005$) than that of the PC group (0.12 ± 0.02 mg MDA/kg). Decreased TBARS may be explained by interactions between lipid and protein oxidation. It has been theorised that MDA may be used as a substrate in one of many possible protein oxidation (coinciding with lipid oxidation) pathways (Xiong, 2000) whereby the concentration of MDA would then decrease as a result. The storage time effect on lipid oxidative stability supported this theory as significant decreases in TBARS levels correlated inversely with the progression of time (Table 4.8). For the NC and LI groups, TBARS levels were lower ($P < 0.001$) at each successive point in time. The TBARS levels of the HI and PC groups were only lower ($P < 0.001$) after 30 days. The interaction between added NaCl level and storage time confirmed this in that the three NaCl added groups with higher ($P < 0.05$) TBARS (0.17 ± 0.03 mg MDA/kg, 0.19 ± 0.03 mg MDA/kg, and 0.18 ± 0.04 mg MDA/kg respectively) than the NC group (0.14 ± 0.02 mg MDA/kg) on day 0, had the most reduced TBARS after day 30 (0.11 ± 0.02 mg MDA/kg, 0.11 ± 0.02 mg MDA/kg, and 0.12 ± 0.02 mg MDA/kg respectively) compared to that of the NC group (0.09 ± 0.01 mg MDA/kg) (Figure 4.3).

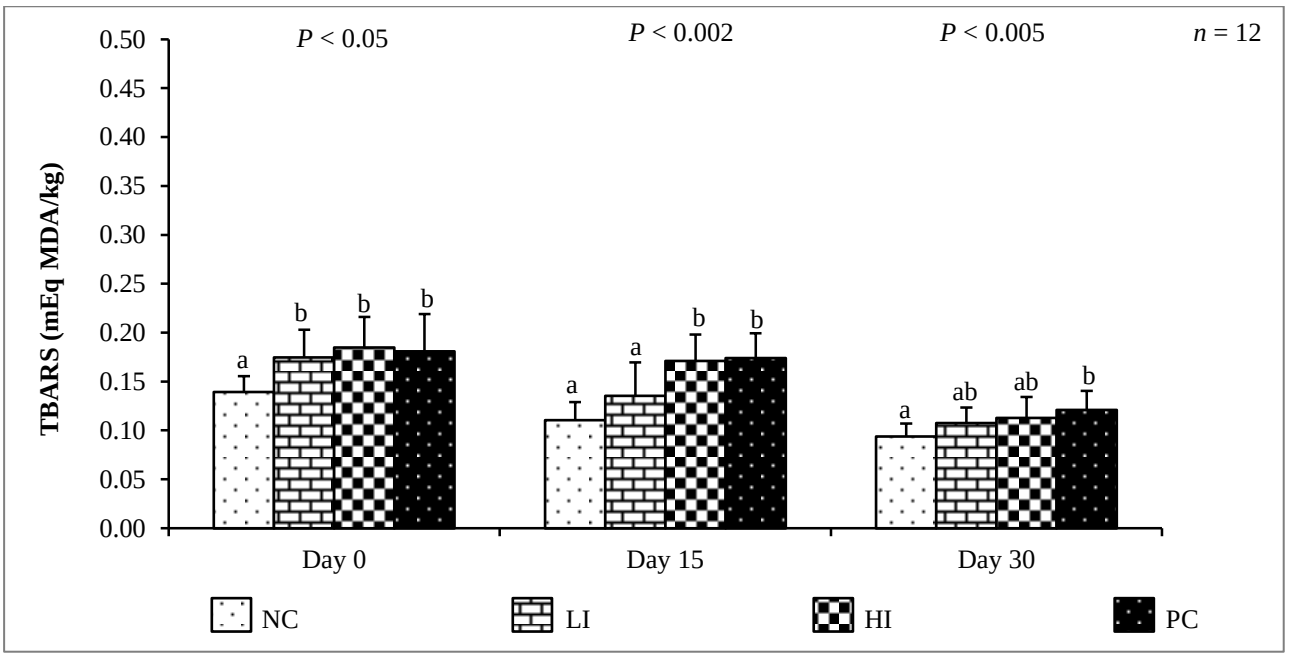

Figure 4.2. The effect of added NaCl level on the TBARS of bacon stored at 4 °C for up to 30 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means.

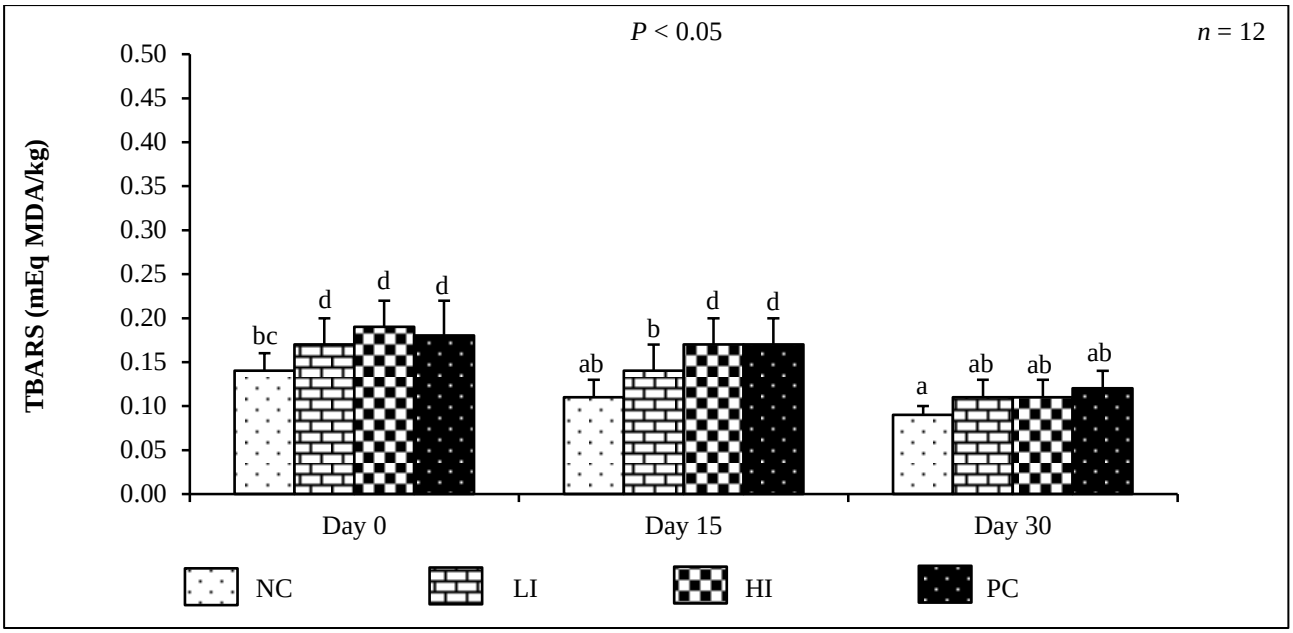

Figure 4.3. The effect of the interaction between added NaCl level and storage time on the TBARS of bacon during storage at 4 °C for up to 30 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

4.3.4. Bacon – Microbial analyses

After manufacturing, the four treatment groups had an average TVC of ~ log 2.50 cfu/g with no significant differences between treatments (Table 4.10). At this stage the bacon products already underwent a 3-day processing period and sampling after manufacturing only took place on the fourth day. The processing period progressed as follows: on the first day the loins were injected and

Table 4.10. The results of the microbial analyses performed on four bacon formulations containing different added NaCl levels.

Day	Treatment	TVC (log cfu/g) n = 12	Sign. level	Coliforms (log cfu/g) n =12	Sign. level	*E. coli* (log cfu/g) n = 12	Sign. level	*S. aureus* (log cfu/g) n =12	Sign. level	Yeasts (log cfu/g) n = 12	Sign. level	Moulds (log cfu/g) n = 12	Sign. level
0	**NC**	2.62 ± 0.33		0.32 ± 0.76		0.23 ± 0.54		0.01 ± 0.01		0.28 ± 0.50		0.48 ± 0.61	
	LI	2.52 ± 0.26	NS	0.21 ± 0.49	NS	0.19 ± 0.45	NS	0.01 ± 0.01	NS	0.22 ± 0.51	NS	0.17 ± 0.39	NS
	HI	2.54 ± 0.44		0.28 ± 0.50		0.19 ± 0.45		0.11 ± 0.38		0.28 ± 0.50		0.73 ± 1.09	
	PC	2.33 ± 0.73		0.11 ± 0.38		0.08 ± 0.29		0.11 ± 0.38		0.62 ± 0.77		0.52 ± 0.81	
15	**NC**	5.43^{b} ± 1.26		0.49 ± 0.90		0.33^{b} ± 0.60		0.01 ± 0.01		0.32 ± 1.11		0.62 ± 0.67	
	LI	5.16^{ab} ± 1.47	$P < 0.05$	0.01 ± 0.01	NS	0.01^{a} ± 0.01	$P < 0.05$	0.01 ± 0.01	NS	0.21 ± 0.49	NS	0.22 ± 0.52	NS
	HI	4.01^{a} ± 1.47		0.11 ± 0.38		0.01^{a} ± 0.01		0.08 ± 0.29		0.30 ± 0.56		0.55 ± 0.85	
	PC	3.96^{a} ± 1.55		0.01 ± 0.01		0.01^{a} ± 0.01		0.08 ± 0.29		0.58 ± 0.89		0.40 ± 0.60	
30	**NC**	6.68 ± 0.23		0.18 ± 0.63		ND		ND		0.70 ± 1.09		0.95 ± 1.25	
	LI	6.87 ± 0.67	NS	0.01 ± 0.01	NS	ND	NSA	ND	NSA	1.76 ± 1.87	NS	0.01 ± 0.01	NS
	HI	7.10 ± 0.56		0.08 ± 0.29		ND		ND		1.10 ± 1.63		0.97 ± 1.28	
	PC	6.70 ± 0.53		0.01 ± 0.01		ND		ND		1.43 ± 1.80		0.88 ± 1.30	

TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*
NC = negative control (0.00% added NaCl); LI = lower intermediate (0.83% added NaCl); HI = higher intermediate (1.66% added NaCl); and PC = higher intermediate (2.50% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
ND = Not detected
NSA = Not statistically analysed
NS = Not significant

stored at 4 °C; on the second day they were smoked and frozen to -18 °C; on the third day they were stored at 4 °C to reach the final tempering temperature of ~ -1°C; before being sliced and packaged on the fourth day as day 0. It was suspected that this process greatly limited microbial metabolism and growth. Therefore, it was accepted that the differences in added NaCl content had little overall effect on the TVC of the four treatment groups. It was only after slicing and packaging and the increase in temperature to 4 °C that the products would have provided environments conducive to the active metabolism and growth of microorganisms. Halfway through the shelf-life, by day 15, significant differences were found between several treatments. The TVC of the NC group of log 5.43 ± 1.26 cfu/g was higher ($P < 0.05$) than both that of the HI group at log 4.01 ± 1.47 cfu/g and that of the PC group at log 3.90 ± 1.55 cfu/g. These results were explained by the fact that after 15 days the levels of added NaCl, the interactions thereof with sodium nitrite and sodium phosphate (Doyle, 2008), the low oxygen environment and low refrigeration temperature were expected to act as a combination of hurdles (Leistner, 1999) that differentially challenged the growth of the microorganisms present. These conditions were also contributed to the selection of a subset of microorganisms, probably dominated by LAB, capable of adapting to the conditions (Greer et al., 1993) and growing to the higher numbers found after 15 days.

After 30 days, there was an overall increase in TVC across all four treatment groups resulting in no significant differences between treatments. All four treatment groups exceeded the limit of log 6 cfu/g (SANS 885, 2011) for TVC after 30 days with that of the HI group being the highest at 7.10 ± 0.56 log cfu/g although upon opening the packets for analyses, no off- or sour flavours were detected. It is conceivable that the lower added NaCl levels of the NC and LI groups resulted in higher counts earlier in the shelf-life and reached a point where the populations decreased again with that of the HI group only peaking close to the end of the 30 day shelf-life. The storage time also significantly affected the TVC of the four treatment groups (Table 4.11). For all four treatment groups the TVC were higher ($P < 0.001$) on day 15 than on day 0, and were again higher ($P < 0.001$) on day 30 than on day 15. These results showed that the TVC eventually increased significantly, regardless of added NaCl level, to the extent that all four treatment groups exceeded hygienic limits at the end of shelf-life. The interaction between added NaCl level and storage time further revealed that, although all four treatments initially had the same TVC, and experienced significant ($P < 0.05$) increases, the increases for the three NaCl added groups were not significantly different to each other at each sampling interval (Figure 4.4).

Low coliform counts were found on day 0 and these counts decreased up to day 30 (Table 4.10). No significant differences were found between any of the treatment groups on the basis of treatment type. Storage time had no significant effect on the coliform counts in part due to the counts being

Table 4.11. The storage time effect over a 30 day shelf-life at 4 °C on the microbial analyses results of four bacon formulations containing different added NaCl levels.

Treat-ment	Storage time	TVC (log cfu/g) *n* = 12	Sign. level	Coliforms (log cfu/g) *n* =12	Sign. level	*E. coli* (log cfu/g) *n* = 12	Sign. level	*S. aureus* (log cfu/g) *n* =12	Sign. level	Yeasts (log cfu/g) *n* = 12	Sign. level	Moulds (log cfu/g) *n* = 12	Sign. level
	Day 0	$2.62^{a} \pm 0.33$		0.32 ± 0.76		0.23 ± 0.54		0.01 ± 0.01		0.28 ± 0.61		0.48 ± 0.61	
NC	**Day 15**	$5.43^{b} \pm 1.26$	$P < 0.001$	0.49 ± 0.90	NS	0.33 ± 0.60	NS	0.01 ± 0.01	NSA	0.32 ± 1.11	NS	0.62 ± 0.67	NS
	Day 30	$6.68^{c} \pm 0.23$		0.18 ± 0.63		ND		ND		0.70 ± 1.09		0.95 ± 1.25	
	Day 0	$2.52^{a} \pm 0.26$		0.21 ± 0.49		0.19 ± 0.45		0.01 ± 0.01		$0.22^{a} \pm 0.51$		0.17 ± 0.39	
LI	**Day 15**	$5.16^{b} \pm 1.47$	$P < 0.001$	0.01 ± 0.01	NS	0.01 ± 0.01	NS	0.01 ± 0.01	NSA	$0.21^{a} \pm 0.49$	$P < 0.005$	0.22 ± 0.52	NS
	Day 30	$6.87^{c} \pm 0.67$		0.01 ± 0.001		ND		ND		$1.76^{b} \pm 1.87$		0.01 ± 0.01	
	Day 0	$2.54^{a} \pm 0.44$		0.28 ± 0.50		0.19 ± 0.45		0.11 ± 0.38		0.28 ± 0.50		0.73 ± 1.09	
HI	**Day 15**	$4.01^{b} \pm 1.47$	$P < 0.001$	0.11 ± 0.38	NS	0.01 ± 0.01	NS	0.08 ± 0.29	NSA	0.30 ± 0.56	NS	0.55 ± 0.85	NS
	Day 30	$7.10^{c} \pm 0.56$		0.08 ± 0.29		ND		ND		1.10 ± 1.63		0.97 ± 1.28	
	Day 0	$2.33^{a} \pm 0.73$		0.11 ± 0.38		0.08 ± 0.29		0.11 ± 0.38		0.62 ± 0.77		0.52 ± 0.81	
PC	**Day 15**	$3.96^{b} \pm 1.55$	$P < 0.001$	0.01 ± 0.01	NS	0.01 ± 0.01	NS	0.08 ± 0.29	NSA	0.58 ± 0.89	NS	0.40 ± 0.60	NS
	Day 30	$6.70^{c} \pm 0.53$		0.01 ± 0.01		ND		ND		1.43 ± 1.80		0.88 ± 1.30	

TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*
NC = negative control (0.00% added NaCl); LI = lower intermediate (0.83% added NaCl); HI = higher intermediate (1.66% added NaCl); and PC = higher intermediate (2.50% added NaCl)
Means with different superscripts in the same column and for the same treatment differ significantly
ND = Not detected
NSA = Not statistically analysed
NS = Not significant

low at certain time intervals with high levels of variation between samples (Table 4.11). In terms of the effect of treatment (Table 4.10) on *E. coli,* the initial numbers present were low and within the hygienic limit of < 10 cfu/g (SANS 885, 2011). After 15 days the counts of the NC group had increased ($P < 0.05$) while that of the other three groups experienced a sharp decrease in *E. coli* counts. After 30 days, no *E. coli* was found in any of the treatment groups. In addition to *E. coli* not being detected in any of the treatment groups on day 30, there were no significant storage time effects between day 0 and day 15 for any treatment group (Table 4.11).

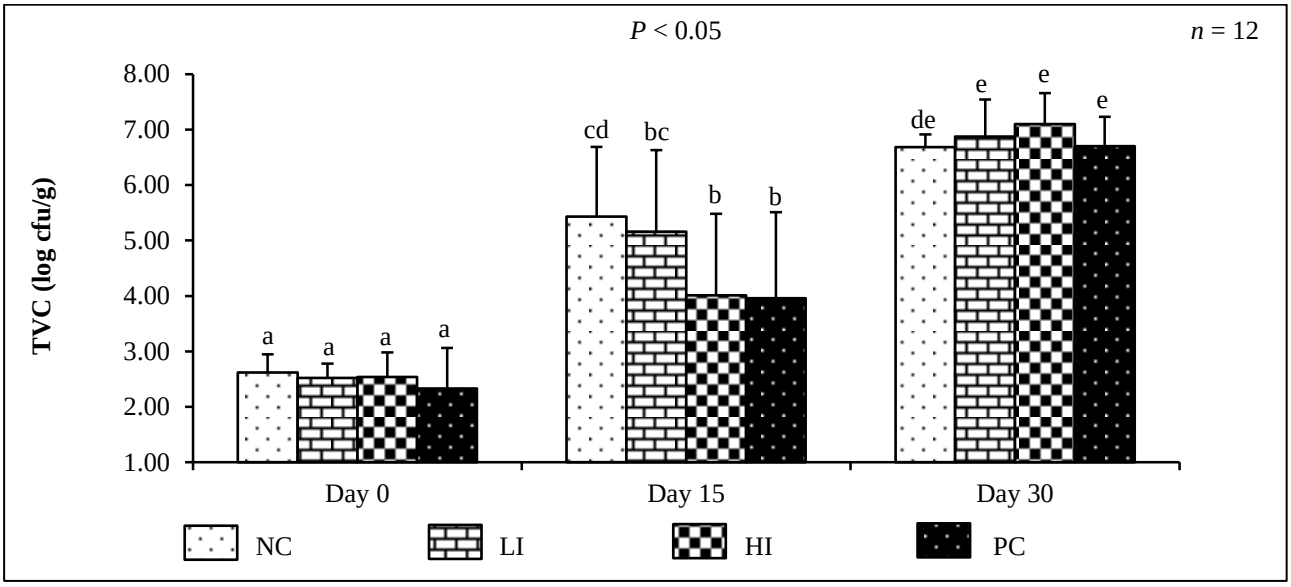

Figure 4.4. The effect of the interaction between added NaCl level and storage time on the TVC of bacon during storage at 4 °C for up to 30 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

Staphylococcus aureus counts were found to be very low on day 0 with slightly lower (NS) counts for the two higher added NaCl groups of HI and PC (Table 4.10). All counts were lower than the < 20 cfu/g limit (SANS 885, 2011). Similar non-significant results were found after 15 days and after 30 days, no *S. aureus* was detected in any of the treatment groups. With the very low *S. aureus* counts on day 0 and day 15, and undetectable levels on day 30, no storage time effect could be assumed beyond that of the *S. aureus* completely dying-off over time regardless of added NaCl level (Table 4.11). It was suspected that the inclusion of sodium ascorbate and sodium nitrite, smoking and vacuum-packaging provided additional hurdles that severely affected the survival of these bacteria. In addition, it was suspected that LAB contributed to the decreased survival of these bacteria as they generally out-compete and dominate the microbiota of meat products under similar conditions (Lücke, 1998).

Yeast and mould counts were very low throughout the 30 days with no significant differences between any of the treatment groups (Table 410). The markedly higher counts of yeasts over that of moulds in the LI, HI and PC groups after 30 days follow the convention that yeasts can be found in greater numbers in meat products (Fleet, 1992; Osei Abunyewa et al., 2000). In contrast to the findings of Aaslyng et al. (2014) where the NaCl content of bacon was reduced to 20 and 25%, the shelf-life of these intermediate added NaCl bacon models (LI and HI) did not appear to suffer any serious defects with added NaCl levels reduced 68% and 33.60%, respectively. Storage time had a significant effect on some of the yeast counts and none of the mould counts (Table 4.11). The yeast counts of the LI group were lower ($P < 0.005$) on day 0 and day 15 than on day 30.

4.3.5. Bacon − Physical analyses: colour

Significant effects due to added NaCl content were observed at every sampling point for every colour parameter (Table 4.12). Lightness indicated by L^* was the highest ($P < 0.001$) in the NC bacon on days 0, 15 and 30, followed by significantly darker bacon at each successively higher added NaCl level. From day 0 to day 15 the L^* of the NC group increased while that of the LI and HI groups decreased so as to be statistically similar. From day 15 to day 30 the L^* of the NC group increased to its highest level, that of the LI group increased again and that of the HI group decreased slightly. Throughout the 30 days, the L^* of the PC group decreased slightly after day 15 and then increased again after day 30 with the smallest variations of any of the four treatment groups. The effect of storage time on L^* was limited to the NC and LI groups (Table 4.13). The NC group experienced a significant ($P < 0.001$) increase in L^* from day 0 up to day 30, while that of the LI group decreased ($P < 0.001$) from day 0 to day 15 and then increased almost back to the original level after day 30. These changes may be linked to changes over time in the pH of these two groups (Table 4.9), resulting in changes in the WHC causing more water to be present on the surface of the bacon, which in turn affected the amount of light reflected (measured as L^*). The interaction between added NaCl level and storage time revealed that L^*-values tended to be lower ($P < 0.001$) and more stable at higher added NaCl over time and vice versa (Table 4.14).

The lightness of meat is reportedly unrelated to the oxidative status of myoglobin (McKenna, Mies, Baird, Pfeiffer, Ellebracht, & Savell, 2005), but closely related to muscle structure (MacDougall, 1982) which affects WHC (Ripoll et al., 2011). In relation to muscle structure, it has been proposed that the light scattering ability of the myofibrils plays a role in lightness due to their expandability (Offer & Trinick, 1983), a theory most probably best suited to meat products with a whole muscle structure such as bacon. The WHC of the NC, LI and HI groups were lower (which was confirmed by higher losses discussed under section 4.3.6.), therefore, the myofibrils would have had greater

Table 4.12. Changes in the colour parameters of four bacon formulations containing different added NaCl levels over a 30 day shelf-life.

Day	Treatment	*L**	Sign. level	*a**	Sign. level	*b**	Sign.	*C**	Sign. level	*H**	Sign. level
		n = 72		*n* = 72		*n* = 72	Level	*n* = 72		*n* = 72	
0	NC	$55.35^{d} \pm 2.99$	$P < 0.001$	$6.95^{b} \pm 1.55$	$P < 0.001$	$5.71^{b} \pm 1.30$	$P < 0.001$	$9.04^{b} \pm 1.83$	$P < 0.001$	$39.34^{c} \pm 5.48$	$P < 0.001$
	LI	$50.45^{c} \pm 3.40$		$7.29^{b} \pm 1.88$		$5.13^{b} \pm 1.88$		$8.99^{b} \pm 2.39$		$34.43^{b} \pm 7.67$	
	HI	$48.12^{b} \pm 4.39$		$5.93^{a} \pm 1.67$		$3.96^{a} \pm 1.56$		$7.23^{a} \pm 1.94$		$33.55^{b} \pm 10.04$	
	PC	$46.02^{a} \pm 3.06$		$6.58^{ab} \pm 1.63$		$3.75^{a} \pm 2.16$		$7.70^{a} \pm 2.31$		$28.10^{a} \pm 9.94$	
15	NC	$57.57^{c} \pm 2.15$	$P < 0.001$	$6.53^{b} \pm 1.36$	$P < 0.001$	$6.33^{c} \pm 1.34$	$P < 0.001$	$9.13^{b} \pm 1.76$	$P < 0.001$	$44.08^{c} \pm 4.70$	$P < 0.001$
	LI	$48.85^{b} \pm 2.02$		$6.99^{b} \pm 2.01$		$5.32^{b} \pm 1.49$		$8.84^{b} \pm 2.27$		$37.50^{b} \pm 7.01$	
	HI	$47.80^{b} \pm 3.60$		$5.65^{a} \pm 1.40$		$4.37^{a} \pm 1.47$		$7.22^{a} \pm 1.71$		$37.25^{b} \pm 8.90$	
	PC	$45.84^{a} \pm 3.14$		$6.72^{b} \pm 1.16$		$4.06^{a} \pm 1.82$		$7.98^{a} \pm 1.62$		$30.08^{a} \pm 9.96$	
30	NC	$60.10^{d} \pm 1.50$	$P < 0.001$	$7.25^{c} \pm 1.58$	$P < 0.001$	$7.72^{c} \pm 0.93$	$P < 0.001$	$10.64^{c} \pm 1.47$	$P < 0.001$	$47.20^{c} \pm 5.83$	$P < 0.001$
	LI	$50.07^{c} \pm 1.75$		$6.47^{b} \pm 1.51$		$6.57^{b} \pm 1.91$		$9.31^{b} \pm 2.08$		$44.94^{bc} \pm 8.27$	
	HI	$47.75^{b} \pm 3.47$		$6.77^{bc} \pm 2.26$		$6.06^{b} \pm 1.84$		$9.23^{b} \pm 2.43$		$42.41^{ab} \pm 9.96$	
	PC	$46.49^{a} \pm 2.21$		$5.42^{a} \pm 1.49$		$4.79^{a} \pm 1.77$		$7.33^{a} \pm 1.97$		$40.65^{a} \pm 10.28$	

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and on the same day differ significantly

NS = Not significant

gaps between individual filaments, increasing light scattering and resulting in higher *L**-values and vice-versa for the PC group with higher WHC.

Meat colour is important in the consumer purchasing decision (Mancini & Hunt, 2005) and redness is the most important aspect of colour for objective pork quality evaluation (Dvorak, Musilova, & Svarcova, 2001). The *a**-values of the bacon groups were found to fluctuate during storage with no consistent deterioration towards the end of shelf-life (Table 4.12). The sodium nitrite added to the brines was attributed to the initial stabilisation of the characteristic cured meat colour under vacuum and through its anti-oxidative nature (Arneth, 2001; Mancini, 2013) maintain the colour stability over time in concert with the anti-oxidative effect of the sodium tripolyphosphate (Trout & Schmidt, 1984). Initially, the NC and LI groups had higher ($P < 0.001$) *a**-values than the HI group and non-significantly higher *a**-values than the PC group. After day 15, the *a**-values of the NC, LI and PC groups were higher ($P < 0.001$) than that of the HI group. After day 30, the results better exhibited the expected outcomes with the highest *a**-value for the NC group, lower ($P < 0.001$) *a**-values for both the LI and HI groups and due to a sharp decrease in redness stability, a lowest *a**-value for the PC group attributed to a high NaCl content that negatively affected redness stability (Andersen et al., 1990; Trout, 1990).

Storage time significantly affected the *a** of all four treatments (Table 4.13). In the NC group, *a** decreased slightly from day 0 to day 15 and then increased to such an extent that the effect was significant ($P < 0.05$) by day 30. The *a** of the LI group decreased continuously with the *a** on day 30 being lower ($P < 0.05$) than that of day 0. In the HI group, *a** also decreased slightly from day 0 to day 15 followed by an increase in *a** that was higher ($P < 0.001$) than both of the previous *a** levels. The PC group was the only group that experienced a slight increase in *a** from day 0 to day 15, followed by a significant ($P < 0.001$) decrease by day 30. The interaction between added NaCl level and storage time revealed that *a**-values tended to be higher ($P < 0.001$) and more stable at no or lower added NaCl levels over time (Figure 4.5). In contrast to the *a**-values, *b**-values consistently increased over time in all four treatment groups. The NC group maintained higher ($P < 0.001$) *b**-values over the other three groups while the PC group maintained the significantly lowest *b**-values of the three NaCl added groups. The increase in *b**-values may have been linked to the different states of myoglobin over time. The *b**-values of all four treatments increased significantly as a function of storage time (Table 4.13). In the NC group the increase in *b** was significant ($P < 0.001$) at each successive time interval. These increases were only significant for the LI ($P < 0.001$), the HI group ($P < 0.001$), and the PC group ($P < 0.001$) between day 0 and day 30. Normally, oxygenation (myoglobin to oxymyoglobin) and oxidation (oxymyoglobin to metmyoglobin) has been shown to increase *b**-values (Fernández-López, Pérez-Alvarez, & Sayas-Barberá, 2000). In

Table 4.13. The storage time effect over a 30 day shelf-life at 4 °C on the colour parameters of four bacon formulations with different added NaCl levels.

Treat.	Storage time	*L** n = 72	Sign. level	*a** n = 72	Sign. level	*b** n = 72	Sign. level	*C** n = 72	Sign. level	*H** n = 72	Sign. level
NC	**Day 0**	$55.35^{a} \pm 2.99$	$P < 0.001$	$6.95^{ab} \pm 1.55$	$P < 0.05$	$5.71^{a} \pm 1.30$	$P < 0.001$	$9.04^{a} \pm 1.83$	$P < 0.001$	$39.35^{a} \pm 5.48$	$P < 0.001$
	Day 15	$57.57^{b} \pm 2.15$		$6.53^{a} \pm 1.36$		$6.33^{b} \pm 1.34$		$9.13^{a} \pm 1.76$		$44.08^{b} \pm 4.70$	
	Day 30	$60.10^{c} \pm 1.50$		$7.25^{b} \pm 1.58$		$7.72^{c} \pm 0.93$		$10.64^{b} \pm 1.47$		$47.20^{c} \pm 5.83$	
LI	**Day 0**	$50.45^{b} \pm 3.40$	$P < 0.001$	$7.29^{b} \pm 1.88$	$P < 0.05$	$5.13^{a} \pm 1.88$	$P < 0.001$	8.99 ± 2.39	NS	$34.43^{a} \pm 7.67$	$P < 0.001$
	Day 15	$48.85^{a} \pm 2.02$		$6.99^{ab} \pm 2.01$		$5.32^{a} \pm 1.49$		8.84 ± 2.27		$37.50^{b} \pm 7.01$	
	Day 30	$50.07^{b} \pm 1.75$		$6.47^{a} \pm 1.51$		$6.57^{b} \pm 1.91$		9.31 ± 2.08		$44.94^{c} \pm 8.27$	
HI	**Day 0**	48.12 ± 4.39	NS	$5.93^{a} \pm 1.67$	$P < 0.001$	$3.96^{a} \pm 1.56$	$P < 0.001$	$7.23^{a} \pm 1.94$	$P < 0.001$	$33.55^{a} \pm 10.04$	$P < 0.001$
	Day 15	47.80 ± 3.60		$5.65^{a} \pm 1.40$		$4.37^{a} \pm 1.47$		$7.22^{a} \pm 1.71$		$37.25^{a} \pm 8.90$	
	Day 30	47.75 ± 3.47		$6.77^{b} \pm 2.26$		$6.06^{b} \pm 1.84$		$9.23^{b} \pm 2.43$		$42.41^{b} \pm 9.96$	
PC	**Day 0**	46.02 ± 3.06	NS	$6.58^{b} \pm 1.63$	$P < 0.001$	$3.75^{a} \pm 2.16$	$P < 0.05$	7.70 ± 2.31	NS	$28.10^{a} \pm 9.94$	$P < 0.001$
	Day 15	45.84 ± 3.14		$6.72^{b} \pm 1.16$		$4.06^{ab} \pm 1.82$		7.98 ± 1.62		$30.08^{a} \pm 9.96$	
	Day 30	46.49 ± 2.21		$5.42^{a} \pm 1.49$		$4.79^{b} \pm 1.77$		7.33 ± 1.97		$40.65^{b} \pm 10.28$	

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and for the same treatment differ significantly

NS = Not significant

this situation, where vacuum-packaging was applied, this had a limited correlation with decreased redness possibly due to an overwhelming contribution made to redness by the formation of nitrosomyoglobin in the presence of sodium nitrite (Mancini, 2013). Display lighting is also reported to contribute significantly to the deterioration of *a**, *b** and *C** (Seyfert, Hunt, Grobbel, Ryan, Johnson, & Monderen, 2006) although it appeared to have little effect on the bacon in this study.

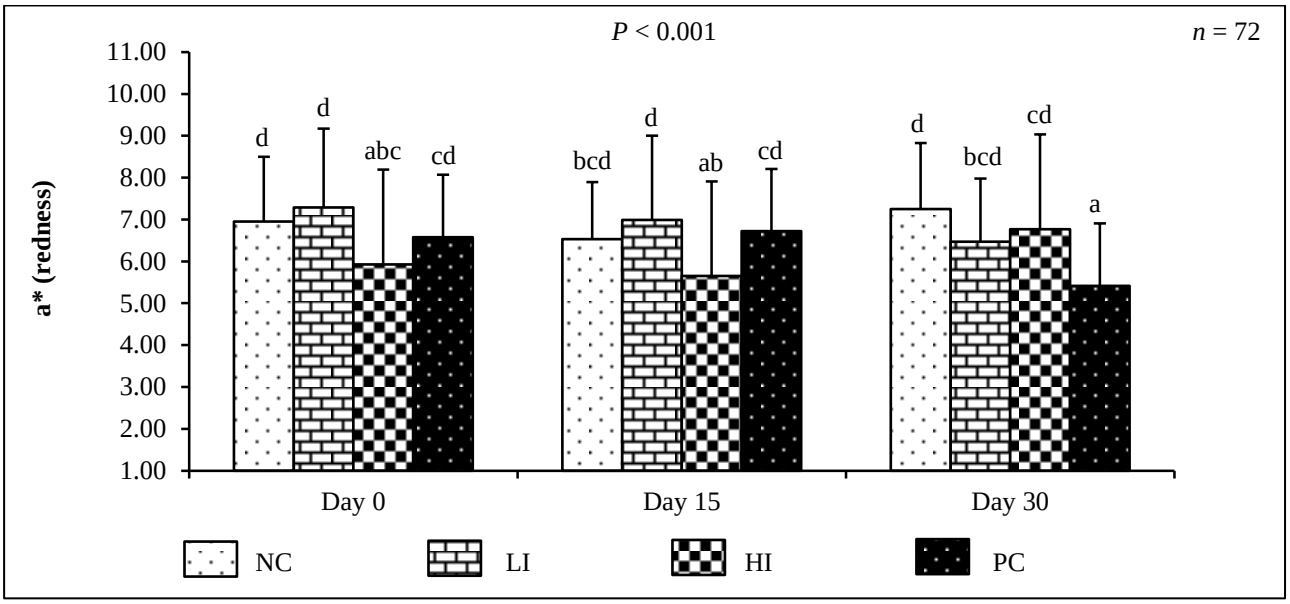

Figure 4.5. The effect of the interaction between added NaCl level and storage time on the *a**-values of bacon during storage at 4 °C for up to 30 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

Chroma of the bacon was determined to reflect the loss in colour brightness with a progression toward discolouration as greying. After day 0 and day, 15 both the NC and LI groups maintained higher ($P < 0.001$) *C**-values compared to the HI and PC groups. After day 30, there was even greater differentiation between treatments. Higher ($P < 0.001$) *C**-values were found for the NC group compared to the other three groups and the *C**-values of the LI and HI groups were also higher ($P < 0.001$) than that of the PC group. The use of both the *a**-values and *b**-values in calculating the *C**-values may have compounded the seemingly random changes in colour to the point that the effect of oxidation linked to NaCl content become discernable. Storage time resulted in significant differences in the *C**-values of the NC and HI groups (Table 4.13). For both of these treatment groups, *C** increased ($P < 0.001$ in both cases) from day 0 to day 30. This resulted in bacon with different added NaCl content experiencing differences in colour brightness at different times.

The interaction between added NaCl level and storage time revealed that in the absence of added NaCl, the bacon colour was the brightest at any given time interval and increased ($P < 0.001$) from day 0 to day 30 (Table 4.14). At the lower intermediate added NaCl level it remained fairly stable and at the higher intermediate added NaCl level it increased ($P < 0.001$) from day 0 to day 30. At the highest added NaCl level there were only minor fluctuations in brightness.

Hue angle represented the purity of colour with *H** nearer to 0° being closer to red and *H** nearer to 90° being closer to yellow. After day 0 the NC group had a greater ($P < 0.001$) *H** over that of the LI and HI groups, which in turn had greater *H** over that of the PC group (Table 4.12). The same level of significant differences ($P < 0.001$) were found after day 15 albeit at greater *H** for all four treatment groups. Hue angle continued to increase to after day 30 with the NC and LI having similar and greater *H** and the HI and PC groups having similar and smaller *H**. Comparing the results of *H** to that of *a** and *b** it was clear that over time, all the H*-values progressed to purer yellow and that added NaCl content was directly proportional to the ability of the treatment group to be inclined towards purer red. The effect of storage time on *H** was confirmed for all four treatment groups (Table 4.13). In the NC and LI groups the *H** increased ($P < 0.001$) at each successive time interval. In the HI and PC groups the *H** were only greater ($P < 0.001$) after 30 days. The interaction between added NaCl level and storage time revealed that *H** and increases in *H** were inversely proportional to added NaCl level over time (Table 4.14). As an example, the *H**-value of the NC group was already greater ($P < 0.05$) on day 15 than on day 0, while that of the LI, HI and PC groups were only slightly larger at the same point in time.

Table 4.14. The effects of the interaction between added NaCl level and storage time on various colour parameters of bacon during storage at 4 °C for up to 30 days.

Day	Treatment	*L**	*C**	*H**
		n = 72	*n* = 72	*n* = 72
0	**NC**	$55.35^{f} \pm 2.99$	$9.04^{bc} \pm 1.83$	$39.35^{de} \pm 5.48$
	LI	$50.45^{e} \pm 3.40$	$8.99^{bc} \pm 2.39$	$34.43^{bc} \pm 7.60$
	HI	$48.12^{c} \pm 4.39$	$7.23^{a} \pm 1.94$	$33.55^{bc} \pm 10.04$
	PC	$46.02^{a} \pm 3.06$	$7.70^{a} \pm 2.31$	$28.10^{a} \pm 9.94$
15	**NC**	$57.57^{g} \pm 2.15$	$9.13^{c} \pm 1.76$	$44.08^{fg} \pm 4.70$
	LI	$48.85^{cd} \pm 2.02$	$8.84^{bc} \pm 2.27$	$37.50^{cd} \pm 7.01$
	HI	$47.80^{bc} \pm 3.60$	$7.22^{a} \pm 1.71$	$37.25^{cd} \pm 8.90$
	PC	$45.84^{a} \pm 3.14$	$7.98^{ab} \pm 1.62$	$30.08^{ab} \pm 9.96$
30	**NC**	$60.10^{h} \pm 1.50$	$10.64^{d} \pm 1.47$	$47.20^{g} \pm 5.83$
	LI	$50.07^{de} \pm 1.75$	$9.31^{c} \pm 2.08$	$44.94^{fg} \pm 8.27$
	HI	$47.75^{bc} \pm 3.47$	$9.23^{c} \pm 2.43$	$42.41^{ef} \pm 9.96$
	PC	$46.49^{ab} \pm 2.21$	$7.33^{a} \pm 1.97$	$40.65^{def} \pm 10.28$
Significance level		$P < 0.001$	$P < 0.001$	$P < 0.05$

NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl)
TVC = total viable count; *L** = lightness; *C** = chroma; and *H** = hue angle
Means with different superscripts in the same column and on the same day differ significantly

4.3.6. Bacon – Sensory analysis

The 75-member consumer panel clearly distinguished the NC group from all three other treatment groups. The sensory rankings of all four attributes for the NC group were lower ($P < 0.001$) than that of the other three groups (Figure 4.6). Sodium was however present at a level of 190.83 ± 62.93 mg/100 g, although this clearly was too low a level to positively influence consumer perception. This was expected and attributed to the major role added NaCl plays in imparting food products with a mainly salty taste (McCaughey, 2007).

With a 68.00% reduction in added NaCl content, the LI group achieved surprisingly high rankings of ~ 6.00 for all four attributes with only taste and overall liking lower ($P < 0.001$) than that of the PC group. The smoky aroma of the bacon was suspected of improving the overall perception of the two low Na treatment groups. It has been reported that these types of aromas may improve the saltiness perception of weak NaCl solutions (Lawrence et al., 2009). With a 33.60% reduction in added NaCl content, the HI group compared favourably to the PC group in terms of sensory attributes with no significant differences between any of the rankings of these two groups. The saltiness ranking of the HI group at 6.71 ± 1.89 was marginally higher than that of the PC group at 6.59 ± 1.79. These results made the rankings of the HI and PC groups almost superimposable with that of the LI group almost running in parallel to the other two groups.

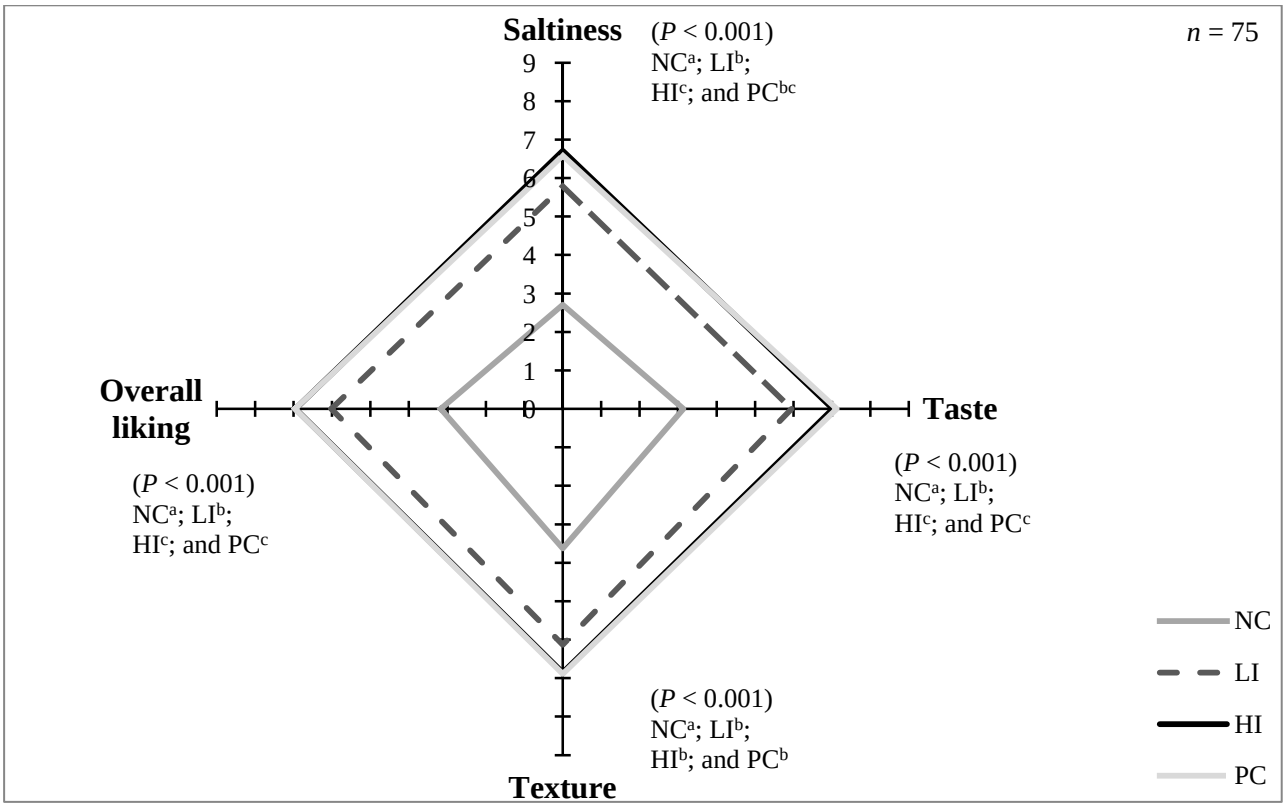

Figure 4.6. Consumer sensory rankings of four bacon treatment groups differing in added NaCl content. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Different letters for the same attribute indicate significant differences.

4.3.7. Polony – Main effects and interactions

Salt level, storage time and replicate were the main effects and salt level with storage time was identified as the main interaction between main effects (Table 4.15). Differences in added NaCl levels had the largest number of significant effects on various parameters evaluated for the polony. Only pH and lipid stability were not significantly affected. Differences in storage time resulted in significant differences for all the chemical parameters and the Warner-Bratzler shear force results. Differences in storage time were not applicable to the chemical properties and composition or to the sensory properties of the polony. Differences between replicates had significant effects on initial moisture content, pH, moisture content and pH over time, lipid stability, TVC and Warner-Bratzler shear force. Similar to the bacon, differences between replicates affected more parameters than differences in storage time. The interaction between salt level and storage time had significant effects limited to only pH over time and Warner-Bratzler shear force.

Table 4.15. ANOVA of the main effects and interactions on various parameters of polony formulated with different added NaCl levels.

Parameter	NaCl level	Storage time	Replicate	NaCl level X Storage time
Chemical properties and composition of polony immediately after manufacturing				
% Ash	$P < 0.001$	NSA	NS	NSA
% NaCl	$P < 0.001$	NSA	NS	NSA
mg NaCl/100 g	$P < 0.001$	NSA	NS	NSA
mg Na/100g	$P < 0.001$	NSA	NS	NSA
% Moisture	$P < 0.001$	NSA	$P < 0.05$	NSA
a_w	$P < 0.001$	NSA	NS	NSA
pH	NS	NSA	$P < 0.001$	NSA
Changes in chemical parameters of polony during storage at 4 ^oC up to 6 months				
% Moisture	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
pH	$P < 0.05$	$P < 0.05$	$P < 0.001$	$P < 0.001$
a_w	$P < 0.001$	$P < 0.05$	NS	NS
TBARS	NS	NS	$P < 0.001$	NS
Changes in the microbiological parameters of polony during storage at 4 ^oC up to 6 months				
TVC	$P < 0.001$	NS	$P < 0.001$	NS
Changes in Warner Bratzler shear force of polony during storage at 4 ^oC up to 6 months				
Shear force (kgf)	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Sensory properties of polony with different NaCl levels				
Taste	$P < 0.001$	NSA	NSA	NSA
Texture	$P < 0.001$	NSA	NSA	NSA
Saltiness	$P < 0.001$	NSA	NSA	NSA
Overall liking	$P < 0.001$	NSA	NSA	NSA

NS = Not significant
NSA= Not Statistically analysed

4.3.8. Polony − Chemical analyses

4.3.8.1. Polony − Ash, NaCl and Na content

The ash content of the four polony treatment groups differed ($P < 0.001$) in response to the added NaCl content (Table 4.16). This was again used to quickly confirm the identity of samples from

different treatment groups. Salt was present at a base level of 89.98 ± 43.09 mg/100 g in the NC group and it was accepted as the total NaCl contribution of all the other formulation components. As was the case for the bacon, consecutive increases in added NaCl led to significant ($P < 0.001$) differences in total NaCl content. The actual Na content of the NC group represented the total Na contribution made by all components in all four formulations, excluding NaCl. The calculated Na content again differed by a wide margin from the actual Na content (35.39 ± 16.95 mg/100 g versus 166.42 ± 15.94 mg/100 g) at a very low NaCl content (NC). The calculated and actual Na values of the intermediate added NaCl groups (LI and HI) were again in close agreement and at high added NaCl content the calculated Na content overestimated the actual Na content.

The formulated total Na content values compiled from the estimated contributions made by each component in the formulation slightly underestimated the total Na content in the case of the NC group. In contrast to the NC group of the bacon, which had a much simpler formulation, the NC polony components contributed more to the total Na content than expected. For the two intermediate added NaCl groups and the PC group the actual Na content was safely overestimated. Similar to the bacon, the Na content of the two intermediate polony groups were in effect considerably lower than the limits they were formulated to closely match. Actual products could in practice be reformulated to better match their limits so as to contain the maximum amount of added NaCl within regulatory limits.

Table 4.16. Salt and Na content of four polony formulations containing different added NaCl levels.

Treatment	NC	LI	HI	PC	Sign. level
% Ash *n* = 12	1.13^{a} ± 0.09	2.45^{b} ± 0.09	3.35^{c} ± 0.21	3.98^{d} ± 0.17	$P < 0.001$
mg NaCl/100 g *n* = 12	89.98^{a} ± 43.09	1352.42^{b} ± 41.25	1807.57^{c} ± 56.55	2432.98^{d} ± 48.14	$P < 0.001$
mg Na/100 g (converted) *n* = 12	35.39^{a} ± 16.95	531.91^{b} ± 16.22	710.92^{c} ± 22.24	956.89^{d} ± 18.93	$P < 0.001$
mg Na/100 g product (actual) *n* = 12	166.42^{a} ± 15.94	575.08^{b} ± 54.84	773.33^{c} ± 59.29	910.83^{d} ± 85.44	$P < 0.001$
mg Na/100 g product (formulated) *n* = 12	~ 126.52	~ 649.69	~ 850.32	~ 1109.94	n.a.
Legislation limits mg Na/100 g	n.a.	650.00	850.00	n.a.	n.a.

NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same row differ significantly
n.a. = Not applicable

4.3.8.2. Polony − pH, a_w and moisture content

The pH of the four polony treatment groups were closely grouped around a pH value of ~ 6.00 after day 0 with no significant differences between any of the four groups (Table 4.17). While meat generally has a pH of around 5.50-5.60 (Jay et al., 2005; Arias, 2012) the pH of the polony was substantially higher. The higher pH was attributed to the inclusion of sodium tripolyphosphate at a concentration of 0.40% (equal to 12 g per 3 kg batch) contributing enough phosphate for a substantial increase in pH. As was the case with the bacon, it did not appear as if added NaCl level had an immediate effect on the pH. After 90 days, a significant ($P < 0.05$) difference was found between the NC group (6.28 ± 0.18) and the PC group (6.02 ± 0.29) with the pH of the former being substantially higher. At this stage it appeared as if the higher added NaCl level contributed to a better buffering capacity against an increase in pH than the NC group. After 180 days, a substantial decrease in the pH of the NC group resulted in it being lower ($P < 0.001$) than that of the three NaCl added treatment groups. This sharp decrease in pH of the NC group strengthens the case for added NaCl contributing considerably to overall buffering capacity of the phosphate. Over the course of 180 days, the pH of any group containing even a little added NaCl (LI at 1.33%), fluctuated within a very narrow range. Further evidence for this phenomenon was found for the storage time effect on pH (Table 4.18). Changes in pH were only observed for the NC group, where the pH increased nonsignificantly from day 0 to day 90 and then decreased ($P < 0.001$) from day 90 to day 180. In terms of the effect of the interaction between added NaCl level and storage time on pH, the NC group experienced the only significant ($P < 0.001$) change in pH between day 90 (6.28 ± 0.18) and day 180 (5.79 ± 0.35) (Figure 4.7).

Table 4.17. Changes in the basic chemical parameters of four polony formulations containing different added NaCl levels over a 180 day shelf-life.

Day	Treatment	pH	Sign. Level	a_w	Sign. level	Moisture content (%)	Sign. level
		n = 12		*n* = 12		*n* = 12	
0	**NC**	6.05 ± 0.24	NS	0.9458^{a} ± 0.0060	$P < 0.001$	73.27^{b} ± 0.85	$P < 0.001$
	LI	6.04 ± 0.11		0.9556^{b} ± 0.0060		72.70^{b} ± 0.61	
	HI	6.02 ± 0.18		0.9473^{a} ± 0.0044		71.74^{b} ± 0.74	
	PC	5.97 ± 0.18		0.9471^{a} ± 0.0044		70.91^{a} ± 1.02	
90	**NC**	6.28^{b} ± 0.18	$P < 0.05$	0.9538^{b} ± 0.0109	$P < 0.001$	72.49^{b} ± 0.93	$P < 0.001$
	LI	6.05^{ab} ± 0.19		0.9539^{b} ± 0.0082		71.70^{ab} ± 1.02	
	HI	6.14^{ab} ± 0.27		0.9454^{a} ± 0.0034		70.89^{a} ± 1.07	
	PC	6.02^{a} ± 0.29		0.9433^{a} ± 0.0050		70.68^{a} ± 1.12	
180	**NC**	5.79^{a} ± 0.35	$P < 0.001$	0.9553 ± 0.0108	NS	71.81^{b} ± 0.94	$P < 0.005$
	LI	6.12^{b} ± 0.09		0.9543 ± 0.0103		71.07^{ab} ± 1.06	
	HI	6.09^{b} ± 0.19		0.9523 ± 0.0054		70.44^{a} ± 1.21	
	PC	6.02^{b} ± 0.11		0.9482 ± 0.0082		70.13^{a} ± 1.27	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
NS = Not significant

Table 4.18. The storage time effect over a 180 day shelf-life at 4 °C on the basic chemical parameters of four polony formulations containing different added NaCl levels.

Treat.	Storage time	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level	TBARS (mEq MDA/kg)	Sign. level
		n = 12		*n* = 12		*n* = 12		*n* = 12	
NC	**Day 0**	$6.05^{ab} \pm 0.24$	$P < 0.001$	$0.9458^{a} \pm 0.0060$	$P < 0.05$	$73.27^{b} \pm 0.85$	$P < 0.01$	0.16 ± 0.03	NS
	Day 90	$6.28^{b} \pm 0.18$		$0.9538^{ab} \pm 0.0109$		$72.49^{ab} \pm 0.93$		0.14 ± 0.05	
	Day 180	$5.79^{a} \pm 0.35$		$0.9553^{b} \pm 0.0108$		$71.81^{a} \pm 0.94$		0.16 ± 0.08	
LI	**Day 0**	6.04 ± 0.11	NS	0.9556 ± 0.0071	NS	$72.70^{b} \pm 0.61$	$P < 0.001$	0.16 ± 0.02	NS
	Day 90	6.05 ± 0.19		0.9539 ± 0.0082		$71.70^{a} \pm 1.02$		0.17 ± 0.03	
	Day 180	6.12 ± 0.09		0.9543 ± 0.0103		$71.07^{a} \pm 1.06$		0.19 ± 0.11	
HI	**Day 0**	6.02 ± 0.18	NS	$0.9473^{a} \pm 0.0044$	$P < 0.01$	$71.74^{b} \pm 0.74$	$P < 0.05$	0.17 ± 0.02	NS
	Day 90	6.14 ± 0.27		$0.9454^{a} \pm 0.0034$		$70.89^{ab} \pm 1.07$		0.16 ± 0.02	
	Day 180	6.09 ± 0.19		$0.9523^{b} \pm 0.0054$		$70.44^{a} \pm 1.21$		0.22 ± 0.14	
PC	**Day 0**	5.97 ± 0.18	NS	0.9471 ± 0.0044	NS	70.91 ± 1.02	NS	0.17 ± 0.02	NS
	Day 90	6.02 ± 0.29		0.9433 ± 0.0050		70.68 ± 1.12		0.16 ± 0.02	
	Day 180	6.02 ± 0.11		0.9482 ± 0.0082		70.13 ± 1.27		0.23 ± 0.15	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same column and for the same treatment differ significantly
NS = Not significant

Significant differences in a_w levels were found after day 0 due to differences in added NaCl content and differences in added meat content to make up for the aforementioned differences (Table 4.17). The a_w of the LI group was higher ($P < 0.001$) than that of HI and PC groups, and unexpectedly higher than that of the NC group. After 90 days, slightly lower a_w levels were found for all three added NaCl groups while that of the NC group increased from day 0 to day 90. This resulted in the a_w of the NC and LI groups being higher ($P < 0.001$) than that of the HI and PC groups. After 180 days, a_w levels of all four groups increased again resulting in no significant differences between any of the treatments. With added NaCl levels of 0.00%, 1.33%, 1.84% and 2.50% these atypical results could not readily be explained. Difficulties in reaching a_w equilibrium have been reported in minced meat products where adsorption and desorption of water failed to reach equilibrium (Motarjemi, 1988). Next to experimental error, differences between experimental values and prediction might be explained by the effect of the osmotic and network pressures of the protein matrix (Tesch, Ramon, Ladyzhinski, Cohen, & Mizrahi, 1999).

It is possible that the further handling and comminution of the samples disrupting the emulsion matrix, before measurements were made and the changes in temperature from 4 °C to 22 °C may have introduced variations that masked the effect of NaCl on a_w. The difference in the equilibrium moisture content between the adsorption and desorption of the water isotherm is known as hysteresis. Main factors that affect the extent of hysteresis include the product composition, isotherm temperature and storage time before isotherm measurement. Hysteresis in high protein foods such as pork has been shown to occur from a a_w of 0.85 and lower (Okos, Campanella, Narsimham, Singh, & Weitnauer, 2006). It is, therefore, conceivable that at different times during the shelf-life of the polonies, varying amounts of hysteresis could have taken place that could have affected the measured a_w to contribute to the atypical results. Storage time also significantly affected the a_w of the NC and HI groups (Table 4.18). The significant increases in a_w of the NC ($P < 0.05$) and HI ($P < 0.001$) groups from day 0 to day 30 provided further evidence for a hysteresis effect taking place over time at certain added NaCl levels. With identical added water content across all four formulations in the form of ice water, the initial differences in moisture content on day 0 (Table 4.17) were attributed to the different amounts of lean beef used in each formulation (Table 4.2). On day 0 there was an almost 2.00% difference in moisture content between the NC and PC corresponding to the 2.50% difference in lean beef content. It is generally accepted that beef and pork contains only about 75% moisture (Heinz & Hautzinger, 2007; Kim, Seong, Cho, Park, Hah, Yu, et al., 2008) making the major effect that beef content had on total moisture content rather unexpected.

Significant ($P < 0.001$) differences were identified in moisture content on day 0 between the NC and LI groups compared to the PC and HI groups (Table 4.17). After 90 days, the HI and PC groups had higher ($P < 0.001$) moisture content than the NC group and this continued to after 180 days. The total loss in moisture between day 0 and day 180 across all four treatment groups were attributed to the water vapour transmission rate (WVTR) of the five-layer nylon casings. The WVTR of these multi-layer, bi-axially orientated, co-extruded, tubular casings can vary between 5-10 g/m^2/24 h and 1.8−3.5 g/m^2/24 h at 90.00 ± 2.00% RH and 37 °C (Savic, 2012). The much smaller surface area, low storage temperature and long storage time of the samples may have contributed to the specific rate of moisture loss. The use of high barrier casings which possess extremely low WVTR are regarded as not being necessary for cooked meat products with a shelf-life of up to 3 months, although for a shelf-life of up to 6 months an even lower WVTR is suggested (Savic, 2012). Water activity may also have played a role in the WVTR of individual treatments; the general rates of moisture loss were not consistent across the four treatments. Moisture loss during the 180 days was also significant within the NC and LI groups. For the NC group, it decreased ($P < 0.001$) from 73.27 ± 0.85% to 71.81 ± 0.94% and for the LI group it decreased ($P < 0.001$) from 72.70 ± 0.61% to 71.07 ± 1.06%.

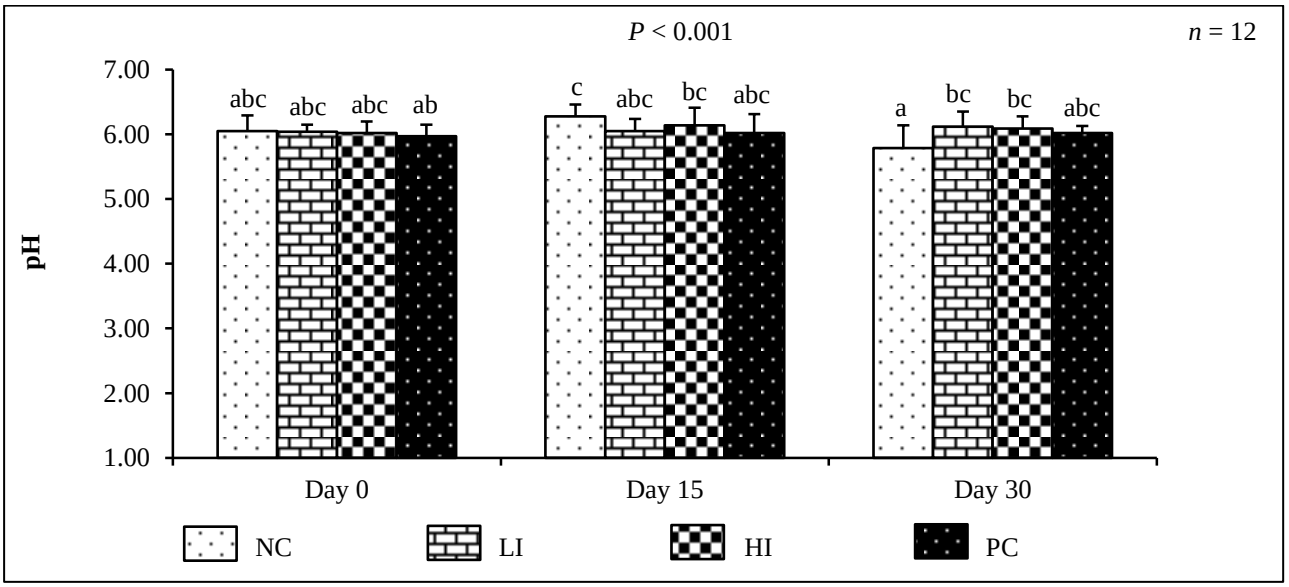

Figure 4.7. The effect of the interaction between added NaCl level and storage time on the pH of polony during storage at 4 °C for up to 180 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

Changes in moisture content over time may have been the result of different initial moisture content levels as a result of different added NaCl levels (Table 4.18). The moisture content of the LI group was lower ($P < 0.001$) after 90 days, that of the NC and HI groups was only lower ($P < 0.01$ and P

< 0.05, respectively) after day 180. Therefore, the WVTR of casings of similar products with low intermediate added NaCl levels may also need to be evaluated against WVTR of the original product to prevent increased moisture loss.

4.3.8.3. Polony − Lipid oxidative stability

All four treatment groups had similar results on day 0 (Figure 4.8) with no significant differences due to the short contact time between NaCl, its co-factors and the meat lipids on producing measurable amounts of MDA. This result was emulated by the day 90 results where no significant effect was observed. Phosphates, sodium ascorbate, nitrite, and spices as used in the formulations have exhibited anti-oxidant activity in various applications (Honikel, 2008; Tangkanakul, Auttaviboonkul, Nigomwit, Lowvitoon, Charoenthamawat, & Trakoontivakorn, 2009; Li, Henning, Zhang, Li, Gao, et al., 2010; Cheng, Wang, Sun, & Ockerman, 2011; Kiliç et al., 2014).

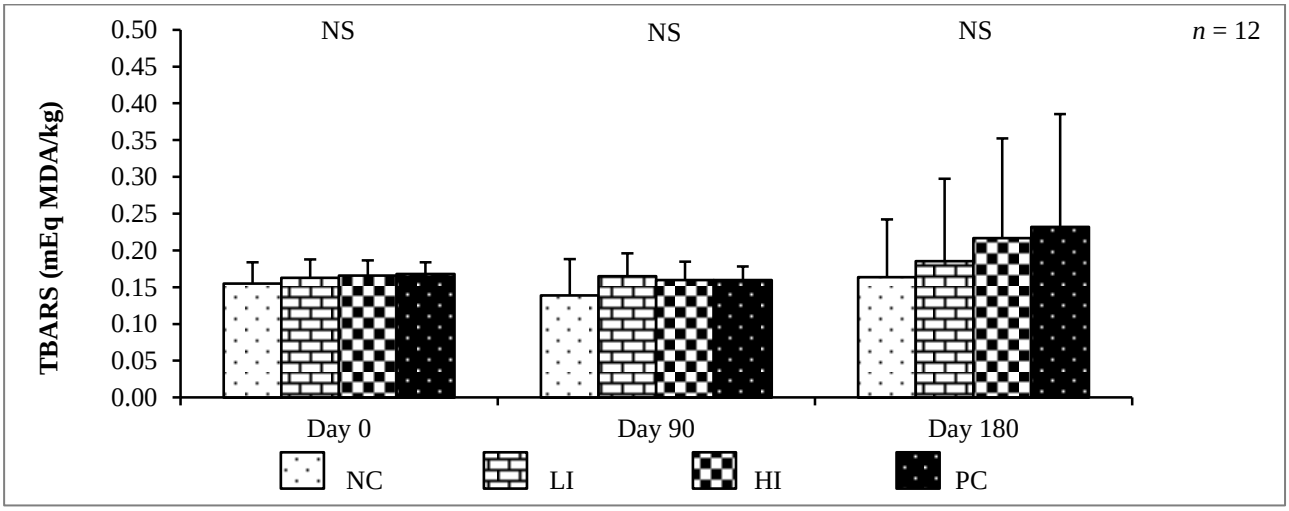

Figure 4.8. The effect of added NaCl level on the TBARS of polony stored at 4 °C for up to 180 days. NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl). Error bars represent standard deviations of means. NS = Not significant.

The role that these other components in the formulation played as anti-oxidants was attributed as the main reason for the consistently low levels of MDA across all four treatments up to day 90. This was reinforced by the low level of variation between samples of a given treatment after day 90 as reflected in the standard deviations. After day 180, a stepwise increase in MDA content was found that was directly proportional to added NaCl content, although this effect was not significant. A greater level of variation between samples of the same treatment group at this stage may have negated the possibility of finding significant differences between the four treatments. The MDA levels of all four treatments were consistently far below the 1 mEq MDA/kg (Gray & Pearson,

1987) and even below the 0.5 mEq MDA/kg (Wood et al., 2008) limits as levels indicative of producing rancid odour and taste detectable by consumers. Storage time had no significant effect on the TBARS of any of the four groups, although the levels were slightly higher after day 180 (Table 4.17).

4.3.9. Polony – Microbial analyses

An immediate effect for different added NaCl levels on TVC of the freshly cooked products was found on day 0 (Table 4.19). The NC group had a higher ($P < 0.001$) TVC of 2.70 ± 0.25 cfu/g compared to the 0.62 ± 1.13 cfu/g TVC of the PC group. This result was expected as NaCl content together with sharp temperature changes (pasteurization followed by quick cooling to 4 °C) are some of the most important hurdles for food preservation (Leistner, 1999). The effectiveness of the heat treatment was limited by the low level of added NaCl in the NC group. Phosphates, especially the polyphosphates, are capable of inhibiting the growth of some microorganisms (including yeasts and moulds) involved in food spoilage (Eillinger, 1972). Without any added NaCl, the ability of the added phosphate to act in this regard may also have been limited. All four treatment groups experienced declines in TVC from day 0 to after day 90; resulting in the TVC in the HI and PC groups being lower ($P < 0.001$) than that of the NC group. Over the entire 180 day shelf-life, the TVC of the two intermediate added NaCl groups with respective reductions in added NaCl content of 46.80% and 36.40% for the LI and HI groups compared favourably to the PC group with no significant differences in TVC. After day 180, TVC increased again for all four treatment groups. Most noticeable were the increases experienced by the HI (log 1.33 ± 2.01 cfu/g) and PC (log 1.63 ± 1.80 cfu/g) groups that were higher than on day 0 and day 90. It was possibly due to changes in the microbial populations of these two products over time which enabled the eventual higher overall growth. The LI group followed suite and after overcoming a decreased TVC after day 90, microbial growth increased to a level sufficiently indistinguishable from that on day 0. Storage time had no significant effect on the TVC of the four groups, in part due to the large degree of variation between counts performed on different samples of the same treatment group (Table 4.20). The TVC across all four treatments remained far below the national microbial limit for processed meat products of < log 7 cfu/g (SANS 885, 2011).

No coliforms, *E. coli*, *S. aureus*, yeasts or moulds were detected in any of the four treatments at any of the sampling times (Table 4.19). The fact that polonies are normally pasteurized to be sold as RTE products contributed greatly to the low microbial detections and good product shelf-life over 180 days. The inactivation temperatures (D-value of 1) of these microbes when vegetative are suggested to be as follows: *E. coli* (70 °C after 5-7 min); *S. aureus* (65 °C after 0.2-2 min); spoilage

Table 4.19. The results of microbial analyses performed on four polony formulations containing different added NaCl levels.

Day	Treatment	TVC (log cfu/g) $n = 12$	Sign. level	Coliforms (log cfu/g) $n = 12$	Sign. level	*E. coli* (log cfu/g) $n = 12$	Sign. level	*S. aureus* (log cfu/g) $n = 12$	Sign. level	Yeasts (log cfu/g) $n = 12$	Sign. level	Moulds (log cfu/g) $n = 12$	Sign. level
0	**NC**	$2.70^{b} \pm 0.25$		ND		ND		ND		ND		ND	
	LI	$1.61^{ab} \pm 1.44$		ND		ND		ND		ND		ND	
	HI	$0.68^{a} \pm 1.23$	$P < 0.001$	ND	NSA	ND	NSA	ND	NSA	ND	NSA	ND	NSA
	PC	$0.62^{a} \pm 1.13$		ND		ND		ND		ND		ND	
90	**NC**	$2.63^{b} \pm 2.26$		ND		ND		ND		ND		ND	
	LI	$0.72^{ab} \pm 1.71$		ND		ND		ND		ND		ND	
	HI	$0.55^{a} \pm 1.89$	$P < 0.05$	ND	NSA	ND	NSA	ND	NSA	ND	NSA	ND	NSA
	PC	$0.42^{a} \pm 1.01$		ND		ND		ND		ND		ND	
180	**NC**	$2.91^{b} \pm 1.29$		ND		ND		ND		ND		ND	
	LI	$0.98^{a} \pm 1.04$		ND		ND		ND		ND		ND	
	HI	$1.33^{ab} \pm 2.01$	$P < 0.05$	ND	NSA	ND	NSA	ND	NSA	ND	NSA	ND	NSA
	PC	$1.63^{ab} \pm 1.80$		ND		ND		ND		ND		ND	

TVC = total viable count; *E. coli = Escherichia coli*; *S. aureus = Staphylococcus aureus*
NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
ND = Not detected
NSA = Not statistically analysed
NS = Not significant

bacteria, yeasts and moulds (65 °C after 0.5-3 min) (Heinz & Hautzinger, 2007). The D-values were calculated for the time period after which only 10% of the original population survived, followed by an identical time period for another 10% and so forth. In effect the full diameter of each polony must have spent a significant time at, or have been close to, the pasteurization end temperature of 72 °C for the undetectable levels of microorganisms reported in the current results.

Table 4.20. The storage time effect over a 180 day shelf-life at 4°C on the TVC of four polony formulations containing different added NaCl levels.

Treatment	Storage time	TVC (log cfu/g) *n* = 12	Significance level
NC	**Day 0**	2.70 ± 0.25	NS
	Day 90	2.63 ± 2.26	
	Day 180	2.91 ± 1.29	
LI	**Day 0**	1.61 ± 1.44	NS
	Day 90	0.72 ± 1.71	
	Day 180	0.98 ± 1.04	
HI	**Day 0**	0.68 ± 1.23	NS
	Day 90	0.55 ± 1.89	
	Day 180	1.33 ± 2.01	
PC	**Day 0**	0.62 ± 1.13	NS
	Day 90	0.42 ± 1.01	
	Day 180	1.63 ± 1.80	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl)
Means with different superscripts for the same treatment differ significantly
NS = Not significant

In all probability the TVC counts were mostly represented by thermotolerant LAB that not only survived the pasteurization temperature, but also led to the increase in TVC over time due to their suitability to the conditions prevalent in the polonies (Dykes et al., 1991; Franz & Holy, 1996; Pérez-Chabela et al., 2008). Commercial polonies do not require a specific shelf-life of 180 days due to their high-volume sales. For some processors this goal serves as an indicator of stability and safety robustness for products that in some instances need to be transported over great distances. During this step these products may be subjected to abusive cold chain conditions and improper stock control and rotation by distribution (Nico de Klerk, South African Meat Processors' Association Technical Committee, personal communication, May 21, 2014).

4.3.10. Polony –Physical analyses: texture

Cross-sectional slices from each group were visually evaluated and compared for differences in appearance and texture (Figure 4.9). The NC group presented a large volume of watery exudate and a number of fat globules (< 3 mm in diameter) between the casings and the polonies, together with a soft, spongy and crumbling texture before slicing. The LI group, and to a much lessor extent, the HI

group, presented wetter cutting surfaces than the PC group. Furthermore, none of the other serious defects were observed for the two intermediate added NaCl groups. Differences in perceivable colour were the result of the angle of incidence of the light and did not signify actual differences in colour.

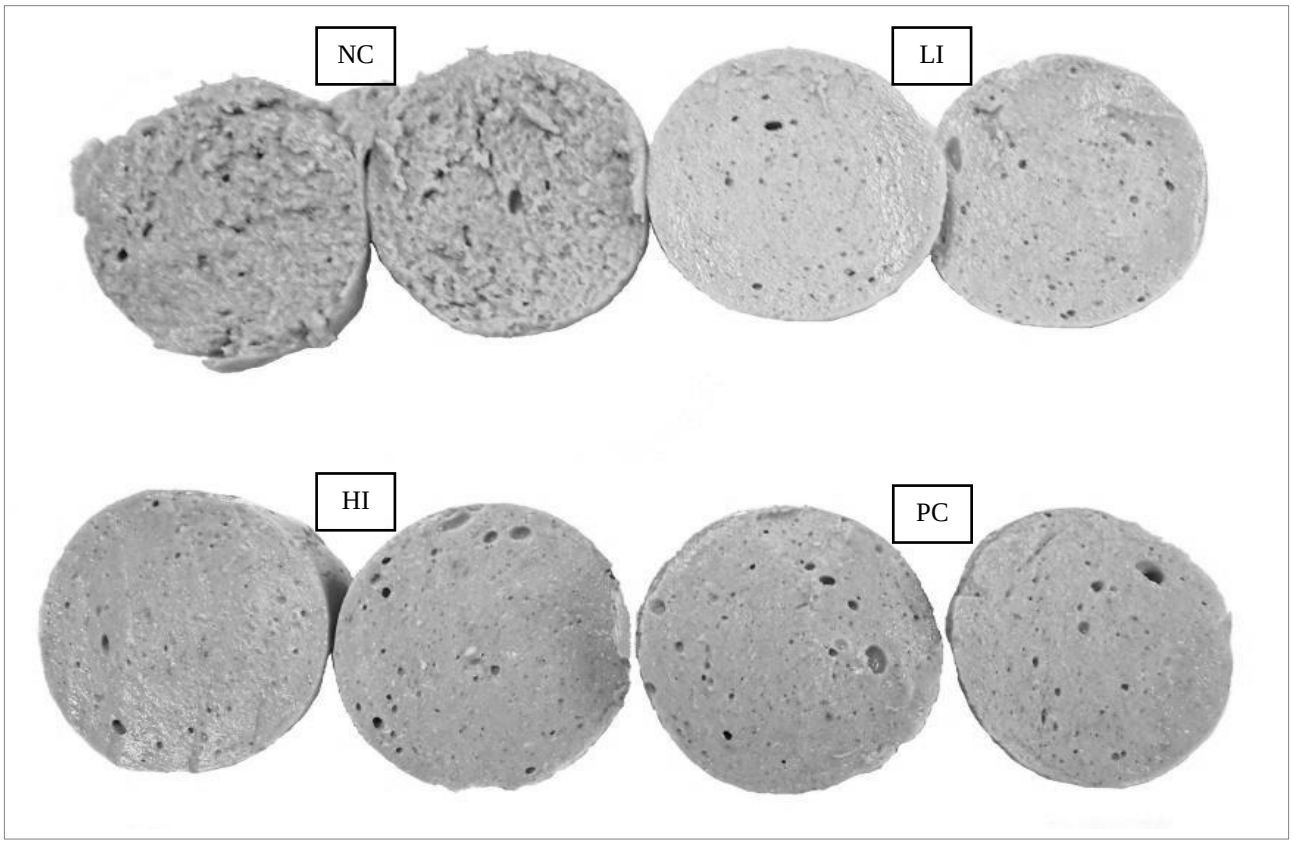

Figure 4.9. Cross-sectional view of four polony models formulated with different added NaCl levels showcasing cutting surface features and texture. NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl).

Significant textural changes were observed over the course of the 180 day shelf-life (Figure 4.10). The first significant ($P < 0.001$) effects were already observed on day 0. This is due to the fact that the main textural changes occur during the salt-assisted solubilisation and extraction of the water-soluble myofibrillar protein during the meat batter preparation and the heat-coagulated protein gel formation during the pasteurization step (Monahan & Troy, 1997; Man, 2007; Xiong, 2007). These processes were influenced by the concentration of NaCl present in the different batters. The quantity of myofibrillar proteins extracted before heating and gelation has been reported to affect the quality and strength of the resulting protein matrix (Tornberg, 2005). On day 0, the two intermediate treatment groups (LI and HI) respectively had the highest ($P < 0.001$) (3.14 ± 0.59 N) and second highest (3.04 ± 0.39) shear force values compared to the NC group (2.55 ± 0.88 N) By day 90 the texture and resulting shear force of the NC group (1.96 ± 0.69 N) deteriorated to such an

extent that it was lower ($P < 0.001$) than that of the three NaCl added groups (3.14 ± 0.59 N, 2.94 ± 0.39 N, and 2.94 ± 0.49 N, respectively).

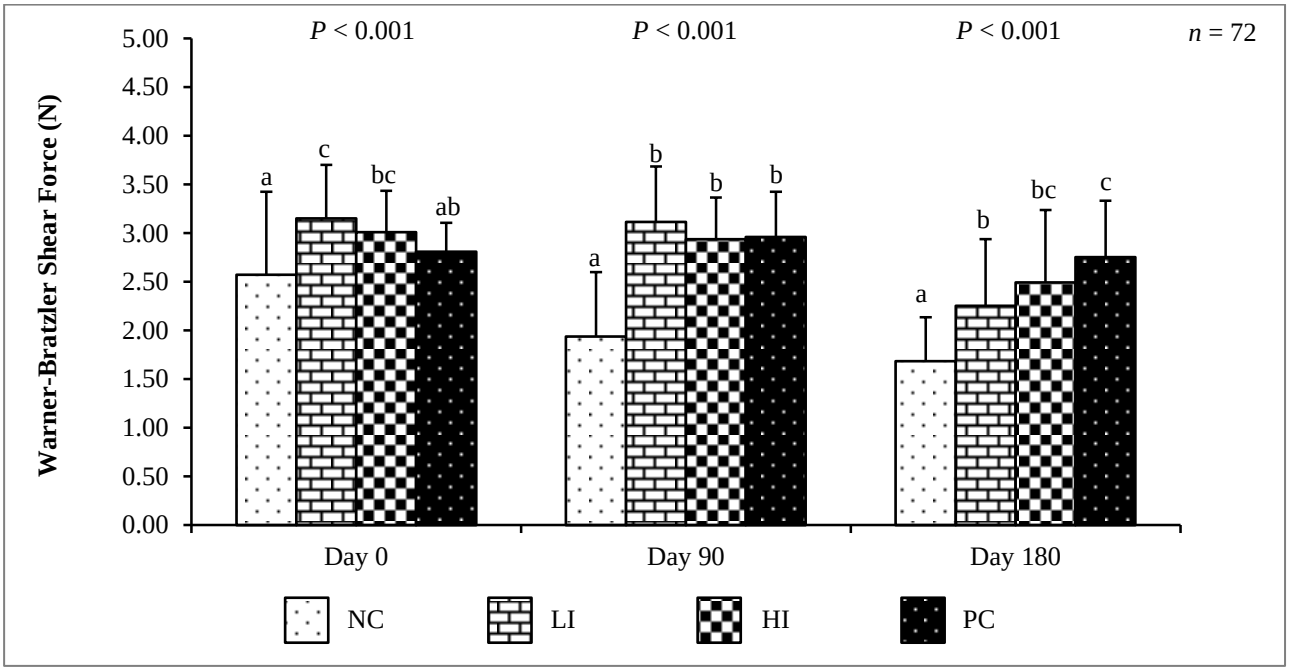

Figure 4.10. The effect of added NaCl level on the Warner-Bratzler shear force values of polony stored at 4 °C for up to 180 days. NC = low intermediate (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means.

By day 180, there was a considerable decrease in firmness across all four treatments. Three treatment groups were found to differ ($P < 0.001$) in terms of textural firmness. The NC group (1.67 ± 0.49 N), had the softest texture, the LI group (2.26 ± 0.69 N) was firmer ($P < 0.001$) and the PC group (2.75 ± 0.59 N) had the firmest texture by the end of the 180 day shelf-life. Of special interest is that the lowest added NaCl level of the LI group resulted in the firmest texture directly after manufacturing and up to 90 days of shelf-life. In contrast to this it has been reported that comminuted meat products become unstable and have poorer texture when they contain less than 1.50% salt in their formulation (Xiong, 2007). Over the complete shelf-life period, the highest added NaCl level of 2.50% (PC) resulted in the most consistent textural firmness of any treatment group. In terms of product consistency this may be more desirable, although it is doubtful if this type of product would reach a shelf-life of close to 180 days in practical terms.

Changes in texture over time were supported by the results of the effect of storage time on Warner Bratzler shear force values (Figure 4.11). The NC group experienced significant ($P < 0.001$) declines in firmness at every time interval (2.55 ± 0.88 N, 1.96 ± 0.69 N, and 1.67 ± 0.49 N). The two intermediate added NaCl groups LI and HI (3.14 ± 0.59 N and 3.04 ± 0.39 N) had very stable

textures up to day 90 (3.14 ± 0.59 N and 2.94 ± 0.39 N) after which both experienced significant ($P < 0.001$ for both) declines in firmness (2.26 ± 0.69 N and 2.45 ± 0.78 N) by day 180. The PC group (2.84 ± 0.29 N) had experienced a slight increase in firmness (2.94 ± 0.49 N) by day 90, thereafter firmness decreased ($P < 0.05$) by day 180 (2.75 ± 0.59 N). The effect of the interaction between added NaCl level and storage time on Warner Bratzler shear force revealed that the two intermediate groups (LI and HI) had firmer ($P < 0.001$) textures (3.14 ± 0.59 N and 3.04 ± 0.39 N) on day 0 (Figure 4.12). The NC group had the least stable texture with lower ($P < 0.001$) firmness at every successive time interval (2.55 ± 0.88 N, 1.96 ± 0.69 N, and 1.67 ± 0.49 N), and the PC group had the most stable texture with no significant differences (2.84 ± 0.29 N, 2.94 ± 0.49 N, and 2.75 ± 0.59 N). With intermediate added NaCl levels, the textures of the LI and HI groups were indistinguishable at every time interval and only lower ($P < 0.001$) after day 180.

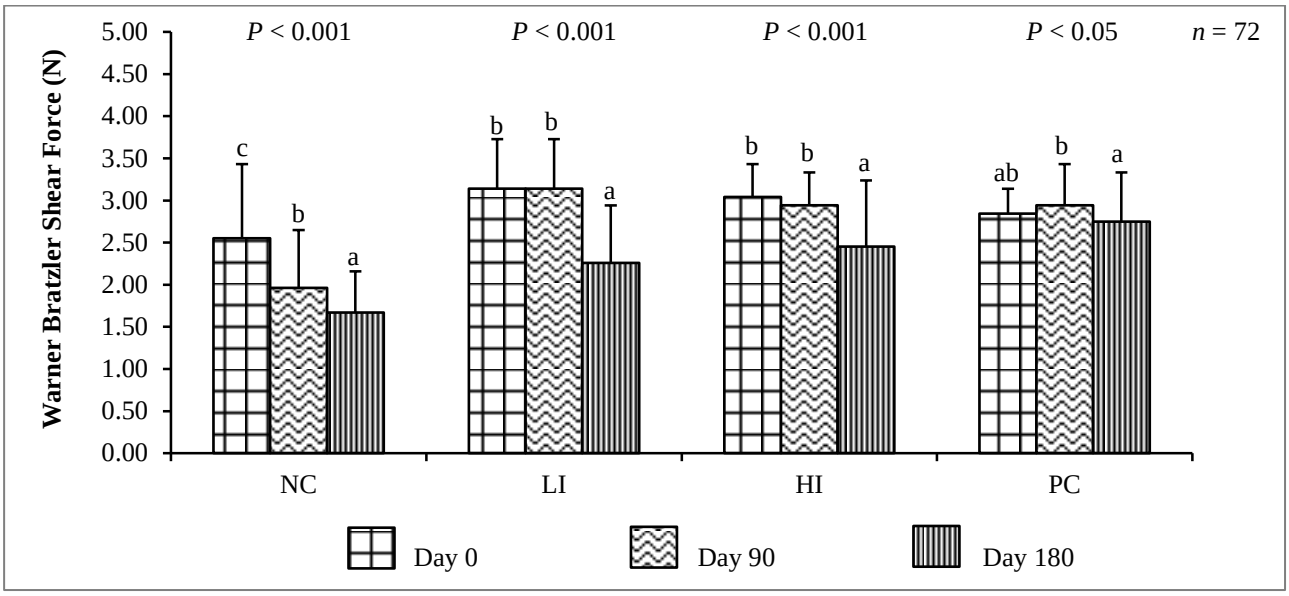

Figure 4.11. The storage time effect over a 180 day shelf-life on the Warner-Bratzler shear force values of polony with different added NaCl levels stored at 4 °C. NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl. Means with different superscripts for the same treatment differ significantly. Error bars represent standard deviations of means.

The effect of different ionic strengths on myosin specifically may explain why the lowest added NaCl polony had the firmest texture and why the highest added NaCl polony had the most stable texture. At low ionic strength (< 0.3 M) myosin molecules self-assemble into long filaments, and if these filaments are especially long, a firmer and more uniform gel is created during heating and at higher ionic strength (> 0.3 M) the myosin self-assemble as loosely dispersed monomers, resulting in a coarser gel (Sharp & Offer, 1992). It is possible that this coarser gel may then be less firm, although more stable overall in resisting degradation of the protein matrix up to 180 days. Actin may also play a role in the differences in gel strength and stability over time. In solution, actin has

been shown to contribute to creating a firmer gel (Yashui, Ishioroshi, & Samejima, 1980). An actomyosin complex with a ratio of 1:15 showed peak gel strength at pH 6.0 and that of myosin alone at pH 6.0 and that of actomyosin at a ratio of 4:1 showed low gel strength (Samejima, Oka, Yamamoto, Asghar, & Yasui, 1986). It may be possible that at higher NaCl concentrations, the ratio of actin to myosin was higher resulting in the softer, albeit overall more stable texture of the PC group compared to the LI group.

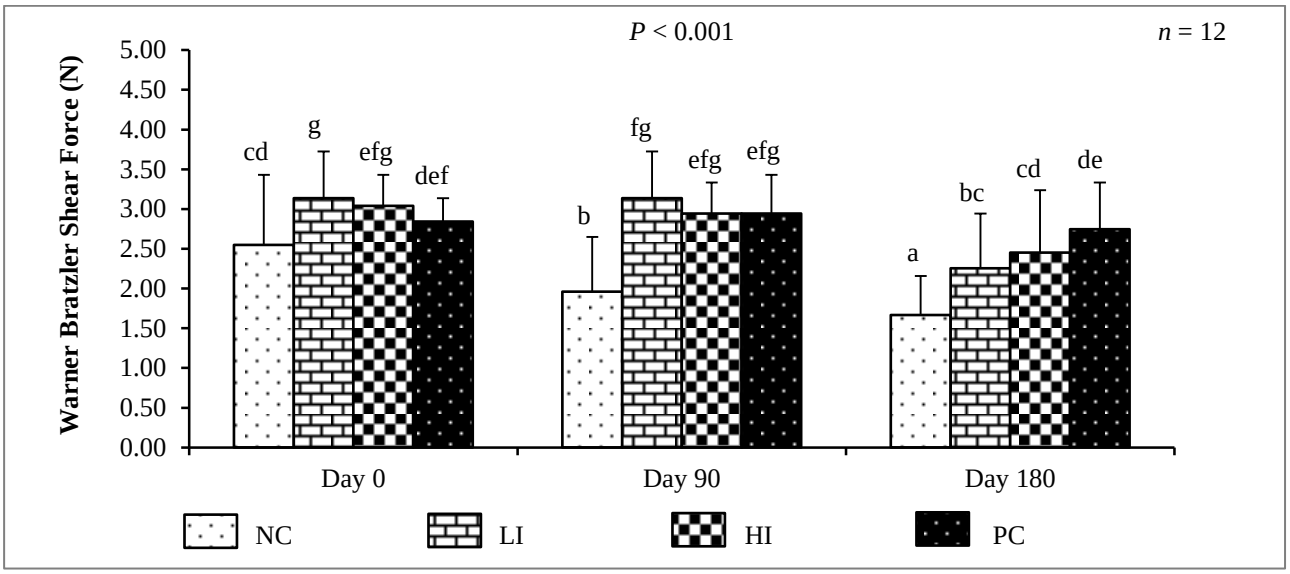

Figure 4.12. The effect of the interaction between added NaCl level and storage time on the Warner-Bratzler shear force values of polony during storage at 4 °C for up to 180 days. NC = negative control (0.00% added NaCl); LI = low intermediate (0.80% added NaCl); HI = high intermediate (1.66% added NaCl); and PC = positive control (2.50% added NaCl). Means with different superscripts differ significantly. Error bars represent standard deviations of means.

4.3.11. Polony – Sensory analysis

The hedonic rankings for all four sensory attributes of the NC group were lower ($P < 0.001$) than that of the other three treatment groups (Figure 4.14) and in contrast to that of the bacon, the rankings were much lower for all four attributes. This result was expected as Na is regarded as being the cation with the most pure salty taste (McCaughey, 2007) and it enhances the taste of certain food components whilst masking or supressing the bitter tastes associated with others (Breslin & Beauchamp, 1995; Kilcast & den Ridder, 2007). Salt was not completely absent from the model, it was present at a level equivalent to 166.42 ± 15.94 mg Na/100 g of polony (Table 4.15). Consumers may not only have found the salty taste to be lacking, but other unfavourable tastes may have become more apparent in the absence of a pleasingly high enough salty taste. Taste quality and intensity represented by taste and saltiness were not the only sensory attributes affected by low added NaCl content. The other sensory properties represented by texture (organoleptic) and overall liking (subjective) respectively, also suffered heavily from a lack of added NaCl. Salty compounds are responsible for not only creating hedonistic perceptions relating to taste and

intensity, but to that of palatability as well (McCaughey, 2007). The evaluation of texture was in agreement with that of the quantitative evaluation through Warner-Bratzler shear force determination on day 0 (Figure 4.10) revealing an unfavourable texture at no added NaCl content (NC). One key difference was that consumers were able to exhibit a more intense response to the effect that a very low Na content had on texture.

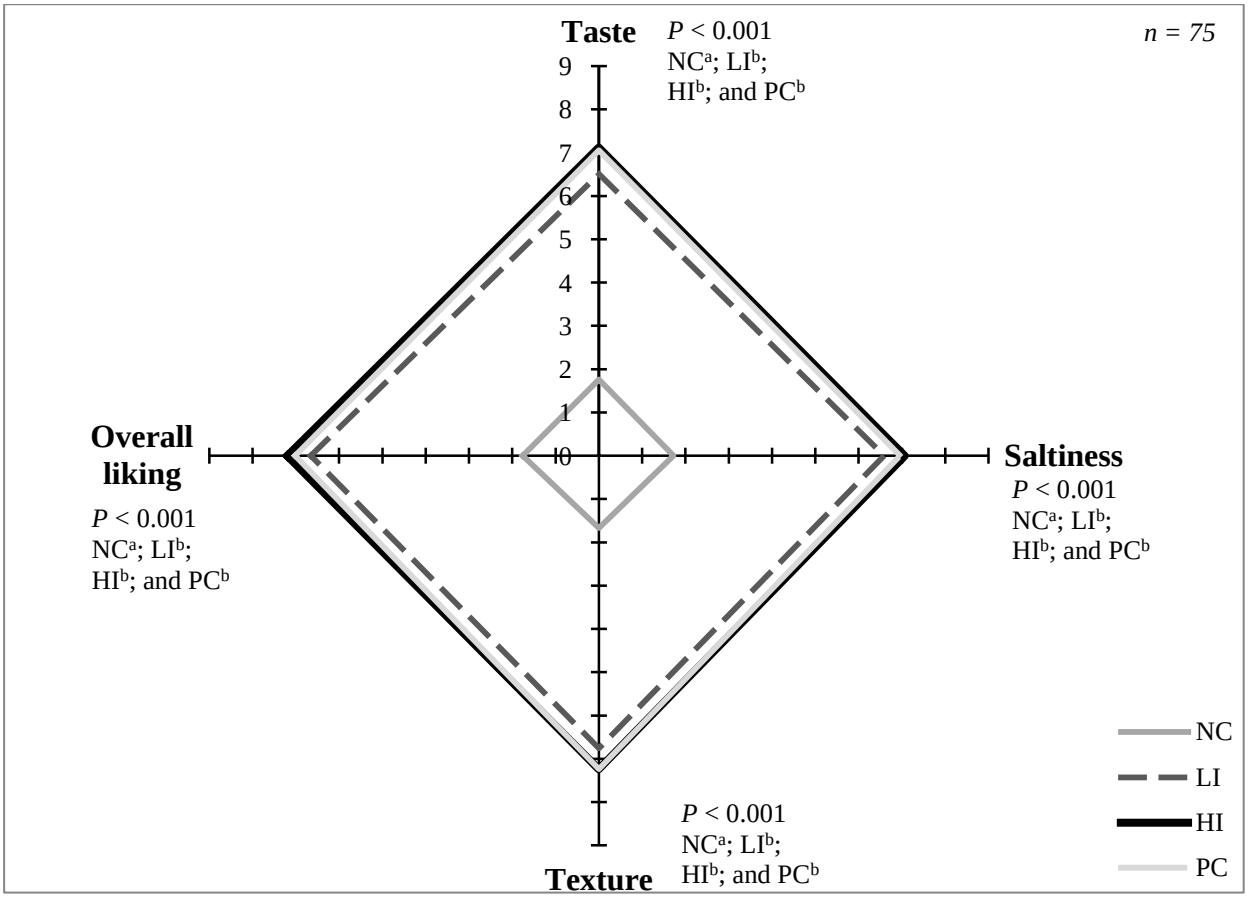

Figure 4.13. Consumer sensory rankings of four polony treatment groups differing in added NaCl content. NC = negative control (0.00% added NaCl); LI = low intermediate (1.33% added NaCl); HI = high intermediate (1.84% added NaCl); and PC = positive control (2.50% added NaCl). Different letters for the same attribute indicate significant differences.

Promising results were found for the two intermediate treatment groups on the basis of all four sensory attributes (Figure 4.13). The LI (46.8% NaCl reduction) and HI (26.4% NaCl reduction) groups managed rankings close to that of the PC group without any significant differences between the sensory attributes of these three groups. The rankings of the HI group specifically appeared to be almost precisely superimposed over that of the PC group's rankings while that of the LI group ran in close parallel to that of the PC group. The rankings of the LI group were consistently lower than that of the two higher NaCl containing groups, indicating that consumers detected a slight discrepancy that they could not associate with any one sensory attribute. The HI group managed

slightly higher rankings for taste, saltiness and overall liking compared to the PC group, indicating that there was room for a significant NaCl reduction without replacement, that consumers actually preferred over the original, higher added NaCl level.

4.3.12. Bangers – Main effects and interactions

Salt level, storage time and replicate were the main effects and salt level with storage time was identified as the main interaction between main effects (Table 4.21). Differences in added NaCl levels followed by differences in replicates had the largest number of significant effects on various parameters. Significant effects of different added NaCl levels on all the losses and sensory properties were most pronounced and those on microbial and colour parameters were most limited. Differences in storage time resulted in significant differences for most chemical, microbial and colour parameters while lipid stability was unaffected. Similarly to the bacon and polony parameters, different replicates of the bangers had more significant effects than differences in storage times on various parameters. In contrast to the ANOVA results of both the bacon and polony, the interaction of salt level and storage time had no significant effects on any of the parameters of the bangers.

4.3.13. Bangers – Refrigeration, thaw, and cooking losses

The NC group had a higher ($P < 0.001$) percentage of thaw loss compared to the other three treatment groups (Figure 4.14). This amounted to 1.84 ± 0.26% thaw loss compared with thaw losses of 0.76 ± 0.10%, 0.74 ± 0.07%, and 0.61 ± 0.07% for the LI, HI, and PC groups, respectively. Owning to the evaporative nature and protein-denaturing effect of cooking, the percentage losses after cooking were much higher compared to thaw losses for all four treatments. For the NC group, cooking losses were higher ($P < 0.001$) than that of the other three groups. Among the three NaCl added groups, the only significant ($P <0.001$) difference in cooking loss was between the LI and HI groups. These results reinforce the involvement of NaCl in extracting the salt-soluble proteins, the formation of a heat-coagulated protein matrix and the eventual entrapment of free water (Monahan & Troy, 1997). Although not significant, it is possible that a very high added salt content of 2.00% also led to a slight increase in cooking loss as a possible result of salting-out. When the percentage total loss, consisting of the sum of thaw and cooking losses was considered, the major losses incurred during cooking were not affected by the relatively low losses incurred during thawing, as the significance of the means remained unchanged.

Table 4.21. ANOVA of the main effects and interactions on various parameters of bangers with different added NaCl levels.

Parameter	NaCl level	Storage time	Replicate	NaCl level X Storage time
Chemical properties and composition of bangers immediately after manufacturing				
% Ash	$P < 0.001$	NSA	NS	NSA
mg NaCl/100 g	$P < 0.001$	NSA	NS	NSA
mg Na/ 100g	$P < 0.001$	NSA	NS	NSA
% Moisture	NS	NSA	$P < 0.01$	NSA
a_w	NS	NSA	$P < 0.001$	NSA
pH	NS	NSA	$P < 0.001$	NSA
Changes in chemical parameters of bangers during storage at 4 ^oC up to 9 days				
% Moisture	$P < 0.005$	$P < 0.005$	$P < 0.001$	NS
pH	NS	$P < 0.001$	$P < 0.001$	NS
a_w	$P < 0.001$	$P < 0.05$	$P < 0.001$	NS
TBARS	$P < 0.005$	NS	$P < 0.001$	NS
Lipid stability of bangers during frozen storage at -18 ^oC up to 180 days				
TBARS	$P < 0.05$	NS	$P < 0.001$	NS
Changes in the microbiological parameters of bangers during storage at 4 ^oC up to 9 days				
TVC	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Coliforms	$P < 0.05$	NS	$P < 0.001$	NS
E. coli	NS	NSA	$P < 0.001$	NS
Yeasts	NS	$P < 0.001$	$P < 0.05$	NS
Moulds	NS	$P < 0.001$	$P < 0.001$	NS
Changes in the colour parameters of bangers during storage at 4 ^oC up to 9 days				
L*	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
a*	NS	$P < 0.001$	$P < 0.001$	NS
b*	P < 0.05	$P < 0.001$	$P < 0.05$	NS
Chroma	NS	$P < 0.001$	$P < 0.001$	NS
Hue angle	$P < 0.05$	NS	$P < 0.001$	NS
Refrigeration, thaw and cooking losses				
Thaw loss (%)	$P < 0.001$	NSA	NSA	NSA
Cooking loss (%)	$P < 0.001$	NSA	NSA	NSA
Total loss (%)	$P < 0.001$	NSA	NSA	NSA
% Refrigeration loss	$P < 0.001$	NSA	NSA	NSA
Sensory properties of bangers with different NaCl levels				
Taste	$P < 0.001$	NSA	NSA	NSA
Texture	$P < 0.001$	NSA	NSA	NSA
Saltiness	$P < 0.001$	NSA	NSA	NSA
Overall liking	$P < 0.001$	NSA	NSA	NSA

NS = Not significant
NSA= Not Statistically analysed

The result of the refrigeration loss revealed a similar trend as for cooking loss. The NC group experienced the highest refrigeration loss compared to the LI and HI groups. Addition of phosphate to the sausage batter may be responsible for increased WHC, even in the range of 1.0-1.5% added NaCl (Rhee & Ziprin, 1987). The synergy and concentration dependence of phosphates with NaCl (Rhee & Ziprin, 1987; Ruusunen & Puolanne, 2005) is probably of greater importance. The markedly lower losses seen in the groups with higher NaCl can probably be ascribed to the combined effects of these various interactions. The results in Figure 4.15 may imply that 1.50% added NaCl consistently resulted in the lowest losses.

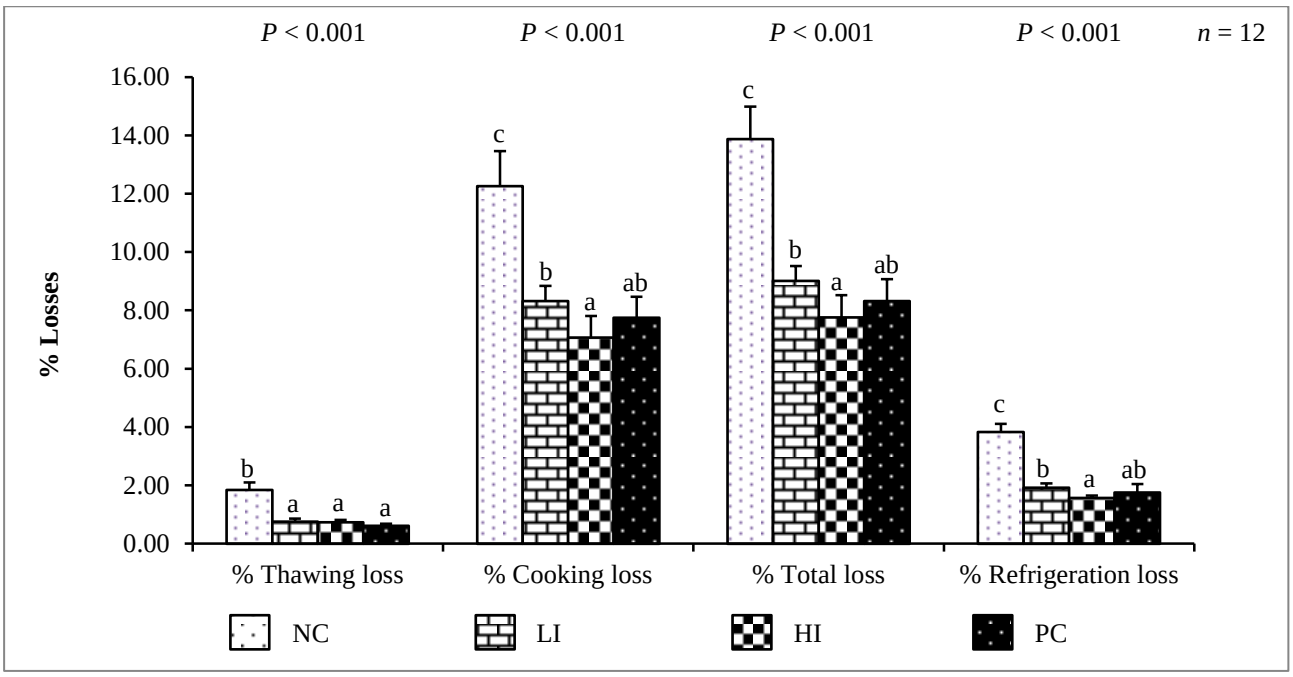

Figure 4.14. Thaw, cooking, total, and refrigeration losses of four banger groups based on added NaCl content. NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). Means with different superscripts for the same parameter differ significantly. Error bars represent standard deviations of means.

4.3.14. Bangers − Chemical analyses

4.3.14.1. Bangers – Ash, NaCl, and Na content

In agreement with the bacon and polony products, the percentage ash of the bangers increased ($P < 0.001$) with increased amounts of added NaCl (Table 4.22), enabling further confirmation of samples belonging to a specific treatment group. Salt was present at a base level of 356.74 ± 81.01 mg/100 g in the NC group. This represented the total NaCl contribution of all the components excluding added NaCl. As was the case for both the bacon and polony, each higher addition of NaCl led to significant ($P < 0.001$) differences in NaCl content. The actual Na content of the NC group (140.32 ± 31.86 mg/100 g) represented the total Na contribution made by all components in all four formulations, excluding added NaCl as source. The calculated Na content closely matched the actual Na content at the lowest level (NC), the lowest added level (LI), and the highest added level (PC). Actual Na content was only overestimated at the highest intermediate level of the HI group (668.34 ± 20.76 mg/100 g versus 610.82 ± 193.02 mg/100 g).

The formulated total Na content of each treatment group compiled from the estimated contributions made by each component in each formulation overestimated the actual Na content in increasing amounts from the NC to HI groups, while for the PC group it was overestimated at a level between the LI and HI groups. Similar to the NC group of the bacon, the components in the NC group of the bangers contributed less to the total Na content than expected. These results were reflected in

comparisons between the actual and formulated Na content of the LI, HI and PC groups, therefore, Na content was safely overestimated. As for the bacon and polony, the Na content of the two intermediate banger groups were in effect considerably lower than the limits they were formulated to. These formulations would also be readjusted to more closely match their corresponding Na limits.

Table 4.22. Salt and Na content of four banger formulations containing different added NaCl levels.

Treatment	NC	LI	HI	PC	Sign. level
% Ash *n* = 12	$1.23^{a} \pm 0.16$	$2.35^{b} \pm 0.15$	$3.03^{c} \pm 0.26$	$3.51^{d} \pm 0.13$	$P < 0.001$
mg NaCl/100 g *n* = 12	$356.74^{a} \pm 81.01$	$1255.09^{b} \pm 73.83$	$1699.31^{c} \pm 52.79$	$2169.72^{d} \pm 64.27$	$P < 0.001$
mg Na/100 g (converted) *n* = 12	$140.31^{a} \pm 31.86$	$493.63^{b} \pm 29.04$	$668.34^{c} \pm 20.76$	$853.35^{d} \pm 25.36$	$P < 0.001$
mg Na/100 g actual) *n* = 12	$140.32^{a} \pm 31.86$	$493.68^{b} \pm 9.04$	$610.82^{c} \pm 193.02$	$853.45^{d} \pm 25.36$	$P < 0.001$
mg Na/100 g product (formulated)	~ 197.67	~ 590.41	~ 786.78	~ 983.15	n.a.
Legislation limits mg Na/100 g	n.a.	600.00	800.00	n.a.	n.a.

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl).
Means with different superscripts in the same row differ significantly.
n.a. = Not applicable

4.3.14.2. Bangers − pH, a_w and, moisture content

The pH of the four banger treatment groups were closely grouped around an average pH of ~ 5.80 after day 0 with no significant differences between treatments (Table 4.23). Similar to the polony, the average pH of the bangers on day 0 were higher than that of meat at 5.50−5.60 (Jay et al., 2005; Arias, 2012). The higher pH values were again attributed to the inclusion of sodium tripolyphosphate, this time at a concentration of 0.30% (equal to 7.5 g per 2.50 kg batch). Similar to the results of the bacon and polony, added NaCl did not significantly affect the pH, although for this product type this was true at every sampling point. The pH values of all four groups did, however, shift over the 9 day shelf-life from ~ 5.80 after day 0, to ~ 5.91 after day 3, to ~ 5.77 after day 6 and finally to 5.65 after day 9.

Storage time significantly affected the pH values of the four treatment groups (Table 4.24). The pH values were higher on day 3 compared to day 9 for the NC ($P < 0.01$), LI ($P = 0.05$), HI ($P < 0.01$), and PC ($P < 0.05$) groups. With the high TVC reported in Table 4.26 it is possible that microbial activity drove the changes in pH. The microbiota of fresh meat consists mainly of Gram-negative, oxidase positive rods, particularly psychrotrophic pseudomonads (Gill, 1982). Enterobacteriaceae may also be present and LAB are present in low numbers. Due to factors such as: a decrease in

oxygen mainly in the middle of sausages due to oxygen permeable casings and packaging; the addition of NaCl and fermentable sugars; the microbial composition shifting to mostly LAB. Some of these organisms are facultative heterofermentative and can produce acetic and lactic acids (Kandler, 1983) which were attributed to decreasing the pH of the bangers. This slow acidification eventually stops at higher pH values as the a_w becomes inhibitory to lactic acid formation, specifically at a_w values in the region of 0.91 (List & Klettner, 1978) similar to that in Table 4.23.

Table 4.23. Changes in the basic chemical parameters of four banger formulations containing different added NaCl levels over a 9 day shelf-life.

Day	Treatment	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level
		n = 12		*n* =12		*n* = 12	
0	**NC**	5.90 ± 0.22	NS	0.9318 ± 0.0080	NS	59.77 ± 1.07	NS
	LI	5.78 ± 0.23		0.9283 ± 0.0145		58.99 ± 1.72	
	HI	5.75 ± 0.17		0.9248 ± 0.0132		59.85 ± 1.31	
	PC	5.75 ± 0.20		0.9226 ± 0.0129		58.64 ± 1.04	
3	**NC**	5.98 ± 0.17	NS	0.9238 ± 0.0132	NS	58.44 ± 0.99	NS
	LI	5.91 ± 0.16		0.9211 ± 0.0111		58.74 ± 0.75	
	HI	5.87 ± 0.18		0.9147 ± 0.0067		58.91 ± 0.88	
	PC	5.86 ± 0.18		0.9197 ± 0.0104		58.77 ± 0.74	
6	**NC**	5.80 ± 0.09	NS	0.9274^{b} ± 0.0073	$P < 0.05$	57.91^{a} ± 0.98	$P < 0.001$
	LI	5.75 ± 0.16		0.9205^{ab} ± 0.0096		59.50^{b} ± 1.40	
	HI	5.76 ± 0.15		0.9133^{a} ± 0.0123		59.55^{b} ± 0.93	
	PC	5.76 ± 0.10		0.9138^{ab} ± 0.0148		58.19^{a} ± 1.02	
9	**NC**	5.73 ± 0.19	NS	0.9366^{b} ± 0.0039	$P < 0.001$	57.77^{a} ± 0.98	$P < 0.05$
	LI	5.62 ± 0.15		0.9232^{a} ± 0.0076		58.30^{ab} ± 0.62	
	HI	5.62 ± 0.13		0.9143^{a} ± 0.0098		59.02^{b} ± 1.11	
	PC	5.63 ± 0.14		0.9171^{a} ± 0.0094		58.41^{ab} ± 0.97	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
NS = Not significant

In terms of a_w, no significant differences were found between any of the four treatment groups on day 0 (Table 4.23). The a_w values of both the NC and LI groups (both 0.93 ± 0.01) were, however, slightly higher than that of the HI and PC groups (both 0.92 ± 0.01). Similarly, no significant differences were found after day 3 between any of the groups. After day 6, the a_w of the NC group at 0.93 ± 0.01 was higher ($P < 0.005$) than that of the HI group at 0.91 ± 0.01. After day 9, the a_w of the NC group was higher ($P < 0.001$) than all three NaCl added groups. Overall, the a_w values of the NC group appeared to decrease, then increase to the same initial level, then to increase again to a new highest level of 0.94 ± 0.01 after day 9.

The a_w of the PC group remained constant and that of the LI and HI groups decreased. The a_w of only the NC group was significantly affected by storage time (Table 4.24). Fluctuations in a_w were observed with the lowest a_w of this group on day 3 being lower ($P < 0.01$) than the highest a_w of the

Table 4.24. The storage time effect over a 9 day shelf-life at 4 °C on the basic chemical parameters of four banger formulations containing different added NaCl levels.

Treat.	Storage time	pH	Sign. level	a_w	Sign. level	Moisture content (%)	Sign. level	TBARS (mEq MDA/kg)	Sign. level
		n = 12		*n* = 12		*n* = 12		*n* = 12	
NC	**Day 0**	$5.90^{ab} \pm 0.22$	$P < 0.01$	$0.9318^{ab} \pm 0.0080$	$P < 0.01$	$59.77^{b} \pm 1.07$	$P < 0.001$	0.77 ± 0.60	NS
	Day 3	$5.98^{b} \pm 0.17$		$0.9238^{a} \pm 0.0132$		$58.44^{a} \pm 0.99$		0.86 ± 0.47	
	Day 6	$5.80^{ab} \pm 0.09$		$0.9274^{ab} \pm 0.0073$		$57.91^{a} \pm 0.98$		0.73 ± 0.41	
	Day 9	$5.73^{a} \pm 0.19$		$0.9366^{b} \pm 0.0039$		$57.77^{a} \pm 0.98$		0.64 ± 0.39	
LI	**Day 0**	$5.78^{ab} \pm 0.23$	$P = 0.005$	0.9283 ± 0.0145	NS	58.99 ± 1.72	NS	0.91 ± 0.58	NS
	Day 3	$5.91^{b} \pm 0.16$		0.9211 ± 0.0111		58.74 ± 0.75		1.37 ± 0.81	
	Day 6	$5.75^{ab} \pm 0.16$		0.9205 ± 0.0096		59.50 ± 1.40		1.27 ± 0.62	
	Day 9	$5.62^{a} \pm 0.15$		0.9232 ± 0.0076		58.30 ± 0.62		1.03 ± 0.63	
HI	**Day 0**	$5.75^{ab} \pm 0.17$	$P < 0.01$	0.9248 ± 0.0132	NS	59.85 ± 1.31	NS	1.00 ± 0.58	NS
	Day 3	$5.87^{b} \pm 0.18$		0.9147 ± 0.0067		58.91 ± 0.88		1.20 ± 0.40	
	Day 6	$5.76^{ab} \pm 0.15$		0.9133 ± 0.0123		59.55 ± 0.93		1.19 ± 0.34	
	Day 9	$5.62^{a} \pm 0.13$		0.9143 ± 0.0098		59.02 ± 1.11		1.08 ± 0.48	
PC	**Day 0**	$5.75^{ab} \pm 0.20$	$P < 0.05$	0.9226 ± 0.0129	NS	58.64 ± 1.04	NS	1.00 ± 0.67	NS
	Day 3	$5.86^{b} \pm 0.18$		0.9197 ± 0.0104		58.77 ± 0.74		1.25 ± 0.74	
	Day 6	$5.76^{ab} \pm 0.10$		0.9183 ± 0.0148		58.19 ± 1.02		1.35 ± 0.76	
	Day 9	$5.63^{a} \pm 0.14$		0.9171 ± 0.0094		58.41 ± 0.97		1.08 ± 0.71	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl)
Means with different superscripts in the same column and for the same treatment differ significantly
NS = Not significant

same group after day 9. In addition to the theories discussed under the a_w results of the polony, another theory might explain the seemingly dynamic a_w reported for the NC group. As part of the formulation, rusk with a high water absorption capacity of 3-4 times its weight (Essien, 2003) was used together with tapioca starch which contributes to decreased cooking losses (Knight & Perkin, 1991; Lyons, Kerry, Morrissey, & Buckley, 1999). It is possible that, due to the starch undergoing cold-water-swelling during mixing and no subsequent heating, a syneresis effect led to small amounts of free water being released that increased the overall a_w only later in the product shelf-life.

No significant differences in moisture content were found after day 0 or day 3(Table 4.23). This reflected the non-significant differences found in a_w values over the same period of time. After day 6, the moisture content of the NC and PC groups were both lower ($P < 0.001$) than that of the two intermediate groups. After day 9, the moisture content of the NC group was lower ($P < 0.05$) than that of the HI group. Storage time also had a significant effect on the decreasing moisture content of the NC group over time (Table 4.24). On day 0, the moisture content was higher ($P < 0.001$) than at any of the other three sampling times. It is possible that, as moisture was being released by a less stable system, the a_w was driven up and with the greater availability of free water, evaporation through the PVC film occurred at a greater tempo than for the other three treatment groups.

4.3.14.3. Bangers − Lipid oxidative stability

In terms of a fresh product shelf-life of 9 days, the results of the four banger treatments are depicted in Figure 4.15. A secondary threshold for the detection of rancidity at 1 mEq MDA/kg (Gray & Pearson, 1987) was applied to the bangers as the lower 0.50 mEq MDA/kg threshold was already surpassed on day 0. Only the TBARS of the NC group remained below the higher threshold throughout the 9 day total fresh product shelf-life. After days 6 and 9, the TBARS of the NC group were lower ($P < 0.05$) than that of the PC group. No significant differences existed between any of the three NaCl added groups. Storage time also had no effect on the lipid stability of the four treatment groups over the 9 day shelf-life at 4°C (Table 4.24). In general, the TBARS of the three NaCl-containing groups possibly peaked on day 3 and day 6. These findings implied that a reduction in added NaCl to a 1.50% or even 1.00% level did not lead to a substantial improvement in lipid oxidative stability under these conditions as the level of SLOP in the intermediate groups was still comparable to that of a 2.00% added NaCl control.

For the frozen bangers, the TBARS of all four treatment groups were below the sensory threshold of 1 mEq MDA/kg on day 0 (Figure 4.16). After day 90, the SLOP levels of only the NC, LI and

PC groups were below the limit. After day 180, secondary lipid oxidation had progressed to such an extent, that only the NC group was found to be below the limit, even though it had also increased from day 90 to day 180 under frozen storage conditions. Of interest was that the HI group and not the PC group had the highest TBARS after day 90. This level was higher ($P < 0.001$) than that of both the NC and LI groups. With increased MDA content across all four groups after day 180, the HI group maintained the highest level of SLOP. The increases experienced by the LI and PC groups meant that this level was only then higher ($P < 0.01$) than that of the NC group. Storage time had no effect on the lipid stability of the four treatment groups over the 180 day shelf-life at -18 °C (Table 4.25). These results from products that were kept either under refrigerated (Figure 4.16) or frozen (Figure 4.17) storage, confirmed the previously reported pro-oxidative nature of salt (Rhee & Ziprin, 2001; Kiliç et al., 2014) leading to the formation of SLOP. The anti-oxidant potential of endogenous enzymes in uncooked meat such as catalase and glutathione peroxidase is believed to be compromised by non-meat additives such as NaCl (Rhee, Smith, & Terrell, 1983). The combination of frozen storage where limited free water is available as a medium for biochemical reactions to take place, together with a possible concentration dependence of the NaCl, may also contribute to explain the high secondary lipid oxidation level of the HI group.

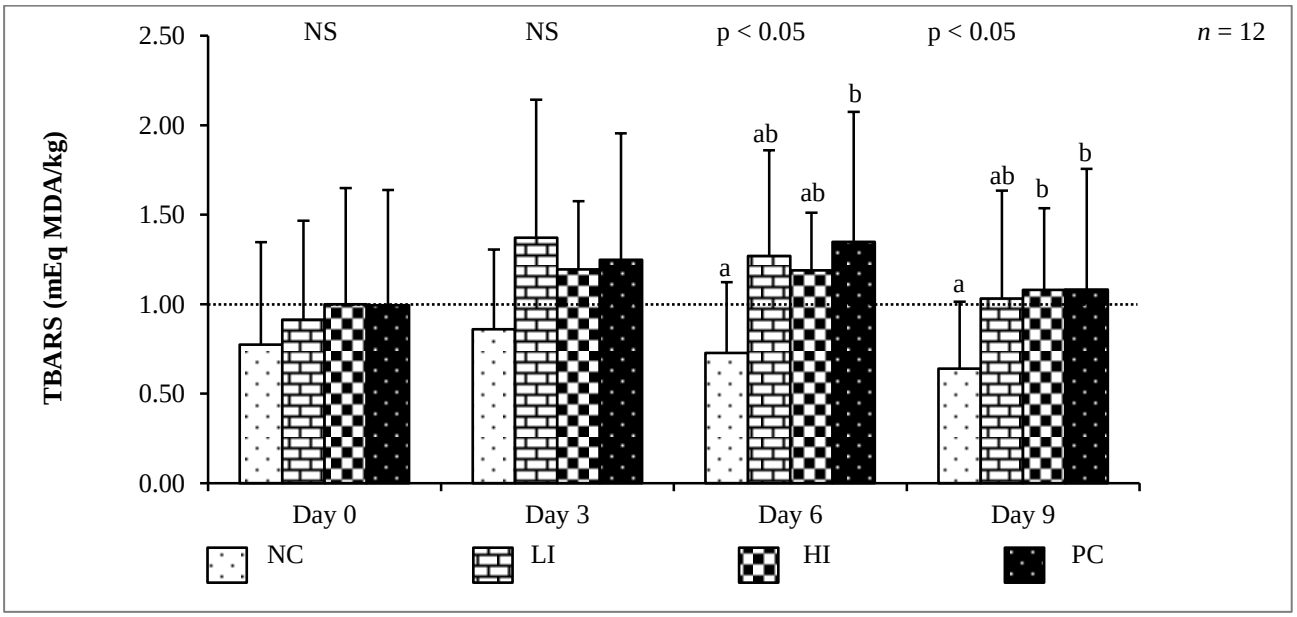

Figure 4.15. The effect of added NaCl level on the TBARS of bangers stored at 4 °C for up to 9 days. NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means. NS = Not significant. Rancidity threshold indicated by the dotted line.

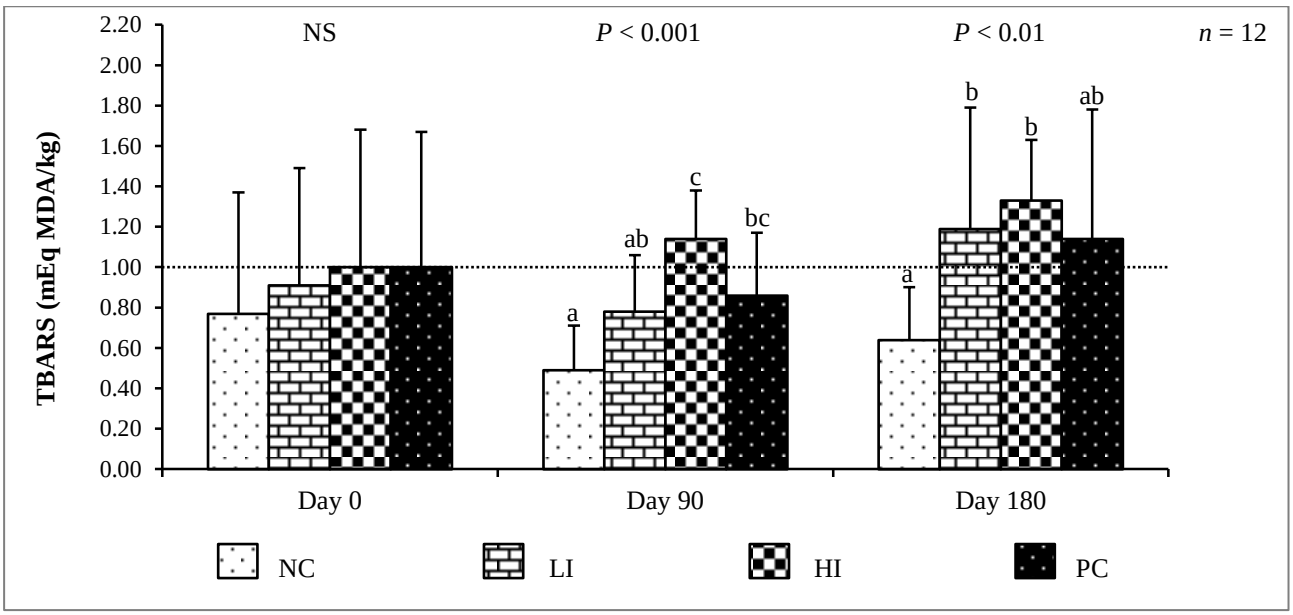

Figure 4.16. The effect of added NaCl level on the TBARS of bangers stored at -18 °C for up to 180 days. NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means. NS = Not significant. Rancidity threshold indicated by the dotted line.

Table 4.25. The storage time effect over a 180 day shelf-life at -18 °C on the TBARS of four banger formulations containing different added NaCl levels.

Treatment	Storage time	TBARS (mEq MDA/kg) *n* = 12	Significance level
NC	**Day 0**	0.77 ± 0.60	NS
	Day 90	0.49 ± 0.22	
	Day 180	0.64 ± 0.26	
LI	**Day 0**	0.91 ± 0.58	NS
	Day 90	0.78 ± 0.28	
	Day 180	1.19 ± 0.60	
HI	**Day 0**	1.00 ± 0.68	NS
	Day 90	1.11 ± 0.24	
	Day 180	1.33 ± 0.30	
PC	**Day 0**	1.00 ± 0.67	NS
	Day 90	0.86 ± 0.31	
	Day 180	1.14 ± 0.64	

NC = negative control (0.00% added NaCl); LI = lower intermediate (1.00% added NaCl); HI = higher intermediate (1.50% added NaCl); and PC = higher intermediate (2.00% added NaCl)
Means with different superscripts for the same treatment differ significantly
NS = Not significant

4.3.15. Bangers − Microbial analyses

No immediate effect of the different NaCl contents on the TVC were noticed on day 0 (Table 4.26). A significant difference ($P < 0.05$) was found after day 3 between the PC and the NC groups. This difference remained constant even as the TVC of all four treatment groups increased. There was, however, no significant difference in TVC between the LI, HI and PC groups from day 3 to day 9. Storage time significantly affected the TVC of the NC, LI and HI groups and not the PC group (Table 4.27). The NC group had higher ($P < 0.001$) TVC on day 6. In contrast, both the

intermediate groups (LI and HI) only had higher ($P < 0.001$ and $P < 0.05$, respectively) TVC on day 9. These findings, therefore, indicate that the salt reduction limits for 2016 and 2019 may be attainable without significant effects on microbial stability. These results are in agreement with Aaslyng et al. (2014), who reported no significant difference in TVC at three NaCl levels of 3.60, 2.80 and 2.00% in hotdog sausages. Sofos (1982c) described faster growth rates for total aerobic counts when salt content of frankfurters was reduced to 1.00%.

Significant effects of added NaCl content on coliform counts were found only for the first 3 days after manufacturing (Table 4.26). During this time counts ranged from log 4.12 ± 0.51 cfu/g for the LI group on day 3 to log 3.43 ± 0.38 cfu/g for the HI group on day 0. On day 0 the coliform counts of the HI group were lower ($P < 0.05$) than that of the NC group. On day 3 only the PC group had slightly lower coliform counts over the two intermediate added NaCl groups. The non-linear decreases and increases from day 3 to day 9 resulted in non-significant differences between the four treatments on day 6 and day 9. Storage time had no effect on the coliform counts of any of the four treatment groups (Table 4.27).

There were no significant differences in *E. coli* counts of the four treatment groups on day 0. In terms of *E. coli*, the initial numbers present were low (Table 4.26) and within the limit of < 10 cfu/g (SANS 885, 2011). On day 3, *E. coli* was only detected in the NC group and at a lower level than on day 0 for the same treatment group. On day 6 and day 9, no *E. coli* was detected in any of the four treatment groups. No storage time effect on *E. coli* counts could be determined (Table 4.27). *Escherichia coli* did, however, become undetectable at all added NaCl levels by day 3, compared to the NC group where it only became undetectable by day 6. As in the case of the *E. coli* counts of the bacon, it was suspected that LAB was able to outcompete the *E. coli* at this low storage temperature and in the presence of additives other than NaCl to which LAB are resistant (Lücke, 1998).

Staphylococcus aureus was not detected in any of the four formulations on any of the time intervals (Table 4.26). Yeast and mould counts were found to steadily increase over the 9 days without any significant differences between treatment groups on any of the days. The counts ranged from over log 4 cfu/g to close to log 7 cfu/g after day 9. In contrast to the treatment effect, storage time significantly affected the yeast and mould counts at every sampling time (Table 4.27). Yeast counts increased at the fastest rate in the HI group with higher ($P < 0.001$) counts at every sampling time. In the LI and PC groups this rate was reduced, although still higher ($P < 0.001$ for both) by day 6 and then again by day 9. Mould counts increased ($P < 0.001$ for all four treatments) from day 0 to day 3 and from day 3 to day 6. Only the mould counts of the

Table 4.26. The results of microbial analyses performed on four banger formulations containing different added NaCl levels.

Day	Treatment	TVC (log cfu/g) $n = 12$	Sign. level	Coliforms (log cfu/g) $n = 12$	Sign. level	*E. coli* (log cfu/g) $n = 12$	Sign. level	*S. aureus* (log cfu/g) $n = 12$	Sign. level	Yeasts (log cfu/g) $n = 12$	Sign. level	Moulds (log cfu/g) $n = 12$	Sign. level
0	**NC**	6.04 ± 0.40	NS	4.09^{b} ± 0.48	$P < 0.05$	0.71 ± 0.79	NS	ND	NSA	4.86 ± 0.38	NS	4.28 ± 0.59	NS
	LI	5.76 ± 0.37		3.92^{ab} ± 0.64		0.70 ± 0.77		ND		4.47 ± 0.59		4.37 ± 0.61	
	HI	5.69 ± 0.58		3.43^{a} ± 0.38		0.32 ± 0.58		ND		4.43 ± 0.51		4.23 ± 0.59	
	PC	5.57 ± 0.73		3.61^{ab} ± 0.54		0.58 ± 0.75		ND		4.56 ± 0.59		4.57 ± 0.35	
3	**NC**	6.24^{b} ± 0.56	$P < 0.05$	3.96^{ab} ± 0.36	$P < 0.05$	0.47 ± 0.90	NSA	ND	NSA	5.12 ± 0.53	NS	5.22 ± 0.51	NS
	LI	5.75^{ab} ± 0.57		4.12^{ab} ± 0.51		ND		ND		5.00 ± 0.45		5.17 ± 0.45	
	HI	5.72^{ab} ± 0.27		3.65^{ab} ± 0.61		ND		ND		4.98 ± 0.35		5.18 ± 0.48	
	PC	5.48^{a} ± 0.72		3.56^{a} ± 0.56		ND		ND		4.74 ± 0.43		5.01 ± 0.58	
6	**NC**	6.80^{b} ± 0.77	$P < 0.05$	3.58 ± 0.70	NS	ND	NSA	ND	NSA	6.01 ± 0.38	NS	5.83 ± 0.35	NS
	LI	6.38^{ab} ± 0.64		3.62 ± 0.71		ND		ND		6.22 ± 0.21		6.12 ± 0.46	
	HI	6.17^{ab} ± 0.63		3.67 ± 0.79		ND		ND		6.31 ± 0.35		6.09 ± 0.37	
	PC	6.04^{a} ± 0.49		3.50 ± 0.70		ND		ND		5.99 ± 0.15		5.87 ± 0.30	
9	**NC**	7.22^{b} ± 0.62	$P < 0.001$	3.84 ± 0.77	NS	ND	NSA	ND	NSA	6.54 ± 0.71	NS	6.43 ± 0.79	NS
	LI	6.73^{ab} ± 0.83		3.43 ± 0.75		ND		ND		6.87 ± 0.64		6.56 ± 0.75	
	HI	6.58^{ab} ± 0.85		3.30 ± 0.69		ND		ND		6.84 ± 0.56		6.53 ± 0.71	
	PC	5.83^{a} ± 1.03		3.44 ± 0.62		ND		ND		6.90 ± 0.50		6.65 ± 0.75	

TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*
NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl)
Means with different superscripts in the same column and on the same day differ significantly
ND = Not detected
NSA = Not statistically analysed
NS = Not significant

NC and PC groups had increased even further ($P < 0.001$ for both) from day 6 to day 9 (Table 4.27). High numbers of these types of microbes are known to cause spoilage (Fleet, 1992), although recently it was suggested that yeasts in particular, do not contribute substantially to meat product spoilage (Nielsen et al., 2008). The relatively low a_w requirements of yeasts and moulds (Jay et al., 2005), the ability of some yeasts to metabolise sodium nitrite (Fleet, 1992) and the oxygen permeability of the PVC film and natural hog casings may have provided an unrestrictive environment for growth. It should be noted that a 9 day shelf-life is highly unlikely and mostly served to illustrate an abusive storage period for this type of uncooked, refrigerated meat product. Although the amount of Na needed to inhibit microbial growth varies according to a number of factors; e.g. pH, temperature, oxygen, fat, and other additives (Doyle & Glass, 2010), it is clear that NaCl concentration plays a major role in microbial control. The concentration of NaCl needed will vary according to the specific type of product and level of processing involved. The current results may shed some light on the type of microbial results that can be expected for similar comminuted, non-heat treated and fresh processed meat products.

4.3.16. Bangers – Physical analysis: colour

High *L**-values were observed for all four treatment groups throughout the 9 day shelf-life (Table 4.28). This was attributed to the high added pork backfat content of 22.50% across all four treatment groups which cause increased lightness in comminuted sausage products (Chin, Lee, & Chun, 2004). No significant differences were observed until after day 6 where the NC and HI groups were darker ($P < 0.01$) than the PC group. After day 9, only the HI group was darker ($P < 0.05$) than the PC group. From day 0 to after day 9, a general decrease in *L**-values were found and this was attributed to a loss of WHC of the myofibrils due to comminution that led to a decrease in light scattering ability (Offer & Trinick, 1983). An initial increase in WHC due to comminution as on day 0 was eventually lost after prolonged storage with no heat treatment by day 9 (Zayas, 1997). Changes on overall moisture content appeared to have been very low (Table 4.23) and therefore, desiccation of the samples were not implicated in contributing to the loss of lightness. Storage time significantly affected the *L**-values of all four treatments (Table 4.29). The NC group was darker ($P < 0.001$) from day 3 onwards. The LI group was darker ($P < 0.005$) only from day 6 onwards, while the HI group was only darker ($P < 0.05$) on day 6. The PC group only became darker ($P < 0.001$) after day 9.

Table 4.27. The storage time effect over a 9 day shelf-life at 4 °C on the microbial parameters of four banger formulations containing different added NaCl levels.

Treat.	Storage time	TVC (log cfu/g) n = 12	Sign. level	Coliforms (log cfu/g) n =12	Sign. level	*E. coli* (log cfu/g) n = 12	Sign. level	Yeasts (log cfu/g) n = 12	Sign. level	Moulds (log cfu/g) n = 12	Sign. level
NC	**Day 0**	$6.04^{a} \pm 0.40$	$P < 0.001$	4.09 ± 0.48	NS	0.71 ± 0.79	NSA	$4.86^{a} \pm 0.38$	$P < 0.001$	$4.57^{a} \pm 0.35$	$P < 0.001$
	Day 3	$6.24^{ab} \pm 0.56$		3.96 ± 0.36		0.47 ± 0.90		$5.12^{a} \pm 0.53$		$5.22^{b} \pm 0.51$	
	Day 6	$6.80^{bc} \pm 0.77$		3.58 ± 0.70		ND		$6.01^{a} \pm 0.38$		$5.83^{c} \pm 0.35$	
	Day 9	$7.22^{c} \pm 0.62$		3.84 ± 0.77		ND		$6.54^{b} \pm 0.71$		$6.43^{d} \pm 0.79$	
LI	**Day 0**	$5.76^{a} \pm 0.37$	$P < 0.001$	3.92 ± 0.64	NS	0.70 ± 0.77	NSA	$4.47^{a} \pm 0.59$	$P < 0.001$	$4.23^{a} \pm 0.59$	$P < 0.001$
	Day 3	$5.75^{a} \pm 0.57$		4.12 ± 0.51		ND		$5.00^{a} \pm 0.45$		$5.17^{b} \pm 0.45$	
	Day 6	$6.38^{ab} \pm 0.64$		3.62 ± 0.71		ND		$6.22^{b} \pm 0.21$		$6.12^{c} \pm 0.46$	
	Day 9	$6.73^{b} \pm 0.83$		3.43 ± 0.75		ND		$6.87^{c} \pm 0.64$		$6.56^{c} \pm 0.75$	
HI	**Day 0**	$5.69^{a} \pm 0.58$	$P < 0.005$	3.43 ± 0.38	NS	0.32 ± 0.58	NSA	$4.43^{a} \pm 0.51$	$P < 0.001$	$4.37^{a} \pm 0.61$	$P < 0.001$
	Day 3	$5.72^{a} \pm 0.27$		3.65 ± 0.61		ND		$4.98^{b} \pm 0.35$		$5.18^{b} \pm 0.48$	
	Day 6	$6.17^{ab} \pm 0.63$		3.67 ± 0.79		ND		$6.31^{c} \pm 0.35$		$6.09^{c} \pm 0.37$	
	Day 9	$6.58^{b} \pm 0.85$		3.30 ± 0.69		ND		$6.84^{d} \pm 0.56$		$6.53^{c} \pm 0.71$	
PC	**Day 0**	5.57 ± 0.73	NS	3.61 ± 0.54	NS	0.58 ± 0.75	NSA	$4.56^{a} \pm 0.59$	$P < 0.001$	$4.28^{a} \pm 0.59$	$P < 0.001$
	Day 3	5.48 ± 0.72		3.56 ± 0.56		ND		$4.74^{a} \pm 0.43$		$5.01^{b} \pm 0.58$	
	Day 6	6.04 ± 0.49		3.50 ± 0.70		ND		$5.99^{b} \pm 0.15$		$5.87^{c} \pm 0.30$	
	Day 9	5.83 ± 1.03		3.44 ± 0.62		ND		$6.90^{c} \pm 0.50$		$6.65^{d} \pm 0.75$	

TVC = total viable count; *E. coli* = *Escherichia coli*

NC = negative control (0.00% added NaCl); LI = lower intermediate (1.00% added NaCl); HI = higher intermediate (1.50% added NaCl); and PC = higher intermediate (2.00% added NaCl)

Means with different superscripts in the same column and for the same treatment differ significantly

ND = Not detected

NSA = Not statistically analysed

NS = Not significant

There were no significant differences in *a**-values as a result of treatment (Table 4.28). It has been reported that during the shelf-life of fresh sausages the products eventually turn brown due to metmyoglobin formation. The brightest red colour may only be stable up to seven days when the sausages are packaged under O_2 inclusive conditions (Schivazappa, Virgili, Bovis, & Pedrelli, 2004). During this study the brightest redness appeared to have declined after day 0 already, although the overall decline was very limited. The effect of storage time on *a**- values confirmed that only the NC and HI groups were affected over time (Table 4.29). The *a**-values of the NC group decreased steadily with lower ($P < 0.001$) redness on day 6 compared to on day 0. For the HI group, this effect was slower with redness only lower ($P < 0.005$) on day 9 than on day 0. The myoglobin was believed to have remained in a relatively high oxygenated state due to the use of PVC overwrapping that was in direct contact with the samples (Carpenter, Cornforth, & Whittier, 2001) and the use of natural hog casings.

There was a general decrease in yellowness from day 0 to day 9 for all four treatment groups (Table 4.28). No significant differences in *b**-values were observed until after day 6. At this point the *b**-value of the HI group was lower ($P < 0.05$) than that of the PC group. After day 9, the *b**-values of all four treatments were so similar to each other that no significant differences were observed. Except for the major contribution made to *b** by the addition of pork backfat to the formulations, there was no clear indication that NaCl level had any effect. In contrast, differences in storage time resulted in significant differences in every treatment group (Table 4.29). Yellowness decreased ($P < 0.001$) in the NC group after day 0 and then remained very stable up to day 9. The two intermediate groups experienced similar significant ($P < 0.001$ for both) decreases in yellowness up to day 9. The PC group had stable *b**-values up to day 3, followed by a significant ($P < 0.001$) decrease by day 6.

Chroma generally decreased from day 0 to day 9 across all four treatments indicating that there was a general decrease in colour brightness over this period. This was confirmed by storage time having significant effects on all four treatments (Table 4.29). In the NC and LI groups, Chroma deteriorated ($P < 0.001$ for both) from day 0 to day 3 after which it remained stable. Chroma was less stable in the HI and PC groups. In the HI group it was lower (P 0.001) by day 3 and again after day 9. In the PC group it was lower ($P < 0.001$) after day 6 and consistently so up to day 9. Hue angle was also largely unaffected by differences in treatments (Table 4.28). After day 9, significant differences were observed. The *H** of the NC and HI groups were smaller ($P < 0.01$) and thus closer to redness compared to that of the PC group which was larger ($P < 0.01$) and more inclined towards yellowness. Significant effects by storage time on Hue angle were limited to only the NC group (Table 4.29). Hue angle was stable up to day 3 and then increased ($P < 0.005$) towards

redness. The addition of sodium metabisulfite, sodium tripolyphosphate, sodium ascorbate, and spices may have exhibited anti-oxidant activities (Gould, 2000; Arneth, 2001; Honikel, 2008; Tangkanakul et al., 2009; Li et al., 2010; Cheng et al., 2011; Mancini, 2013; Kiliç et al., 2014) to such an extent that the concentration dependent pro-oxidative effects of NaCl were sufficiently limited.

4.3.17. Bangers – Sensory analysis

The 75-member consumer panel consistently ranked the NC group lower ($P < 0.001$) than the other three treatment groups for all four of the chosen sensory attributes (Figure. 4.18). For taste, texture and overall liking, no significant differences in rankings were found between the LI, HI and PC groups, indicating that consumers could not detect any differences in terms of these attributes between the different treatments. Included in the banger formulations were a great variety of spices believed to have largely contributed in maintaining the overall product liking amongst consumers. Consumers were able to detect and indicate the negative effect that low NaCl content had on the texture (McCaughey, 2007) of the bangers. Furthermore, they were able to distinguish between the LI and PC groups in terms of saltiness with a preference for the highest NaCl containing bangers. These results confirm that there is a limit to the amount of NaCl that can be removed from a given product before it becomes unpalatable to consumers (Beauchamp, 2009).

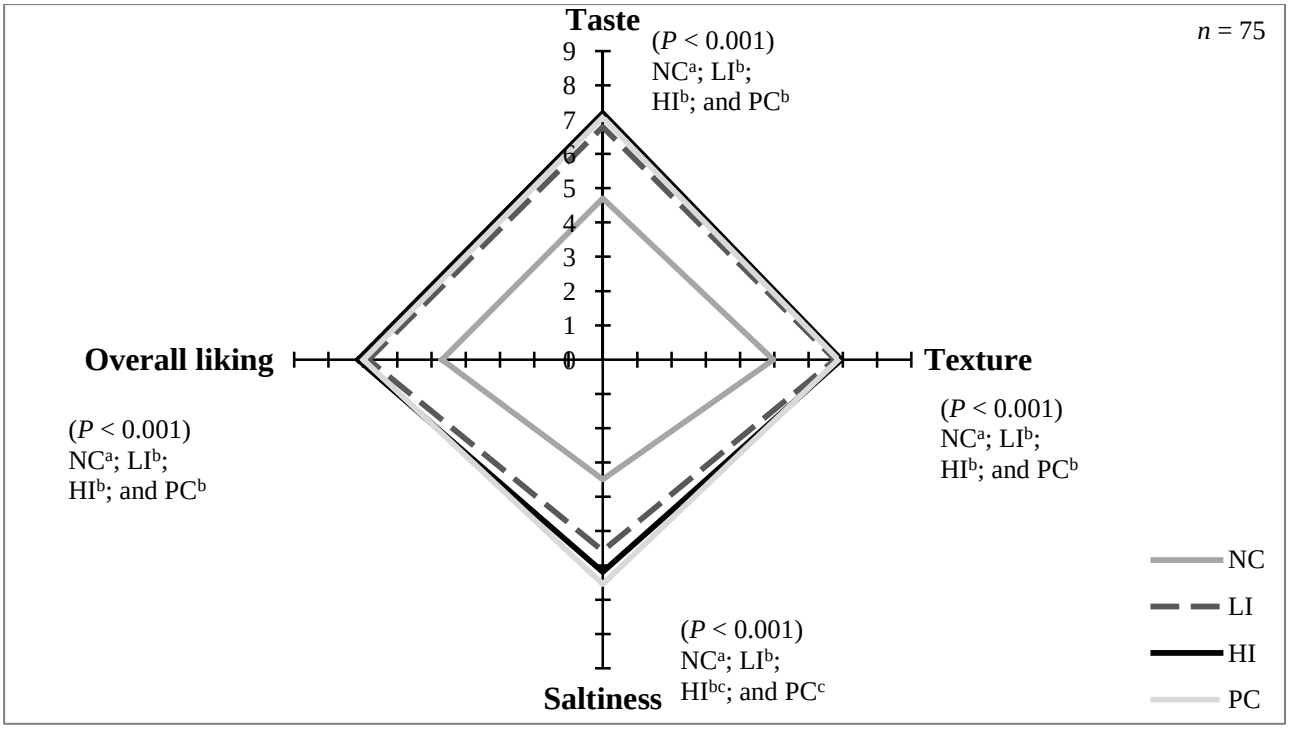

Figure. 4.17. Consumer sensory rankings of four banger treatment groups differing in added NaCl content. NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). Different letters for the same attribute indicate significant differences.

Table 4.28. Changes in the colour parameters of four banger formulations containing different added NaCl levels over a 9 day shelf-life.

Day	Treatment	*L** n = 72	Sign. level	*a** n = 72	Sign. level	*b** n = 72	Sign. level	*C** n = 72	Sign. level	*H** n = 72	Sign. level
0	**NC**	64.81 ± 2.86	NS	9.69 ± 1.06	NS	15.85 ± 0.77	NS	18.60 ± 0.90	NS	58.59 ± 2.95	NS
	LI	63.89 ± 2.49		9.58 ± 0.63		15.66 ± 0.54		18.37 ± 0.46		58.54 ± 2.12	
	HI	63.70 ± 2.60		9.72 ± 1.05		15.60 ± 0.47		18.41 ± 0.57		58.12 ± 3.12	
	PC	65.13 ± 1.52		9.75 ± 0.87		15.74 ± 0.69		18.53 ± 0.72		58.23 ± 2.60	
3	**NC**	63.21 ± 1.99	NS	9.32 ± 1.14	NS	15.10 ± 0.80	NS	17.75 ± 1.17	NS	58.41 ± 2.44	NS
	LI	62.77 ± 1.47		9.01 ± 0.84		14.97 ± 0.61		17.48 ± 0.79		59.00 ± 2.20	
	HI	62.21 ± 1.68		9.24 ± 1.37		14.61 ± 0.59		17.31 ± 1.11		57.85 ± 3.29	
	PC	63.13 ± 1.34		9.27 ± 0.92		14.85 ± 0.61		17.52 ± 0.94		58.09 ± 1.88	
6	**NC**	61.29^{a} ± 1.71	$P < 0.01$	8.61 ± 0.73	NS	14.07^{ab} ± 0.71	$P < 0.05$	16.50 ± 0.88	NS	58.56 ± 1.74	NS
	LI	61.55^{ab} ± 1.98		8.88 ± 0.71		14.14^{ab} ± 0.73		16.70 ± 0.94		57.92 ± 1.43	
	HI	60.43^{a} ± 2.15		8.89 ± 1.02		13.98^{a} ± 0.49		16.59 ± 0.61		57.60 ± 3.33	
	PC	63.26^{b} ± 1.16		8.70 ± 0.93		14.69^{b} ± 0.65		17.08 ± 0.98		59.46 ± 1.92	
9	**NC**	61.00^{ab} ± 2.22	$P < 0.05$	9.04 ± 1.38	NS	14.17 ± 0.90	NS	16.82 ± 1.48	NS	57.63^{a} ± 2.50	$P < 0.01$
	LI	61.85^{ab} ± 1.43		8.27 ± 0.87		14.09 ± 0.49		16.35 ± 0.82		59.67^{ab} ± 1.98	
	HI	60.60^{a} ± 2.13		8.63 ± 1.05		14.12 ± 0.60		16.57 ± 0.94		58.67^{a} ± 2.65	
	PC	62.89^{b} ± 1.08		8.09 ± 0.82		14.64 ± 0.67		16.74 ± 0.92		61.14^{b} ± 1.85	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and on the same day differ significantly

NS = Not significant

Table 4.29. The storage time effect over a 9 day shelf-life at 4 °C on the colour parameters of four banger formulations with different added NaCl levels.

Treat.	Storage time	*L** *n* = 72	Sign. level	*a** *n* = 72	Sign. level	*b** *n* = 72	Sign. level	*C** *n* = 72	Sign. level	*H** *n* = 72	Sign. level
NC	**Day 0**	$65.13^{b} \pm 1.52$	$P < 0.001$	$9.75^{c} \pm 0.87$	$P < 0.001$	$15.74^{b} \pm 0.69$	$P < 0.001$	$18.53^{b} \pm 0.72$	$P < 0.001$	$58.23^{a} \pm 2.60$	$P < 0.005$
	Day 3	$63.13^{a} \pm 1.34$		$9.27^{bc} \pm 0.92$		$14.85^{a} \pm 0.61$		$17.52^{a} \pm 0.94$		$58.09^{a} \pm 1.88$	
	Day 6	$63.26^{a} \pm 1.16$		$8.70^{ab} \pm 0.93$		$14.69^{a} \pm 0.65$		$17.08^{a} \pm 0.98$		$59.46^{ab} \pm 1.92$	
	Day 9	$62.89^{a} \pm 1.08$		$8.09^{a} \pm 0.82$		$14.64^{a} \pm 0.67$		$16.74^{a} \pm 0.92$		$61.14^{b} \pm 1.85$	
LI	**Day 0**	$63.70^{b} \pm 2.60$	$P < 0.005$	9.72 ± 1.05	NS	$15.60^{c} \pm 0.47$	$P < 0.001$	$18.41^{b} \pm 0.57$	$P < 0.001$	58.12 ± 3.12	NS
	Day 3	$62.21^{ab} \pm 1.68$		9.24 ± 1.37		$14.61^{b} \pm 0.59$		$17.31^{a} \pm 1.11$		57.85 ± 3.29	
	Day 6	$60.43^{a} \pm 2.15$		8.89 ± 1.02		$13.98^{a} \pm 0.49$		$16.59^{a} \pm 0.61$		57.60 ± 3.33	
	Day 9	$60.60^{a} \pm 2.13$		8.63 ± 1.05		$14.12^{ab} \pm 0.60$		$16.57^{a} \pm 0.94$		58.67 ± 2.65	
HI	**Day 0**	$63.89^{b} \pm 2.49$	$P < 0.05$	$9.58^{b} \pm 0.63$	$P < 0.005$	$15.66^{c} \pm 0.54$	$P < 0.001$	$18.37^{c} \pm 0.46$	$P < 0.001$	58.54 ± 2.12	NS
	Day 3	$62.77^{ab} \pm 1.47$		$9.01^{ab} \pm 0.84$		$14.97^{b} \pm 0.61$		$17.48^{b} \pm 0.79$		59.00 ± 2.20	
	Day 6	$61.55^{a} \pm 1.98$		$8.88^{ab} \pm 0.71$		$14.14^{a} \pm 0.73$		$16.70^{ab} \pm 0.94$		$57. 92 \pm 1.43$	
	Day 9	$61.85^{ab} \pm 1.43$		$8.27^{a} \pm 0.87$		$14.09^{a} \pm 0.49$		$16.35^{a} \pm 0.82$		59.67 ± 1.98	
PC	**Day 0**	$64.81^{b} \pm 2.86$	$P < 0.001$	9.69 ± 1.06	NS	$15.85^{b} \pm 0.77$	$P < 0.001$	$18.60^{c} \pm 0.90$	$P < 0.001$	58.59 ± 2.59	NS
	Day 3	$63.21^{ab} \pm 1.99$		9.32 ± 1.14		$15.10^{b} \pm 0.80$		$17.75^{bc} \pm 1.17$		58.41 ± 2.44	
	Day 6	$61.29^{ab} \pm 1.71$		8.61 ± 0.73		$14.07^{a} \pm 0.71$		$16.50^{a} \pm 0.88$		58.56 ± 1.74	
	Day 9	$61.00^{a} \pm 2.22$		9.04 ± 1.38		$14.17^{a} \pm 0.90$		$16.82^{ab} \pm 1.48$		57.63 ± 2.50	

NC = negative control (0.00% added NaCl); LI = low intermediate (1.00% added NaCl); HI = high intermediate (1.50% added NaCl); and PC = positive control (2.00% added NaCl). *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and for the same treatment differ significantly

NS = Not significant

Salt may affect the flavour of foods in other ways than just contributing a salty taste. In agreement with the results of the polony, it appeared as if the masking effect of added NaCl (Kilcast & den Ridder, 2007) may have contributed to the overall higher liking consumers had for the three NaCl containing groups. Of the three product types, the NC group of the bangers achieved the most consistently higher rankings than the NC groups of the other two products. This was attributed to the use of many spices which improved the overall sensory experience (Salles, 2006; Doyle, 2008) even with no added NaCl and the comminuted texture of a sausage product that did not rely greatly on good protein extraction for its overall texture.

4.4. Conclusions

A major concern when the NaCl content of any processed meat product is reduced is the well documented negative effect such as step might have on the microbial stability of an affected product. The results from this study found this effect to be greatly dependent on the type of product involved and to be more limited than expected. The use of intermediate added NaCl levels in bacon led to microbial parameters that were agreeable with the original NaCl content. Due to the particular processing of bacon involving the use of nitrite, smoking, freezing, thawing, and vacuum packaging, a number of microbial hurdles remained intact at intermediate added NaCl levels to successfully challenge spoilage and potential pathogenic microorganisms. The microbial shelf-life of the two intermediate added NaCl polony models was also indistinguishable from the original NaCl level model. The fact that this type of product is sealed, then heat-treated and contains a number of components with anti-microbial properties, made it particularly suitable for Na reduction without negatively affecting microbial shelf-life. The results of the intermediate added NaCl bangers gave similar results to that of the bacon and polony. No substantial defects as a result of intermediate added NaCl levels on various microbial parameters were identified. Although the results of the general microbial parameters were very favourable, further investigation of the survivability and growth of specific spoilage microorganisms and potential pathogens related to these products would be advisable.

The intermediate added NaCl levels had very little effect on basic chemical parameters of the bacon such as pH, a_w and moisture content. The pH was the only parameter to be significantly affected and only at the end of the bacon shelf-life. The pH of all the polony treatment groups increased after manufacturing due to the added phosphate without being negatively affected by lower added salt content. Over time, the intermediate added NaCl levels together with the original added NaCl level had the most stable pH. No effects on a_w and moisture content were found. In contrast to the pH of

the bacon and polony, added NaCl level had no effect on the pH of any of the banger formulations. Added NaCl level only affected the a_w of the intermediate groups from day 6 to day 9. Moisture content was similarly only affected from day 6 onwards. The overwrap packaging was most probably an important factor in contrast to the much less permeable vacuum and nylon casing packaging of the bacon and polony, respectively. Due to the inconsistency of these results, greater evaluation may be needed to confirm that the use of intermediate added NaCl levels may actually have such limited effects on these particular parameters and to test the validity of some of the proposed theories.

In the absence of other replacement strategies that could contribute to the non-volatile mineral content of a particular sample, determining the ash content quickly and cheaply helped to identify samples from different treatments as well as serving as a preparation step for AAS. Atomic absorption spectroscopy was confirmed to be more precise than the titrimetric method with regard to accurately determining Na content which is very important from a regulatory point of view. The titrimetric method disregards all other sources of sodium not originally associated with chloride and in a complex product with any number of Na containing sources, may lead to significant discrepancies between the labelled and actual values.

The negative control groups revealed baseline Na content that could not simply be explained by the Na content of the meat and fat as main components. This “hidden” Na contributed by many other components in the different formulations was in most cases overestimated during formulation. The actual Na levels of the intermediate groups were consistently lower than the regulatory and theoretical limits they were formulated to match. These formulations were kept the same for all replicates to limit the introduction of new variations. In a real world situation, these levels could be fine-tuned to better match the limits thereby maximising the Na content, with the accompanied benefits of Na and NaCl whilst still adhering to the regulatory limits aimed at limiting the daily Na consumption.

As one of its most reported negative effects, the pro-oxidative effect of salt on the lipid oxidative stability of meat products was expected to reveal the greatest variation between treatment groups with different added salt levels. For the bacon, the negative control consistently had the lowest, and the positive control, the highest level of lipid oxidation. The lipid oxidation levels in the intermediate groups fluctuated between these two extremes. In terms of detectable rancidity development, all the treatments maintained levels far below the detection threshold. For the polony, no significant improvement of lipid oxidative stability by intermediate added salt levels were found with lipid oxidation levels consistently far below the detectability of rancidity threshold. It was

ascribed to the low concentration of reactive substances across all four treatment groups. It was also concluded that a number of other components limited lipid oxidation to such an extent that even between the highest and lowest added salt levels, no significant effects were found. For the bangers, differences in lipid oxidation were found during both short-term refrigerated and long-term frozen storage, although there was no consistently direct correlation between added salt content and the level of lipid oxidation. The upper detection limit for rancidity was soon reached during the fresh product shelf-life of all three salt-containing treatment groups. This was also reflected by the results at the end of the frozen storage shelf-life. Differences in packaging were also believed to have played a role in the different lipid oxidation levels of the different products. While the bacon was vacuum-packaged and the polony batters filled into nylon casings, the bangers were overwrapped with PVC film in EPS trays. This resulted in a significant headspace over the bangers, containing oxygen rich air which may have promoted oxidation reactions. With the exception of butcheries, commercial frozen bangers are already packaged in moulded trays which may or may not contain modified atmospheres that may limit these oxidation reactions.

Added NaCl level had a number of significant effects on the colour and colour stability of the bacon. The lightness parameter appeared to be highly dynamic over the shelf-life period and a correlation with changes in WHC was drawn. Redness followed a similar dynamic with no consistent deterioration most probably in relation to the cured meat colour development characteristic of this product type. Yellowness increased over time although the perceivable effect was largely hidden by the cured meat colour. These interactions were reflected in the Chroma and hue angle results. The lightness and yellowness of the bangers was substantially higher than that of the bacon due to the incorporated pork backfat. The higher intermediate group was significantly darker than the positive control and lightness generally decreased from directly after manufacturing to the end of shelf-life, probably due to a loss in WHC and the resulting light scattering ability. Redness remained stable in all four treatments probably more due to the oxygen permeable packaging than the anti-oxidant effect of components in the formulations. Chroma only generally decreased over time and the hue angle of the higher intermediate group was substantially greater than that of the positive control at the end of shelf-life. It was concluded that added salt level had very limited effect on banger colour compared to that of bacon.

The observable textures of the intermediate polony groups only revealed wetter cutting surfaces compared to the positive control. Shear force data revealed that the intermediate groups had textures that could rival that of the positive control directly after manufacturing. In the middle of the shelf-life, these textures were significantly firmer than that of the negative control. Whilst the textures of all four treatments deteriorated by the end of shelf-life, that of the higher intermediate group still

compared favourable to the positive control. The two intermediate bacon treatment groups experienced cooking losses similar to that of the negative control while total losses for these two groups were only midway between that of the two control groups. Losses were better controlled in the two intermediate banger groups. In terms of thaw, cooking, refrigeration and total losses, these groups were both comparable to that of the positive control groups.

As expected, the negative control groups were clearly disliked by consumers due to the very low salt taste and accompanied negative effects on other organoleptic properties while the positive controls were well received. The higher intermediate group bacon was indistinguishable from the positive control and even the low salt intermediate received considerable sensory rankings. The negative control of the polony was the most unacceptable of all three negative controls and this best exemplified the important contributions made by salt to organoleptic properties other than just taste. The two intermediate salt levels again compared favourably to a positive control group with even the lower intermediate group found to be indistinguishable. A slightly lower than current added salt level may actually have been preferred. Both the intermediate added salt groups of the bangers compared very well with the positive control with only the saltiness ranking of the lower intermediate significantly lower than that of the positive control. The inclusion of a number of spices was believed to have largely masked the greatly reduced salt content of this group.

The use of two intermediate added NaCl levels specific to product type as sole Na reduction strategy delivered promising results. Basic chemical, microbial, sensory and lipid oxidative stability analyses results were by far the most encouraging results in favour of straight forward Na reduction without any replacement. Discrepancies in contrast to positive control levels were found for other parameters such as colour, yields and losses. It is possible that by initially constant reformulation, the intermediate groups of each product type may better meet specific regulatory limits thus resulting in higher total salt and Na content that may further improve on these discrepancies. Increasing the use of already critical and multi-functional additives such as phosphates may also address some of these issues, although this might be regarded as a controversial approach. These scenarios might avoid situations where “one-size-fits-all” solutions need to be considered that may exceed the replacement requirements and that may introduce new challenges such as off-flavours and tastes, and unknown, “scientific-sounding” names on originally simpler product labels. One of the greatest challenges to these findings is the degree of success this approach will have in large scale production. It is, however, believed that salt reduction as sole approach to Na reduction should be evaluated for similar meat products as it may limit the increased production costs and possibly negative consumer reaction associated with the use of salt and Na replacers.

CHAPTER 5

THE EFFECTS OF SALT/SALT REPLACER COMBINATIONS ON THE CHEMICAL, MICROBIAL, SENSORY, AND TECHNOLOGICAL PARAMETERS OF PORK SAUSAGES

ABSTRACT

This study evaluated the effect of a 1.00% added NaCl level in conjunction with various sodium replacers on the thawing, cooking and refrigeration losses; a_w; lipid oxidative, microbial and colour stability; and sensory quality of pork sausages. The following treatments were added (w/w) to the 1.00% basal NaCl: 1.00% NaCl with 1.00% KCl; 1.00% NaCl with 1.00% K-gluconate; 0.80% NaCl with 0.80% KCl and 1.00% YE; and 1.00% NaCl with 0.80% KCl and 0.20% K-lactate. The goal was to best match the quality characteristics of a 2.00% Na content in pork banger sausages while complying with the final total sodium content limit of local regulations set to become mandatory in the near future. Basic chemical parameters and lipid oxidative stability were mostly unaffected by the replacers, although significant effects were observed for other parameters. The use of KCl maintained the highest lightness colour score, lowest TVC and best consumer acceptability. Potassium gluconate had poor taste and overall consumer liking. The addition of YE increased both TVC and yellowness colour score. The low concentration of K-lactate increased TVC and improved redness stability over time. The use of KCl as the best candidate for Na replacement was confirmed for use in pork bangers. Final Na content levels were in the region of 600 mg/100 g product. Potassium content was greatly increased from 230 mg/100 g in the original formulation to 390-600 mg/100 g in a food product not normally associated with being a major dietary source of potassium.

Keywords: sodium replacers, pork bangers, shelf-life, potassium chloride, potassium gluconate, yeast extract, potassium lactate

5.1. Introduction

In recent decades the increased consumption of processed foods containing high levels of sodium (Na) has changed the perception of dietary salt. It is now considered a potential health threat (Doyle, 2008). There is a progressive increase in the blood pressure levels of individuals and increasing prevalence of age-associated hypertension across populations which appears to be

directly correlated to Na intake (Dickinson & Havas, 2007). The World Health Organization has recommended that salt intake or Na intake be limited to <5 g/day or <2 g/day, respectively (WHO, 2007). When Na reductions are made in commercial products, it is usually driven by policy, as is the case in South Africa with the government being the driving force behind current Na reductions (Department of Health, 2013). Contributing to this is a need to be more competitive with having healthier products in the market (Appel et al., 2012). This occurs regardless of whether consumers are aware of, and understand either the reductions or labelling thereof (Kim et al., 2012).

A number of reviews have described the approaches taken to achieve Na reduction which includes: reformulation to lower salt and/or Na content, sometimes by gradual reductions or "reduction-by-stealth"; replacement of salt and/or Na with other compounds either free from Na or contributing smaller amounts of Na; and the alteration of current, or incorporation of new processing techniques (Ruusunen & Puolanne, 2005; Desmond, 2006; Albarracín, Sánchez, Grau, & Barat, 2011; Wallis & Chapman, 2012; Kloss, Meyer, Graeve, & Vetter, 2015).

From the results of the previous chapter where consumers could not discriminate between reduced NaCl of three product types (bacon, polony and pork bangers), it was concluded that there is room for straight-forward Na reduction to substantially lower Na levels, although the greater the reduction, the more uncertain the end result on quality and stability may be. It was also found that while one product type may work well with highly reduced Na content, another product may exhibit defects at lower reduced Na content. With the nutritional and health aspect in mind, the original importance of NaCl in meat quality and safety cannot be ignored. In developing countries, maintenance of a robust product shelf-life is critical. For example, 70% of poor urban households in South Africa already suffer from severe food insecurity (Frayne, Battersby-Lennard, Fincham & Haysom, 2009). Food wastage through spoilage can further exacerbate this problem. In South Africa, an estimated 2 million tonnes of meat products were produced in 2007 and, of this, over 544 thousand tonnes were wasted before reaching the consumer, as waste generated from production up to, and including, distribution (Oelofse & Nahman, 2013).

Maintaining current saltiness levels with the aid of replacers may avoid the situation where consumers need to "learn" to like lower saltiness. It may also avoid situations where consumers switch to competitors' products where the original taste profile might be better maintained (Bobowski et al., 2015). The focus of most literature has been to compare different replacer formulations by combining one or more replacers with NaCl over gradients in concentration. There have been no reports of using replacers to improve a reduced NaCl level previously shown to provide mostly positive results, to a point where it is indistinguishable from the original NaCl level.

A final consideration with regard to the implementation of replacers is the cost of these salt/sodium replacers in relation to the low cost of NaCl. Sodium replacement at levels of of 20-30% may increase raw material costs by 5-30% (Dötsch et al., 2009). Therefore, cheap but highly effective replacements have to be sought in an effort to reduce the overall dependence on high added NaCl levels.

The aim of this chapter was to investigate the potential use of various Na replacers in lowering the total Na content in a pork banger sausage model as described for Class 7 or "Comminuted, uncured, no or partial heat treated products" (SANS 885, 2011), whilst maintaining, or even improving, the overall stability and sensory quality. Bacon was immediately excluded as a potential model for this chapter due to its current exclusion from the Na reduction regulations. Polony was excluded as a model due to its status as a heat-treated product which would have limited oberservations in terms of the microbial parameters as well as the colour paramters due to the use of erythrosine BS as colourant. Comparisons were drawn against a current Na usage level and final regulatory Na limit on the basis of:

1. Chemical and microbial parameters,
2. Sensory acceptance and colour, and
3. Refrigeration, thaw, and cooking losses.

5.2. Materials and methods

5.2.1. Sourcing of lean meat, backfat, additives and spices

Sourcing of all lean meat, pork backfat, additives and spices was conducted as described in Chapter 4, pp. 66, 70-71 of this thesis.

5.2.2. Formulation of the banger batters

Six treatments groups were formulated for use in this study (Tables 5.1 and 5.2). A positive control with 2.00% added NaCl and a baseline level with only 1.00% added NaCl as the lowest added NaCl similar to the NaCl level of most of the formulations were used. The third formulation involved a 50% replacement of NaCl with KCl as a very simple replacement formulation. The fourth formulation involved a 50% replacement of NaCl with potassium gluconate (K-gluconate) without the addition of KCl to study the specific effects K-gluconate may have on various parameters. The fifth formulation involved the use of a 50% replacement of NaCl with KCl and the inclusion of yeast extract (YE) for the evaluation of its effect on colour, lipid oxidative stability, microbial

stability and sensory acceptability specifically and to determine if it is required to mask the use of KCl. The sixth formulation involved the use of potassium lactate (K-lactate) as a salt of an organic acid together with KCl as an ionic analogue for NaCl with specific interest on its effect on microbial stability.

Table 5.1. Sodium contributions towards total Na content of six banger formulations with different added NaCl and/or replacer levels.

		Sodium contribution by component (mg/100 g)					
	Treatment	**BL**	**PC**	**NaK**	**NaKGlu**	**NaKYE**	**NaKKlac**
Formulation component	**Baseline formulation**[†]	197.04	196.41	196.41	196.41	196.03	196.41
	NaCl[††]	393.37	786.74	393.37	393.37	314.70	393.37
	KCl	n.a.	n.a.	0.00	n.a.	0.00	0.00
	K-gluconate	n.a.	n.a.	n.a.	0.00	n.a.	n.a.
	Yeast extract[*]	n.a.	n.a.	n.a.	n.a.	90.00	n.a.
	K-lactate	n.a.	n.a.	n.a.	n.a.	n.a.	0.00
	Total Na (mg/100 g)	590.41	983.15	589.78	589.78	600.73	589.68

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

[†]Depending on percentage lean meat included to make up 100%

[††]Depending on percentage NaCl included to make up 100%

[*]Determined through AAS to contain the equivalent of 23% w/w NaCl

n.a. = not applicable

Table 5.2. The composition of six banger batters formulated with different added NaCl and/or replacer levels.

Composition (%)	**BL**	**PC**	**NaK**	**NaKGlu**	**NaKYE**	**NaKKlac**
Lean pork 90/10	55.81	54.81	54.81	54.81	54.21	54.81
Pork backfat	22.50	22.50	22.50	22.50	22.50	22.50
Ice water	15.00	15.00	15.00	15.00	15.00	15.00
Rusk	3.50	3.50	3.50	3.50	3.50	3.50
Spices and additives	1.39	1.39	1.39	1.39	1.39	1.39
Starch	0.80	0.80	0.80	0.80	0.80	0.80
NaCl	1.00	2.00	1.00	1.00	0.80	1.00
KCl	n.a.	n.a.	1.00	n.a.	0.80	0.80
K-gluconate	n.a.	n.a.	n.a.	1.00	n.a.	n.a.
Yeast extract (YE)	n.a.	n.a.	n.a.	n.a.	1.00	n.a.
K-lactate	n.a.	n.a.	n.a.	n.a.	n.a.	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

The basis of formulation for each banger batter was a percentage of NaCl plus replacer(s) equivalents to 2.00% of the original formulation as equal to 2.00% NaCl in the original formulation (Table 5.1) on a weight-per-weight basis. The first formulation only included 2.00% added NaCl as

the positive control treatment and the second formulation included only 1.00% added NaCl as the baseline treatment. The 1.00% level was selected as the baseline group as this was the lowest level of added NaCl to be used in conjunction with replacers in the other four treatments. From the results of Chapter 4, the effect that this level would have on various parameters of the bangers, were already known and it was expected that the results of the replacer-added formulations would fall within a range between the 2.00% and 1.00% added NaCl levels. Consistent with the previous chapter, the total Na content of the 1.00% added NaCl treatment was expected to be ~ 590.41 mg/100 g which was within the Na content limit of no more than 600 mg Na/100 g by 30 June 2019 (Department of Health, 2013) as described in Chapter 3 on p. 49. The disparity between formulated Na content and actual Na content was not addressed as this would have resulted in an increase in the amount of NaCl to be added to the 1.00% added NaCl formulation thereby creating a variation in results. Pertaining to this study, it was also accepted that the baseline formulation, without NaCl would have a total Na content of ~ 196.03 to ~ 197.04 mg/100 g product depending on the percentage of meat in the formulation. Thus, at 2.00% added NaCl, 786.74 mg Na/100 g would be contributed to the final product plus ~ 196.41 mg Na/100 g (baseline formulation Na content) resulting in ~ 983.15 mg Na/100 g in the final formulation. At 1.00% added NaCl, 393.37 mg Na/100 g would be contributed to the final product plus ~ 197.04 mg Na/100 g resulting in ~ 590.41 mg Na/100 g in the final formulation (below 2019 target of 600 mg/100 g).

The third formulation consisted of a 1:1 ratio of 1.00% added NaCl and 1.00% added KCl with the following total Na content: 196.41 mg Na/100 g (baseline formulation Na content) + 393.37 mg Na/100 g (1.00% NaCl) + 0.00 mg Na/100 g (1.00% KCl) = 589.78 mg Na/100 g (below 2019 target of 600 mg/100 g). The fourth formulation consisted of a 1:1 ratio of 1.00% added NaCl and 1.00% added K-gluconate with the following total Na content: 196.41 mg Na/100 g (baseline formulation Na content) + 393.37 mg Na/100 g (1.00% NaCl) + 0.00 mg Na/100 g (1.00% K-gluconate) = 589.78 mg Na/100 g (below 2019 target of 600 mg/100 g). The fifth formulation consisted of a 0.80:0.80:1 ratio of 0.80% added NaCl, 0.80% added KCl and 1.00% YE with the following total Na content: 196.03 mg Na/100 g (baseline formulation Na content) + 314.70 mg Na/100 g (0.8% NaCl) + 0.00 mg Na/100 g (0.80% KCl) + 90.00 mg Na/100 g (1.00% YE) = 600.73 mg Na/100 g (just over 2019 target of 600 mg/100 g). The sixth and final formulation consisted of a 1:0.80:0.20 ratio of 1.00% added NaCl, 0.80% added KCl and 0.10% K-lactate with the following total Na content: 196.41 mg Na/100 g (baseline formulation Na content) + 393.37 mg Na/100 g (0.80% NaCl) + 0.00 mg Na/100 g (0.10% K-lactate) = 589.68 mg Na/100 g (below 2019 target of 600 mg/100 g). The percentage of NaCl had to be reduced to 0.80% to accommodate the 23% w/w NaCl contribution made by the YE and still limit total Na to less than 600 mg/100 g. To

maintain a 1:1 ratio with KCl, the KCl also had to be reduced to 0.80%. The six formulations were henceforth named: BL for the baseline group, PC for the positive control, NaK for the first replacer formulation, NaKGlu for the second replacer formulation, NaKYE for the third replacer formulation, and NaKKlac for the fourth replacer formulation.

In order to maintain a 2.50 kg batch size across all six treatments, the difference in added NaCl and replacers was compensated for by adjusting the amount of lean pork used from 55.81% for the BL group to 54.21% for the NaKYE group (Table 5.2). The NaKYE group had to contain less lean meat than any other group to accommodate the addition of 1.00% YE. The spice and additive pack of each treatment group was identical and contained the same components as described in Chapter 4 on p. 71.

5.2.3. Manufacturing of the bangers

Three separate replicates were manufactured at least one month apart. This was done to negate variations in raw materials and processing and environmental conditions. A single replicate consisted of all six treatments. A further fourth replicate was manufactured specifically for sensory analysis and a fifth replicate was manufactured for determining losses. The manufacturing of the bangers then proceeded as described in Chapter 4 on p. 73.

5.2.4. Sampling

Sample handing, storage and selection proceeded as described in Chapter 4 on p. 74.

5.2.5. Refrigeration, thaw and cooking losses

Refrigeration, thaw and cooking losses were carried out as for the bangers in Chapter 4 on p. 75

5.2.6. Chemical analyses

For the determination of K in addition to that of Na (Chapter 3, pp. 51−52), 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. All other chemical analyses were carried out and applied as for the bangers in Chapter 4 as described on pp. 75−76.

5.2.7. Microbial analyses

The microbial analyses procedures were identical to those described in Chapter 4 on p. 78.

5.2.8. Physical analysis

Colour analysis was carried out as for the bangers in Chapter 4 on p. 78−79.

5.2.9. Consumer sensory evaluation

Consumer sensory analysis was carried out as for the bangers in Chapter 4 on p. 81.

5.2.10. Statistical analyses

Two experimental designs were used consisting of a 6x4x3 (n = 72) factorial design for the fresh bangers and a 6x3x3 factorial design for the frozen bangers. The first factorial design was made up out of six formulations (BL, PC, NaK, NaKGlu, NaKYE and NaKKlac), four sampling intervals (days 0, 3, 6 and 9) and three replicates per treatment group per sampling interval. The second factorial design was made up out of six formulations (BL, PC, NaK, NaKGlu, NaKYE and NaKKlac), three sampling intervals (days 0, 90 and 180) and three replicates per treatment group per sampling interval. Statistical analyses were carried out as described in Chapter 4 on p. 81. Statistically significant variables were visualized in a 2-dimensional space by Principal Component Analysis (PCA) (NCSS, 2007).

5.3. Results and discussion

5.3.1 Salt replacer rationale

A number of salt replacers to be used in conjunction with NaCl were considered. The use of the following replacers was based on their relative cost, previous use in literature, current commercial use and availability. Potassium chloride was selected as the first replacer due to its simplicity, low cost of around R20.00/kg and availability (Cobus Ferreira, BT Enterprises Johannesburg, personal communication, April 29, 2015). Potassium chloride at a 2% inclusion level may result in metallic and bitter aftertastes (Campagnol et al., 2011). Using KCl in combination with NaCl was believed to minimize these tastes and even to enhance saltiness (Keast & Hayes, 2011). Guárdia et al. (2006) found no significant difference in consumer preference of 100% NaCl over 50% NaCl and 50% KCl in fermented sausages. It was believed that in a similarly flavourful sausage type such as bangers, this result could be replicated.

Sodium gluconate (Na-gluconate) and K-gluconate were considered as options for the second replacer. These products are reportedly good salt replacers and relatively cheap at round R38/kg. A total salt reduction of 40% is reportedly achievable without any negative effect on shelf-life or taste.

Inclusion levels (w/w) of between 1.00 and 2.50% are used depending on the total salt reduction needed (Cobus Ferreira, BT Enterprises Johannesburg, personal communication, April 29, 2015). Commercial products such as sub4salt® from Jungbunzlauer (Ladenburg GmbH, Germany) and Gloconal® K-G from Corbion (Amsterdam, The Netherlands) consist of blends involving K-gluconate in combination with other components such as NaCl and KCl. Due to the proprietary nature of such products, the exact compositions are, however, unknown. It was decided to create a new blend with known composition for this study. Sodium gluconate was immediately excluded due to an estimated Na content of 105.40 mg/g which would have been too high for inclusion at 1.00% in a 1:1 ratio to NaCl. It was also found that although multiple commercial products exists containing K-gluconate, no peer-reviewed articles could be found describing its use in meat products except for an original patent filing by Bolin, Bacus, & Barhaug (1983).

Yeast extract was also included due to its flavour enhancing and masking abilities (Brandsma, 2006) with regard to masking negative flavours with the use of KCl (Campagnol et al., 2011). Commercial products such Provesta® (Hamburg, Germany) and Maxarome® (DSM, Johannesburg, South Africa) exist although the composition of these products were also unknown. A pure yeast extract containing only autolyzed yeast with a known NaCl content (23% w/w) normally used as additive to spice blends, was used (Crown National, Johannesburg, South Africa).

The final replacer selected was K-lactate. In meat products, K-lactate is used to enhance flavour and extend shelf-life. Its use is, however, limited by its bitter taste (Brewer et al. 1991; Gou et al., 1996; according to Guárdia et al., 2006). Sensory preference results have indicated no significant preference between using this salt in combination with KCl for a 50% NaCl reduction versus 100% NaCl (Guárdia et al., 2006; 2008). For this study, K-lactate was sourced as Corbion Purac Purasal® HiPure P Plus with a known composition of 78% potassium-L-lactate and 22% water as a syrupy liquid (Lionheart Chemical Enterprises, Johannesburg, South Africa).

5.3.2. Main effects and interactions

Salt and/or replacer level, storage time and replicate were the main effects and salt and/or replacer level with storage time was identified as the main interaction between main effects (Table 5.3). The largest number of significant effects on various parameters was due to differences in added NaCl and/or replacer levels, followed by differences in replicates and then differences in storage time. Significant effects of different added NaCl and/or replacer levels were most pronounced for the chemical properties and composition of the bangers, colour parameters and refrigeration, thaw and cooking losses. Differences in storage time resulted in significant differences in most chemical

parameters, TBARS during frozen storage, most microbial parameters and all colour parameters. Differences in replicates resulted in significant differences in all chemical parameters and TBARS during frozen storage, most of the microbial and colour parameters, and some of the chemical properties and composition of the bangers. The interaction between NaCl and/or replacer levels and storage time resulted in significant differences for the TVC and all the colour parameters.

Table 5.3. ANOVA of the main effects and interactions of various parameters of bangers with different added and/or replacer levels.

Parameter	NaCl and /or replacer level	Storage time	Replicate	NaCl and/or replacer level X Storage time
Chemical properties and composition of bangers immediately after manufacturing				
% Ash	$P < 0.001$	NSA	NS	NSA
% NaCl	$P < 0.001$	NSA	NS	NSA
g NaCl/100 g	$P < 0.001$	NSA	NS	NSA
mg Na/100g	$P < 0.001$	NSA	NS	NSA
mg K/100 g	$P < 0.001$	NSA	NS	NSA
% Moisture	NS	NSA	$P < 0.001$	NSA
a_w	$P < 0.001$	NSA	$P < 0.001$	NSA
pH	NS	NSA	$P < 0.001$	NSA
Changes in chemical parameters of bangers during storage at 4 ^oC up to 9 days				
% Moisture	NS	NS	$P < 0.001$	NS
pH	NS	$P < 0.001$	$P < 0.001$	NS
a_w	$P < 0.001$	$P < 0.01$	$P < 0.001$	NS
TBARS	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Lipid stability of bangers during frozen storage at -18 ^oC up to 90 days				
TBARS	$P < 0.01$	$P < 0.001$	$P < 0.001$	NS
Changes in the microbiological parameters of bangers during storage at 4 ^oC up to 9 days				
TVC	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$
Coliforms	$P < 0.001$	NS	$P < 0.001$	NS
E. coli	NS	$P < 0.001$	$P < 0.001$	NS
S. aureus	NSA	NSA	NSA	NSA
Yeasts	NS	$P < 0.001$	$P < 0.001$	NS
Moulds	NS	$P < 0.001$	$P < 0.001$	NS
Changes in the colour parameters of bangers during storage at 4 ^oC up to 9 days				
L*	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$
a*	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
b*	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Chroma	$P < 0.001$	$P < 0.001$	$P < 0.005$	$P < 0.001$
Hue angle	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Refrigeration, thaw and cooking losses				
Thaw loss (%)	$P < 0.001$	NSA	NSA	NSA
Cooking loss (%)	$P < 0.001$	NSA	NSA	NSA
Total loss (%)	$P < 0.001$	NSA	NSA	NSA
% Refrigeration loss	$P < 0.001$	NSA	NSA	NSA
Sensory properties of bangers with different NaCl levels and/or replacer combinations				
Aroma	NS	NSA	NSA	NSA
Taste	$P < 0.05$	NSA	NSA	NSA
Texture	NS	NSA	NSA	NSA
Saltiness	NS	NSA	NSA	NSA
Overall acceptability	$P < 0.05$	NSA	NSA	NSA

TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*
NS = Not significant
NSA= Not Statistically analysed

5.3.3. Refrigeration, thaw, and cooking losses

Thaw and cooking losses were measured on the bangers that were initially frozen and the total losses were the sum of these two sets of results. Refrigeration loss was only measured after 9 days on the bangers kept at 4 °C. The PC group had the lowest ($P < 0.001$) thaw loss of 0.61 ± 0.07% followed by a higher ($P < 0.001$) thaw loss of 0.76 ± 0.10% for the BL group (Table 5.4). All four replacer formulations had similar and higher ($P < 0.001$) thaw losses compared to the two NaCl-only treatment groups. The thaw loss of the four groups varied between a minimum of 1.11 ± 0.11% for the NaKGlu group and a maximum of 1.21 ± 0.10% for the NaKYE group. These levels were regarded as acceptable as there were no signs of any exudate between the samples and the packaging material nor any pooling of fluid between the batters and the casings.

Both the BL (8.32 ± 0.51%) and PC (7.76 ± 0.71%) groups had higher ($P < 0.001$) cooking losses compared to that of the other four groups. The NaKGlu group (6.73 ± 0.38%) had higher ($P < 0.001$) cooking losses compared to both the NaKYE (5.96 ± 0.84%) and NaKKlac (5.27 ± 0.49%) groups. These results were mostly reflected by the total losses of all six treatment groups, although significance levels changed. The PC group had lower ($P < 0.001$) total losses than the BL group, similar to that of the NaKGlu group. The total losses of the NaK, NaKGlu, and NaKYE groups did not differ significantly from each other, but were higher ($P < 0.001$) than that of the NaKKlac group. The increases in losses incurred during cooking were mostly attributed to the protein-denaturing and subsequent evaporative effect of the high cooking temperature (Viera, Diaz, Martinéz, & García-Cachán, 2009; Leygonie, Britz, & Hoffman, 2012).

In contrast to the results of Chapter 4 on pp. 118−119, the BL and PC groups had the highest percentages of cooking and total losses. These results were difficult to explain with reference to the positive effects of added NaCl on the WHC and lower eventual losses (Rhee et al., 1987; Monahan & Troy, 1997). It is possible that greater cooking losses were incurred due to lower levels of gelatinization of the starch from the added rusk and tapioca leading to smaller amounts of water and melted fat being captured inside the sausage models. Factors such as moisture content (free and bound), solute content (ionic and anionic), the presence of other biomaterials, and processing and pre-treatment conditions may affect the temperature at which gelatinization occurs (Ghiasi, Hoseney, & Varriano-Marston, 1982; Sablani, 2009). Of interest is that the losses incurred by the four replacer formulations, were quite low and comparable to that of the 1.66% added NaCl treatment in Chapter 4.

The lower cooking losses of the replacer groups may be explained by the effect on gelatinization as previously stated. Various compounds are capable of decreasing the gelatinization temperature of

starches thereby increasing the amount of moisture and fat captured by the system (Sablani, 2009). It may, therefore, have been possible that lower added NaCl levels in combination with different Na replacers may have exerted overall positive effects on cooking losses, thereby also leading to lower total losses. The NaKKlac treatment had lower ($P < 0.001$) cooking and total losses than the NaKGlu treatment indicating a better overall WHC capacity and possibly gelatinization of starch. This may also be attributed to the ability of K-lactate to act as a humectant (Igoe & Hui, 1996) thereby increasing the WHC, not in the traditional sense as with the effects of NaCl and phosphates on the meat proteins (Offer et al., 1989; Van Laack, 1999), but of the sausage batter itself.

Table 5.4. Thaw, cooking, total, and refrigeration losses of six banger formulations based on different added NaCl and/or replacer levels.

Treatment	% Thaw loss	% Cooking loss	% Total loss	% Refrigeration loss
	$n = 12$	$n = 12$	$n = 12$	$n = 12$
BL	$0.76^{b} \pm 0.10$	$8.32^{d} \pm 0.51$	$9.02^{d} \pm 0.50$	$1.92^{b} \pm 0.14$
PC	$0.61^{a} \pm 0.07$	$7.76^{d} \pm 0.71$	$8.32^{c} \pm 0.74$	$1.79^{ab} \pm 0.26$
NaK	$1.17^{c} \pm 0.05$	$6.09^{bc} \pm 0.31$	$7.19^{b} \pm 0.33$	$1.72^{a} \pm 0.07$
NaKGlu	$1.11^{c} \pm 0.11$	$6.73^{c} \pm 0.38$	$7.76^{bc} \pm 0.35$	$1.88^{ab} \pm 0.09$
NaKYE	$1.21^{c} \pm 0.10$	$5.96^{b} \pm 0.84$	$7.10^{b} \pm 0.82$	$1.75^{a} \pm 0.09$
NaKKlac	$1.15^{c} \pm 0.10$	$5.27^{a} \pm 0.49$	$6.35^{a} \pm 0.55$	$1.72^{a} \pm 0.10$
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts in the same column differ significantly.

Significant differences in refrigeration loss were observed between the six treatment groups (Table 5.4). The BL group had higher ($P < 0.01$) refrigeration losses compared to the NaK, NaKYE and NaKKlac treatment groups. Refrigeration losses were also the lowest ($P < 0.001$) in the NaK, NaKYE, and NaKKlac groups. In general, refrigeration losses were higher than thaw losses. This may be explained by multiple theories. In whole muscle, the crystallisation of water to ice disrupts the muscle structure, freeing a greater amount of fluid which adds to the growth of the ice crystals causing even greater muscle structure disruption. During thawing, all this moisture returns to a fluid state and due to the damaged muscle structure, is not completely reabsorbed with possibly a substantial amount of fluid purged from the meat as thaw losses (James & James, 2010). In a comminuted product such as bangers, the muscle structure has already been disrupted, although the positive effect of especially NaCl and phosphates improves the WHC of the meat proteins (Offer et al., 1986; Sofos, 1986). The sausage batters were also stuffed into hog casings, further limiting the expulsion of moisture from the bangers. Greater losses found after refrigeration may also have the result of moisture having more time to pass through the casings into the head space around the

sample and into the absorbent pad inside the trays or even through evaporation through the PVC films used to overwrap the samples.

5.3.4. Chemical analyses

5.3.4.1. Ash, NaCl, Na and K content

The mineral content of the six treatment groups were determined as the percentage ash present after preparing the samples for AAS (Table 5.5). Significant ($P < 0.001$) differences were found between the six groups depending on the residual free mineral content of the specific formulation. The BL group had the lowest ash content due to its 1.00% added NaCl content without any incorporation of replacers. The PC, NaK, NaKYE, and NaKKlac treatments all had similar ash contents. Some K may be lost during ashing (Qi, Li, Han, Zuo, Gao, Wang, et al., 2016), although the retained total ash of NaK was similar to that of PC where only NaCl was added to the batter. It was concluded that lower percentages of ash in other treatment groups would then had to have been the result of lower proportions of K to other lower ash-forming compounds in the added replacers. This was exemplified by the NaKYE group which only had a slightly lower ash content than PC and NaK, due in part to the high ash content of YE (Halász & Lásztity, 1991) which has been reported to be as high as 11.26% of the original YE (Suwanapong, Khongsay, Laopaiboon, Jaisil, & Laopaiboon, 2013). A better example of this was the lower ($P < 0.001$) percentage ash found for the NaKGlu treatment group. This was the result of the small percentage of K in the overall molar mass of K-gluconate. The chemical formula for K-gluconate is $C_6H_{11}KO_7$ and with a molar mass for K of 39.10 g/mol (Wieser, Holden, Coplen, Böhlke, Bergland, Brand, et al., 2013), it only constitutes 16.69% of the molar mass of K-gluconate. This is considerably less than the 52.45% that the K contributes to the molar mass of KCl.

Significant ($P < 0.001$) differences in NaCl content were found depending on the added NaCl content (0.80%, 1.00% or 2.00%). The PC group had a higher ($P < 0.001$) NaCl content than the NaK group, which had a higher ($P < 0.001$) NaCl content than both the NaKYE and NaKKlac groups, which in turn had higher ($P < 0.001$) NaCl contents than both the BL and NaKGlu groups.

Naturally, the Na content values converted from the NaCl content determined by the Volhard titration method mirrored these significance levels to the same extent. A comparison of this set of values with the values of the Na content revealed major discrepancies. The first was that the converted values of the BL and PC groups both underestimated the actual Na content of these groups: 450.14 ± 12.35 mg Na/100 g versus 582.81 ± 34.60 mg Na/100 g for the BL group;

Table 5.5. Salt and Na content of six banger formulations with different added NaCl and/or replacer levels.

Treatment	BL	PC	NaK	NaKGlu	NaKYE	NaKKlac	Sign. level
% Ash *n* = 12	$2.24^{a} \pm 0.24$	$3.59^{c} \pm 0.22$	$3.52^{c} \pm 0.28$	$2.58^{b} \pm 0.20$	$3.39^{c} \pm 0.17$	$3.50^{c} \pm 0.32$	$P < 0.001$
mg NaCl/100 g *n* = 12	$1144.51^{a} \pm 31.39$	$2036.38^{d} \pm 55.50$	$1877.89^{c} \pm 29.04$	$1134.08^{a} \pm 32.32$	$1726.15^{b} \pm 66.26$	$1767.37^{b} \pm 113.34$	$P < 0.001$
mg Na/100 g (converted) *n* = 12	$450.14^{a} \pm 12.35$	$800.91^{d} \pm 21.83$	$738.57^{c} \pm 11.42$	$446.03^{a} \pm 12.71$	$678.90^{b} \pm 26.06$	$695.11^{b} \pm 44.58$	$P < 0.001$
mg Na/100 g (actual) *n* = 12	$582.81^{a} \pm 34.60$	$906.15^{b} \pm 37.71$	$585.49^{a} \pm 51.22$	$597.95^{a} \pm 64.76$	$595.68^{a} \pm 33.76$	$605.02^{a} \pm 42.35$	$P < 0.001$
mg Na/100 g (formulated) *n* = 12	590.41	983.15	589.78	589.78	600.73	589.68	n.a.
mg K/100 g *n* = 12	$229.54^{a} \pm 11.17$	$250.35^{a} \pm 10.47$	$567.58^{cd} \pm 55.38$	$393.16^{b} \pm 39.55$	$537.37^{c} \pm 26.81$	$601.70^{d} \pm 42.64$	$P < 0.001$
Regulatory Na limit (2019)				600 mg/100 g			

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

Means with different superscripts in the same row differ significantly

n.a. = not applicable

and 800.91 ± 21.83 mg Na/100 g versus 906.15 ± 37.71 mg Na/100 g for the PC group. This indicated that there was more Na present in both treatment groups, not originally associated with chloride, which could not be explained by the Volhard method results, but only through the direct Na measurements with AAS. The second round of discrepancies involved the NaK, NaKYE, and NaKKlac groups where the converted NaCl values overestimated the actual Na content. This was explained by the fact that these groups contained additional chloride associated with K in the form of KCl: 1.00% KCl in NaK, 0.80% KCl in NaKYE, and 0.80% KCl in NaKKlac. The additional chloride reacted with the other reagents during the Volhard method titrations and incorrectly presented a higher than actual NaCl content. The latter discrepancies were expected beforehand and served as motivation for comparing the two analytical techniques. As can be seen from a comparison of the results, the use of the Volhard method to indirectly determine the Na content of current and future reduced-Na products cannot be supported and this method have to be replaced with a more direct method of determining Na content, such as AAS.

Overall, the formulated Na content compared favourably with the actual Na content, illustrating a relative ease in estimating the Na contributions made by various components in the formulations. Only the formulated Na content of ~ 983.15 mg Na/100 g of the PC group overestimated the actual Na content of 906.15 ± 37.71 mg Na/100 g. All five reduced Na treatments had actual Na levels very close to the final regulatory limit of 600 mg Na/100 g. With the inclusion of K in various treatments, significant differences in K content were observed. The BL and PC groups had the lowest K content followed by a higher ($P < 0.001$) K content in the NaKGlu group. Higher ($P < 0.001$) K content was found for the NaK, NaKYE and NaKKlac groups with NaKKlac having the highest overall K content, due to the use of two sources of K, namely KCl and K-lactate. Increasing the K content of meat products has the added benefit of contributing to higher K content in the diet. In South Africa, only 8% of the population have been demonstrated to meet or exceed the recommended K intake of 3330 mg/day (Charlton et al., 2005) and at a level of 500-600 mg K/day, this was comparable to a 100 g of tree nuts, spinach or cabbage (WHO, 2012). Increasing K consumption may help to create a K-rich diet which may further reduce blood pressure (Abernethy, 1979; Geleijnse et al., 2003).

5.3.4.2. pH, a_w, and moisture content

No significant differences were found between any of the six treatment groups after any of the four time intervals in terms of pH (Table 5.6). The pH values of the six treatment groups were grouped around an average pH of 5.85 after day 0. Similar to what was reported in Chapter 4, this average pH was higher than that of meat at 5.50-5.60 (Jay et al., 2005; Arias, 2012). This was attributed to

the inclusion of sodium tripolyphosphate at a level of 0.30% (equal to 7.5 g per 3.50 kg batch of sausage batter). Storage time significantly affected the pH of only the NaKYE and NaKKlac groups with fluctuations in pH observed for both of these two groups over the 9 day shelf-life (Table 5.6). The pH of the NaKYE group was higher ($P < 0.05$) on day 6 than on day 3. The NaKKlac group had a longer sustained fluctuation with a higher ($P < 0.005$) pH on day 6 than on day 3 and then a lower ($P < 0.005$) pH again on day 9.

Table 5.6. Changes in the pH, a_w and moisture content of six banger formulations with different added NaCl and/or replacer levels at 4 °C over a 9 day shelf-life.

Day	Treatment	pH *n* = 12	Sign. level	a_w *n* = 12	Sign. level	Moisture content (%) *n* = 12	Sign. level
0	**BL**	5.79 ± 0.20	NS	0.9387^{b} ± 0.0063	$P < 0.001$	59.05 ± 0.81	NS
	PC	5.81 ± 0.15		0.9284^{a} ± 0.0067		58.65 ± 0.75	
	NaK	5.78 ± 0.20		0.9383^{b} ± 0.0057		59.25 ± 0.54	
	NaKGlu	5.90 ± 0.06		0.9408^{b} ± 0.0056		58.93 ± 0.64	
	NaKYE	5.88 ± 0.10		0.9390^{b} ± 0.0047		59.22 ± 1.16	
	NaKKlac	5.95 ± 0.08		0.9360^{b} ± 0.0058		59.35 ± 1.05	
3	**BL**	5.82 ± 0.26	NS	0.9382^{ab} ± 0.0057	$P < 0.05$	58.92 ± 0.73	NS
	PC	5.81 ± 0.22		0.9328^{a} ± 0.0039		58.69 ± 0.75	
	NaK	5.84 ± 0.22		0.9386^{ab} ± 0.0040		59.04 ± 0.71	
	NaKGlu	5.83 ± 0.22		0.9374^{ab} ± 0.0096		58.62 ± 1.16	
	NaKYE	5.85 ± 0.19		0.9370^{ab} ± 0.0061		58.23 ± 1.16	
	NaKKlac	5.87 ± 0.20		0.9403^{b} ± 0.0037		58.81 ± 1.15	
6	**BL**	5.92 ± 0.23	NS	0.9416 ± 0.0055	NS	58.92 ± 0.86	NS
	PC	5.89 ± 0.25		0.9370 ± 0.0053		58.15 ± 0.79	
	NaK	5.94 ± 0.21		0.9401 ± 0.0033		58.50 ± 0.54	
	NaKGlu	5.96 ± 0.18		0.9412 ± 0.0059		58.33 ± 0.73	
	NaKYE	6.02 ± 0.13		0.9418 ± 0.0059		58.73 ± 1.30	
	NaKKlac	6.09 ± 0.14		0.9411 ± 0.0070		58.59 ± 1.16	
9	**BL**	5.82 ± 0.19	NS	0.9386^{b} ± 0.0057	$P < 0.001$	59.23 ± 0.92	NS
	PC	5.80 ± 0.18		0.9303^{a} ± 0.0050		58.25 ± 1.24	
	NaK	5.86 ± 0.16		0.9386^{b} ± 0.0044		58.64 ± 0.98	
	NaKGlu	5.84 ± 0.16		0.9399^{b} ± 0.0041		58.44 ± 0.92	
	NaKYE	5.88 ± 0.13		0.9415^{b} ± 0.0071		58.26 ± 1.28	
	NaKKlac	5.90 ± 0.15		0.9456^{b} ± 0.0109		58.90 ± 1.31	

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

Means with different superscripts in the same column and on the same day differ significantly

NS = Not significant

Generally, the BL and PC treatments resisted changes in pH better than the four replacer formulations. This was attributed to the limited NaCl content of, and the addition of replacers to the four replacer groups which may have had a slightly negative effect on the added sodium

tripolyphosphate to buffer against fluctuations in pH of especially the NaKGlu and NaKKlac groups. Normally, greater amounts of added NaCl enhance the functionality of phosphates (Ruusunen et al., 2002; Ruusunen & Puolanne, 2005). Potassium lactate is also known to be neutral in pH even though it is the salt of lactic acid, a weak organic acid. This property makes both sodium and potassium lactates suitable for low acid foods such as meat and poultry products (Joshi & Thakur, 2000).

The effect of added NaCl and/or replacer level on a_w was observed on day 0 with the water activity of the PC group lower ($P < 0.001$) than that of the NC and four replacer formulations (Table 5.6). Ionic substances such as NaCl, decrease a_w more than other classes of substances (Mossel & Thomas, 1988). This is of great importance in hampering the growth of spoilage and pathogenic microorganisms (Doyle & Glass, 2010). Of special interest was the fact that the replacement of 1.00% NaCl with 1.00% KCl in the NaK group resulted in a a_w of 0.9383 ± 0.0057. This was similar to the a_w of the 1.00% added NaCl of the BL group and higher ($P < 0.001$) than the a_w of the PC group at 0.9284 ± 0.0067. Potassium chloride has a lower ionic strength and a slightly higher a_w than NaCl. It has been suggested that, to maintain the same a_w of a 2.00% NaCl level, a NaCl-KCl mixture should be included at a level of 2.50% (Pearson & Gillett, 1996).

After day 3, the a_w of the PC group was lower ($P < 0.05$) than that of the NaKKlac group. After day 6 there were no significant differences in a_w of any of the six treatment groups. The a_w of all six groups appeared to have increased to values higher than on day 3 and day 6 for all six treatment groups. At the end of the 9 day shelf-life, the a_w of all six treatment groups had changed again with that of the PC group again lower ($P < 0.001$) than that of the other five treatments. The a_w of the BL, PC, NaK, NaKGlu and NaKYE groups decreased from day 6 to day 9 while that of the NaKKlac treatment group increased to its highest level yet (0.9456 ± 0.0109). Similar increases in a_w reported for the bangers in Chapter 4 on p. 122 were ascribed to a syneresis effect taking place in the starch of the rusk and possibly that of the tapioca. Due to the long storage without heating and subsequent gelatinization, it was theorised that free water was eventually expressed leading to increased a_w. The NaKKlac group had the highest increase in a_w after day 3 and an even higher increase after day 9. It appeared as if this particular replacer combination contributed the most to an overall increase in a_w. This may also be attributed to the hygroscopic nature of K-lactate making it a humectant suitable for use in food products (Igoe & Hui, 1996). Differences in storage time significantly affected the a_w of the PC and NaKKlac groups (Table 5.7). The a_w of the PC group was higher ($P < 0.005$) on day 6 than on day 0 and then lower ($P < 0.05$) again on day 9. For the NaKKlac group this effect was somewhat limited with the a_w only higher ($P < 0.05$) on day 9 compared to on day 0.

Table 5.7. The storage time effect over a 9 day shelf-life at 4°C on the basic chemical parameters of six banger formulations with different added NaCl and/or replacer levels.

Treat-ment	Day	pH	Sign. Level	a_w	Sign. level	Moisture content (%)	Sign. level
		n = 12		*n* = 12		*n* = 12	
BL	**0**	5.79 ± 0.20	NS	0.9387 ± 0.0063	NS	59.05 ± 0.81	NS
	3	5.82 ± 0.26		0.9382 ± 0.0057		58.92 ± 0.73	
	6	5.92 ± 0.23		0.9416 ± 0.0055		58.92 ± 0.86	
	9	5.82 ± 0.19		0.9386 ± 0.0057		59.23 ± 0.92	
PC	**0**	5.81 ± 0.15	NS	0.9284^{a} ± 0.0067	*P* < 0.005	58.65 ± 0.80	NS
	3	5.81 ± 0.22		0.9328^{ab} ± 0.0039		58.69 ± 0.75	
	6	5.89 ± 0.25		0.9370^{b} ± 0.0053		58.15 ± 0.79	
	9	5.80 ± 0.18		0.9303^{a} ± 0.0050		58.25 ± 1.24	
NaK	**0**	5.78 ± 0.20	NS	0.9383 ± 0.0057	NS	59.25 ± 0.54	NS
	3	5.84 ± 0.22		0.9386 ± 0.0040		59.04 ± 0.71	
	6	5.94 ± 0.21		0.9401 ± 0.0033		58.50 ± 0.54	
	9	5.86 ± 0.16		0.9386 ± 0.0044		58.64 ± 0.98	
NaKGlu	**0**	5.90 ± 0.06	NS	0.9408 ± 0.0056	NS	58.93 ± 0.64	NS
	3	5.83 ± 0.22		0.9374 ± 0.0096		58.62 ± 1.16	
	6	5.96 ± 0.18		0.9412 ± 0.0059		58.33 ± 0.73	
	9	5.84 ± 0.16		0.9399 ± 0.0041		58.44 ± 0.92	
NaKYE	**0**	5.88^{ab} ± 0.10	*P* < 0.05	0.9390 ± 0.0047	NS	59.22 ± 1.16	NS
	3	5.85^{a} ± 0.19		0.9370 ± 0.0061		58.23 ± 1.16	
	6	6.02^{b} ± 0.13		0.9418 ± 0.0059		58.73 ± 1.30	
	9	5.88^{ab} ± 0.13		0.9415 ± 0.0071		58.26 ± 1.28	
NaKKlac	**0**	5.95^{ab} ± 0.08	*P* < 0.005	0.9360^{a} ± 0.0058	*P* < 0.05	59.35 ± 1.05	NS
	3	5.87^{a} ± 0.20		0.9403^{ab} ± 0.0037		58.81 ± 1.15	
	6	6.09^{b} ± 0.14		0.9411^{ab} ± 0.0070		58.59 ± 1.16	
	9	5.90^{a} ± 0.15		0.9456^{b} ± 0.0109		58.90 ± 1.31	

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

Means with different superscripts in the same column and on the same day differ significantly

NS = Not significant

No significant differences in moisture content were found between any of the six treatment groups after any of the four time intervals (Table 5.6). This was expected as the total amount of ice water added to all six treatments remained consistent at 15.00% (Table 5.2). The total amount of lean pork varied only between the BL and NaKYE treatments by 1.60% due to their specific formulations, although this had no significant effect on moisture content after day 0 or any other sampling day. Similarly, storage time had no significant effect on the moisture content of any of the six treatment groups (Table 5.7).

5.3.4.3. Lipid oxidative stability

The lipid oxidative stability of all six treatment groups was very acceptable throughout the entire 9 day shelf-life of the fresh sausages (Figure 5.1). All six groups had SLOP levels far below the 1.00 mEq MDA/kg (Gray & Pearson, 1987) and even the 0.50 mEq MDA/kg (Wood et al., 2008) detection thresholds for rancidity. Salt added at levels of 2.00% and 1.00% as the only variable in

either the PC or BL group, only showed a slight pro-oxidative effect by day 9. This is in keeping with its reported pro-oxidative synergy with heme and non-heme iron (Rhee & Ziprin, 2001; Kiliç et al., 2014) and its own pro-oxidative capability at inclusion levels between 0.50-5% (Rhee & Ziprin, 2001). After day 6, the TBARS of all six treatment groups appeared to have decreased momentarily before increasing again to the levels found after day 9. As discussed in Chapter 4 on pp. 89-90, this might have been indicative of a possible link with protein oxidation where MDA may reportedly act as a substrate in protein oxidation (Xiong, 2000).

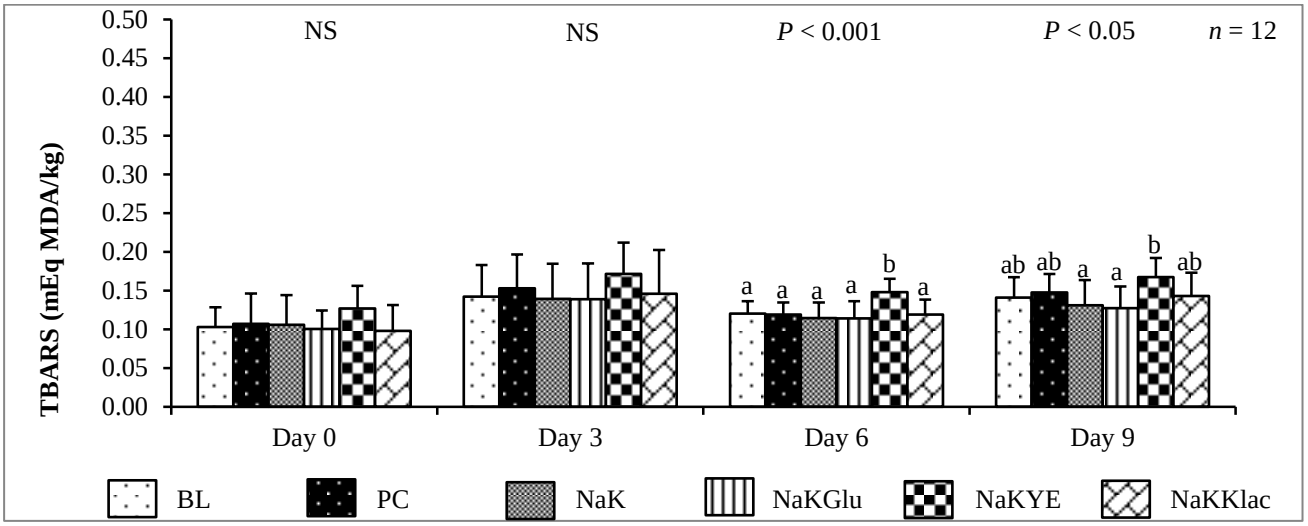

Figure 5.1. The effect of added NaCl and/or replacers on the TBARS of bangers stored at 4 °C for up to 9 days. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means.

Significant differences were only observed after day 6 where the NaKYE group had higher (P < 0.001) TBARS compared to all the other groups including the PC group. After day 9, increases in TBARS were found for all six treatment groups with that of the NaKYE group significantly (P < 0.05) higher than that of the NaK and NaKGlu treatment groups. In all probability, the higher TBARS of this group was brought about by the inclusion of 1.00% YE in the formulation. Even at the sampling times where there were no significant differences, the TBARS of the NaKYE group was higher than that of the rest. The higher TBARS of this group on day 0 indicate that certain components in the extract may have contributed directly to the higher overall TBARS. Generally, dried yeast biomass is high in protein, nucleic acid, ash and vitamin content, has moderate carbohydrate content and low lipid content (Halász & Lásztity, 1991). Dried yeast contain on average only 4-10% lipids of which 20-50% is triglycerides, 1-15% is mono- and diglycerides and 1-20% free fatty acids. Oxidation during processing of the yeast, of the mono- and diglycerides and especially the free fatty acids, may have contributed significantly to the TBARS content of the

extract as added to the NaKYE formulation, explaining the marginally higher TBARS result of this group on day 0.

In terms of vitamin content, yeast is known to be a major source of the B-vitamin group (Halász & Lásztity, 1991) and thiamine (B_1), riboflavin (B_2), nicotinic acid (B_3) and folic acid (B_9) have been shown to have pro-oxidative effects on linoleic acid peroxidation at concentrations of 2.5 μM to 2.5 mM (Higashi-Okai, Nagino, Yamada, & Okai, 2006). Pork backfat as added to the bangers at a level of 22.50% (Table 5.2), contains linoleic acid in the region of 14.9-10.6% of total lipids at a backfat thickness of 8-16 mm (Wood & Enser, 1997) although this percentage can vary depending on other factors such as feeding regime, breed and sex. It is possible that the pro-oxidative effect of these vitamins may have contributed to the slight increase in TBARS over time. Free amino acids in the yeast extract may also have played a role in the higher level of lipid oxidation of NaKYE. Regardless of how yeast extracts are prepared, they are characterized by a high content of free amino acids (Munch, Hoffman, & Schieberle, 1997). The aromatic amino acids: phenylalanine, tyrosine, and tryptophan are susceptible to oxidation (Genot, Berton, & Roper, 2013) and cysteine has been shown to be converted to a thiol radical by other free radicals in a powdered, encapsulated lipid system (Park, Murakami, & Matsumura, 2005).

Storage time significantly affected the TBARS of most of the treatment groups over a 9 day shelf-life at 4 °C, with the exception of the NaK group (Figure 5.2). The BL, PC, NaKYE and NaKKlac groups shared a similar pattern of SLOP formation where the TBARS were significantly ($P < 0.005$ and $P < 0.01$ only for NaKKlac) higher on day 3 and day 9 than on day 0. For the NaKGlu group, TBARS were only significantly ($P < 0.05$) higher on day 3 than on day 0. These results indicated that the low added NaCl group and the four replacer groups tended to follow a similar pattern of SLOP formation to that of the PC group.

The SLOP levels of the six treatment groups remained very low and stable throughout storage at -18 °C (Figure 5.3), similar to the SLOP levels at 4 °C for up to 9 days. Similar to the TBARS results of the fresh product shelf-life, the TBARS of all six treatment groups under frozen storage at -18 °C remained far below the detection thresholds of 0.50 mEq MDA/kg (Wood et al., 2008) and 1.00 mEq MDA/kg (Gray & Pearson, 1987) for rancidity detection. The only significant effect was found at the end of the 180 day shelf-life where the TBARS value of the NaKYE group was significantly ($P < 0.05$) higher than that of both the NaK and NaKGlu groups. The highest TBARS values for NaKYE of 0.16 ± 0.02 mEq MDA/kg on day 90 and 0.16 ± 0.01 mEq MDA/kg on day 180, were close to the highest TBARS levels of the same treatment group at 0.17 ± 0.04 mEq MDA/kg on day 3 and 0.17 ± 0.02 mEq MDA/kg on day 9. The inclusion of YE at 1.00% led to the

highest TBARS values, although over much longer time intervals (90 and 180 days) and under frozen storage conditions, there appeared to have been no meaningful increases in TBARS beyond that of the normal added NaCl level of 2% (w/w). This was confirmed by the results of the effect of storage time over a 180 days at -18 °C on the TBARS of the six banger formulations (Figure 5.4). The NaKYE group had a similar rate of SLOP formation to that of the PC and NaKGlu groups with significantly ($P < 0.01$ for NaKYE) higher TBARS only found after day 180. It was concluded that the pro-oxidative effect of the YE appeared to have been dependant on the availability of free, unfrozen water for the effect to have been exerted. In contrast, in the BL, NaK and NaKKlac groups, TBARS were already significantly ($P < 0.005$ for all three) higher after day 90.

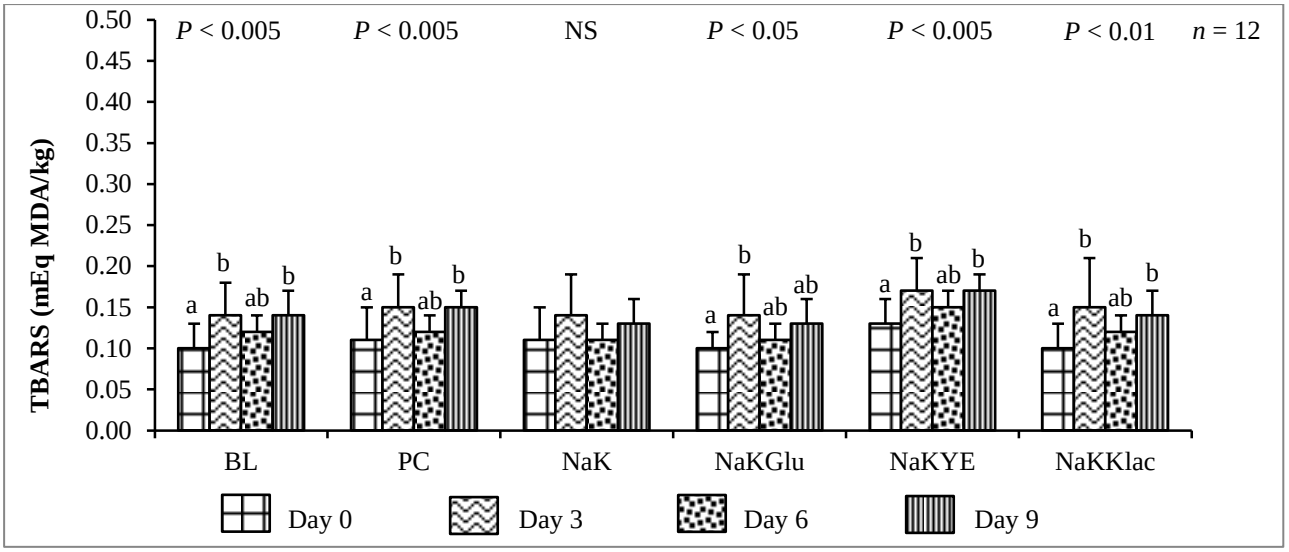

Figure 5.2. The storage time effect over a 9 day shelf-life at 4 °C on the TBARS of six banger formulations containing different added NaCl and/or replacer levels. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts for the same treatment differ significantly. Error bars represent standard deviations of means.

For both sets of TBARS results, it appeared as if the anti-oxidant activity of the added phosphates, sodium ascorbate, sodium nitrite and spices (Honikel, 2008; Tangkanakul et al., 2009; Li et al., 2010; Cheng et al., 2011; Kiliç et al., 2014) may have limited the pro-oxidant effect of NaCl at the 1.00% (BL, NaK, NaKGlu & NaKlac) and 2.00% (PC) inclusion levels. Potassium chloride (Rhee et al., 1983) and K-lactate (Tan & Shelef, 2002; Seyfert et al., 2006) also do not exhibit the pro-oxidative effect of NaCl. During the 9 day fresh product shelf-life and the 180 day frozen product shelf-life, there was a trend for the NaKGlu treatment to have very low TBARS values, although this was only significant after day 180. Gluconic acid of which K-gluconate is the potassium salt, has been reported to be an anti-oxidant found in honey (Gheldof, Wang, & Engeseth, 2002). It was theorized that in the aqueous environment of the sausage batter, the K-gluconate might have

dissociated to form free gluconic acid that might have exhibited an anti-oxidant effect, although, no anti-oxidant activity for K-gluconate has been reported in meat products to date.

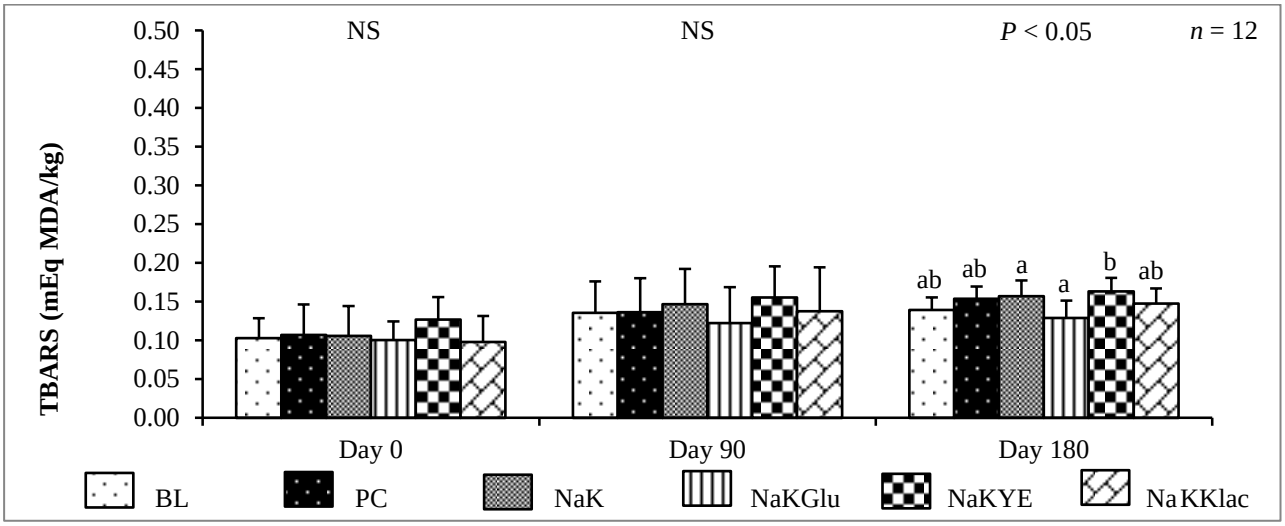

Figure 5.3. The effect of added NaCl and/or replacers on the TBARS of bangers stored at -18 °C for up to 180 days. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means.

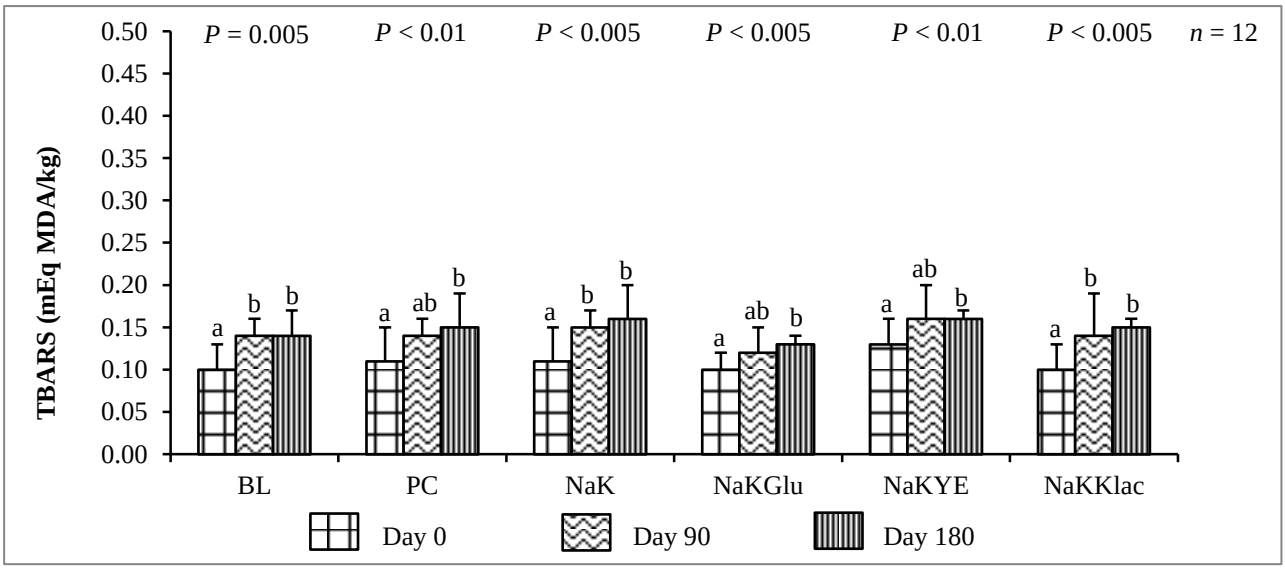

Figure 5.4. The storage time effect over a 180 day shelf-life at -18 °C on the TBARS of six banger formulations containing different added NaCl and/or replacer levels. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts for the same treatment differ significantly. Error bars represent standard deviations of means.

5.3.5. Microbial analyses

There was an immediate effect on TVC on day 0 by banger formulation type (Table 5.8). The TVC of the NaKYE group at log 4.81 ± 0.59 cfu/g was significantly ($P < 0.001$) higher than that of the

five other treatment groups. This result was theorised to have been effected in three possible ways. The first involves the microbial load of the YE itself. It is possible that due to a combination of factors, the YE may have contained viable microorganisms. Spores and vegetative cells may remain viable for months or years in dried foods (Finn, Condell, McClure, Amézquita, & Fanning, 2013). The second theory involves the role YE plays in providing microorganisms with growth factors that may increase their numbers. Yeast extract is commonly used in various culture media as a source of carbon and nitrogen for microorganisms with few nutritional requirements, but more often than not it is specifically added to media for the growth of fastidious microorganisms that require specific vitamins, peptides and amino acids (da Silva, Taniwaki, Junqueira, Silveira, do Nascimento, & Gomes, 2012). Other components consistent in all the formulations included sodium ascorbate, sodium tripolyphosphate, sodium metabisulfite, and various spices that may have collectively exhibited antimicrobial activity that was then somewhat limited by the incorporation of YE. The third theory involves the changes made to NaCl and KCl inclusion levels to accommodate the extra Na contributed by the YE without exceeding the 600 mg Na/100 g limit. Reducing both inclusion levels from 1.00% to only 0.80% resulted in a combined NaCl equivalent of 1.60% added NaCl. If this alone improved the growth of microorganisms, then the BL group with only 50.00% of the original 2.00% added NaCl level would also have had much higher overall TVC. This was however, only marginally higher than that of the PC group. The addition of YE was again attributed to improving the growth of the microorganisms in conjunction with the NaCl at only 50.00% of the original level.

After day 3, the TVC of the NaKYE treatment decreased by ~ 1 log unit possibly due to the antimicrobial action of various formulation components overcoming the initial contribution to growth by the YE addition. After day 6, the TVC of the PC and NaK groups were significantly ($P < 0.001$) lower than that of the NaKGlu, NaKYE and NaKKlac groups. The TVC of the NaKYE group at 5.40 ± 1.14 log cfu/g was again the highest of all six treatment groups possibly due to a shift in population favouring microorganisms better suited to overcome any antimicrobial activity and better suited to make use of the added YE. It appeared as if the partial replacement of NaCl with 50.00% K-gluconate could not maintain a lower TVC as when only NaCl and NaCl with KCl were used.

After 9 days, the TVC of the NaKYE and NaKKlac groups were significantly ($P < 0.001$) higher than all four other treatment groups. Potassium lactate at a high concentration of 3% (w/w) has been shown to markedly reduce aerobic plate, *E. coli* and coliform counts in minced beef in the absence of added NaCl (Pohlman, Dias-Morse, Quilo, Brown Jr., Crandall, Baublits, et al., 2007). In fresh ground pork, 2% (w/w) K-lactate in combination with either 1% or 2% (w/w) NaCl more

Table 5.8. Changes in the microbial parameters of six banger formulations with different added NaCl and/or replacer levels at 4 °C over a 9 day shelf-life.

Day	Treatment	TVC (log cfu/g) *n* = 12	Sign. level	Coliforms (log cfu/g) *n* = 12	Sign. level	*E. coli* (log cfu/g) *n* = 12	Sign. level	*S. aureus* (log cfu/g) *n* = 12	Sign. level	Yeasts *n* = 12	Sign. level	Moulds *n* = 12	Sign. level
0	**BL**	$3.85^{a} \pm 0.80$	$P < 0.001$	2.25 ± 0.62	NS	0.64 ± 1.16	NS	ND	NSA	3.59 ± 0.71	NS	0.67 ± 0.85	NS
	PC	$3.56^{a} \pm 0.83$		2.10 ± 0.88		0.66 ± 0.97		ND		3.48 ± 0.59		0.83 ± 0.79	
	NaK	$3.58^{a} \pm 0.44$		1.90 ± 0.97		0.31 ± 0.73		ND		3.57 ± 0.72		0.58 ± 0.86	
	NaKGlu	$3.64^{a} \pm 0.45$		1.86 ± 0.77		0.60 ± 0.90		ND		3.66 ± 0.79		0.87 ± 0.81	
	NaKYE	$4.81^{b} \pm 0.59$		1.59 ± 1.03		0.58 ± 0.86		ND		3.64 ± 0.80		0.71 ± 0.76	
	NaKKlac	$3.77^{a} \pm 0.46$		1.47 ± 1.20		0.35 ± 0.65		ND		3.62 ± 0.87		0.51 ± 0.78	
3	**BL**	3.50 ± 0.59	NS	2.25 ± 0.56	NS	0.28 ± 0.50	NS	ND	NSA	4.28 ± 0.74	NS	0.38 ±0.57	NS
	PC	3.06 ± 0.46		1.76 ± 0.64		0.08 ± 0.29		ND		3.56 ± 0.31		0.59 ± 0.63	
	NaK	3.36 ± 0.34		1.87 ± 0.91		0.42 ± 0.63		ND		4.16 ± 0.89		0.67 ± 1.01	
	NaKGlu	3.60 ± 0.58		1.51 ± 1.17		0.25 ± 0.45		ND		4.24 ± 0.97		0.33 ± 0.65	
	NaKYE	3.81 ± 1.36		1.34 ± 1.05		ND		ND		4.10 ± 0.83		0.47 ± 0.90	
	NaKKlac	3.85 ± 0.54		1.29 ± 0.88		ND		ND		4.13 ± 0.76		0.36 ± 0.54	
6	**BL**	$3.66^{ab} \pm 0.71$	$P < 0.001$	2.04 ± 0.73	NS	ND	NSA	ND	NSA	5.62 ± 0.92	NS	1.88 ± 2.77	NS
	PC	$2.78^{a} \pm 0.26$		1.06 ± 1.04		ND		ND		5.34 ± 0.65		1.67 ± 2.46	
	NaK	$3.08^{a} \pm 0.47$		1.76 ± 0.90		ND		ND		5.54 ± 0.82		1.74 ± 2.58	
	NaKGlu	$4.37^{bc} \pm 0.38$		1.67 ± 0.90		ND		ND		5.61 ± 0.74		1.79 ± 2.65	
	NaKYE	$5.40^{d} \pm 1.14$		1.33 ± 1.03		ND		ND		5.39 ± 0.99		1.80 ± 2.67	
	NaKKlac	$5.08^{cd} \pm 1.31$		1.18 ± 1.03		ND		ND		5.42 ± 0.63		1.70 ± 2.55	
9	**BL**	$3.74^{a} \pm 0.85$	$P < 0.001$	$2.33^{b} \pm 0.57$	$P < 0.05$	ND	NSA	ND	NSA	6.34 ± 0.85	NS	2.22 ± 3.29	NS
	PC	$2.98^{a} \pm 0.43$		$1.18^{a} \pm 1.01$		ND		ND		6.26 ± 1.00		1.98 ± 2.92	
	NaK	$3.81^{a} \pm 0.24$		$1.80^{ab} \pm 0.97$		ND		ND		6.49 ± 0.64		2.04 ± 3.01	
	NaKGlu	$3.87^{a} \pm 0.23$		$1.91^{ab} \pm 0.80$		ND		ND		6.65 ± 0.72		2.18 ± 3.22	
	NaKYE	$5.38^{b} \pm 1.14$		$1.58^{ab} \pm 0.80$		ND		ND		6.57 ± 0.62		2.14 ± 3.16	
	NaKKlac	$5.23^{b} \pm 1.35$		$1.03^{a} \pm 1.00$		ND		ND		6.78 ± 0.73		2.68 ± 3.05	

TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

Means with different superscripts in the same column and on the same day differ significantly

ND = Not detected

NSA = Not statistically analysed

NS = Not significant

effectively delayed spoilage than when used without NaCl (Tan & Shelef, 2002). In this case, the very low concentration of K-lactate in the presence of 1% NaCl, had the opposite effect by promoting microbial growth. The TVC of the NaKGlu group decreased to a level still higher than that of the BL, PC and NaK groups, although no longer significantly so. For the most part, the inclusion of: NaCl and KCl at 0.80% and YE at 1.00%; and the inclusion of NaCl at 1.00%, KCl at 0.80% and K-lactate at 0.20% negatively affected the overall microbial stability of the affected banger formulations.

Storage time significantly affected the TVC of the PC, NaK, NaKGlu, NaKYE and NaKKlac treatment groups (Table 5.9). The TVC of the PC group was lower ($P < 0.01$) on day 6 than on day 0. For the NaK group, a significant ($P < 0.001$) decrease in TVC was also found after day 6, followed by a significant ($P < 0.001$) increase after day 9. The TVC of the NaKGlu group was slightly lower on day 3 than on day 0, higher ($P < 0.001$) on day 6 and then lower ($P < 0.001$) again on day 9. For the NaKYE group, the TVC decreased from day 0 to day 3 and then increased ($P < 0.005$) after day 6 and remained at this level up to day 9. In the NaKKlac group, the TVC remained constant up to day 3 after which it increased ($P < 0.001$) by day 6 and remained at this level up to day 9.

Effectively, only the TVC of the NaKYE and NaKKlac groups showed any real deterioration. The effect of the interaction between added NaCl and/or replacer level and storage time confirmed this with significant effects limited to these two groups (Figure 5.5). On day 0, the NaKYE group already had higher ($P <0.001$) TVC than the PC, NaK and NaKGlu groups. On day 6 both groups had similar higher ($P < 0.001$) counts than the BL, PC and NaK groups. By day 9, the TVC of both groups were higher ($P < 0.001$) than all four of the other groups. For the NaKYE group, this was shown by the higher ($P < 0.001$) TVC (Table 5.8) and for the NaKKlac group this was shown by the significant ($P < 0.001$) increase in TVC from day 0 to day 6 onwards (Table 5.9).

No significant effects on coliform counts were found during the first 6 days of shelf-life (Table 5.8). The coliform counts of the two NaCl-only treatments, BL and PC were slightly higher and there was a trend for the BL group to have higher coliform counts over the first 6 days. Only after day 9, the coliform count of BL was higher ($P < 0.05$) than that of the PC and NaKKlac groups. The 2.00% NaCl content of the PC group and the combination of 1.00% NaCl, 0.80% KCl and 0.20% K-lactate in the NaKKlac group clearly challenged the growth and survival of coliforms. Potassium lactate has been reported to be as effective as sodium lactate in controlling the growth of both aerobes and anaerobes in meat and with greater effect in combination with other antimicrobials (Shelef, 1994). In terms of the effect of storage time on coliform counts, this effect was found to be

Table 5.9. The storage time effect over a 9 day shelf-life at 4 °C on the microbial analyses results of six banger formulations with different added NaCl and/or replacer levels.

Treatment	Day	TVC (log cfu/g) n = 12	Sign. level	Coliforms (log cfu/g) n =12	Sign. level	*E. coli* (log cfu/g) n = 12	Sign. level	Yeasts (log cfu/g) n = 12	Sign. level	Moulds (log cfu/g) n = 12	Sign. level
BL	**0**	3.85 ± 0.80	NS	2.25 ± 0.62	NS	0.64 ± 1.16	NSA	3.48^{a} ± 0.59	$P < 0.001$	0.67 ± 0.85	NS
	3	3.50 ± 0.59		2.25 ± 0.56		0.28 ± 0.50		3.56^{a} ± 0.31		0.38 ± 0.57	
	6	3.66 ± 0.71		2.04 ± 0.73		ND		5.34^{b} ± 0.65		1.88 ± 2.77	
	9	3.74 ± 0.85		2.33 ± 0.57		ND		6.26^{c} ± 1.00		2.22 ± 3.29	
PC	**0**	3.56^{b} ± 0.83	$P < 0.01$	2.10 ± 0.88	NS	0.66 ± 0.97	NSA	3.59^{a} ± 0.71	$P < 0.001$	0.83 ± 0.79	NS
	3	3.06^{ab} ± 0.46		1.76 ± 0.64		0.08 ± 0.29		4.28^{a} ± 0.74		0.59 ± 0.63	
	6	2.78^{a} ± 0.26		1.06 ± 1.04		ND		5.62^{b} ± 0.92		1.67 ± 2.46	
	9	2.98^{ab} ± 0.43		1.18 ± 1.01		ND		6.34^{b} ± 0.85		1.98 ± 2.92	
NaK	**0**	3.58^{bc} ± 0.44	$P < 0.001$	1.90 ± 0.97	NS	0.31 ± 0.73	NSA	3.57^{a} ± 0.72	$P < 0.001$	0.58 ± 0.86	NS
	3	3.36^{ab} ± 0.34		1.87 ± 0.91		0.42 ± 0.63		4.16^{a} ± 0.89		0.67 ± 1.01	
	6	3.08^{a} ± 0.47		1.76 ± 0.90		ND		5.54^{b} ± 0.82		1.74 ± 2.58	
	9	3.81^{c} ± 0.24		1.80 ± 0.97		ND		6.49^{c} ± 0.64		2.04 ± 3.01	
NaKGlu	**0**	3.64^{a} ± 0.45	$P < 0.001$	1.86 ± 0.77	NS	0.60 ± 0.90	NSA	3.66^{a} ± 0.79	$P < 0.001$	0.87 ± 0.81	NS
	3	3.60^{a} ± 0.58		1.51 ± 1.17		0.25 ± 0.45		4.24^{a} ± 0.97		0.33 ± 0.65	
	6	4.37^{b} ± 0.38		1.67 ± 0.97		ND		5.61^{b} ± 0.74		1.79 ± 2.65	
	9	3.87^{a} ± 0.23		1.91 ± 0.80		ND		6.65^{c} ± 0.72		2.18 ± 3.22	
NaKYE	**0**	4.81^{ab} ± 0.59	$P < 0.005$	1.59 ± 1.03	NS	0.58 ± 0.86	NSA	3.64^{a} ± 0.80	$P < 0.01$	0.71 ± 0.76	NS
	3	3.81^{a} ± 1.36		1.34 ± 1.05		ND		4.10^{a} ± 0.83		0.47 ± 0.90	
	6	5.40^{b} ± 1.14		1.33 ± 1.03		ND		5.39^{b}± 0.99		1.80 ± 2.67	
	9	5.38^{b} ± 1.14		1.58 ± 0.80		ND		6.57^{c} ± 0.62		2.14 ± 3.16	
NaKKlac	**0**	3.77^{a} ± 0.46	$P < 0.001$	1.47 ± 1.20	NS	0.35 ± 0.65	NSA	3.62^{a} ± 0.87	$P < 0.001$	0.51^{ab} ± 0.78	$P < 0.05$
	3	3.85^{a} ± 0.54		1.29 ± 0.88		ND		4.13^{a} ± 0.76		0.36^{a} ± 0.54	
	6	5.08^{b} ± 1.31		1.18 ± 0.86		ND		5.42^{b} ± 0.63		1.70^{ab} ± 2.55	
	9	5.23^{b} ± 1.35		1.03 ± 1.00		ND		6.78^{c} ± 0.73		2.68^{b} ± 3.05	

TVC = total viable count; *E. coli* = *Escherichia coli*; BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts in the same column and for the same treatment differ significantly. ND = Not detected. NSA = Not statistically analysed. NS = Not significant.

a bacteriostatic effect with no significant increases or decreases in coliform counts for any of the six treatment groups over the 9 day shelf-life (Table 5.9).

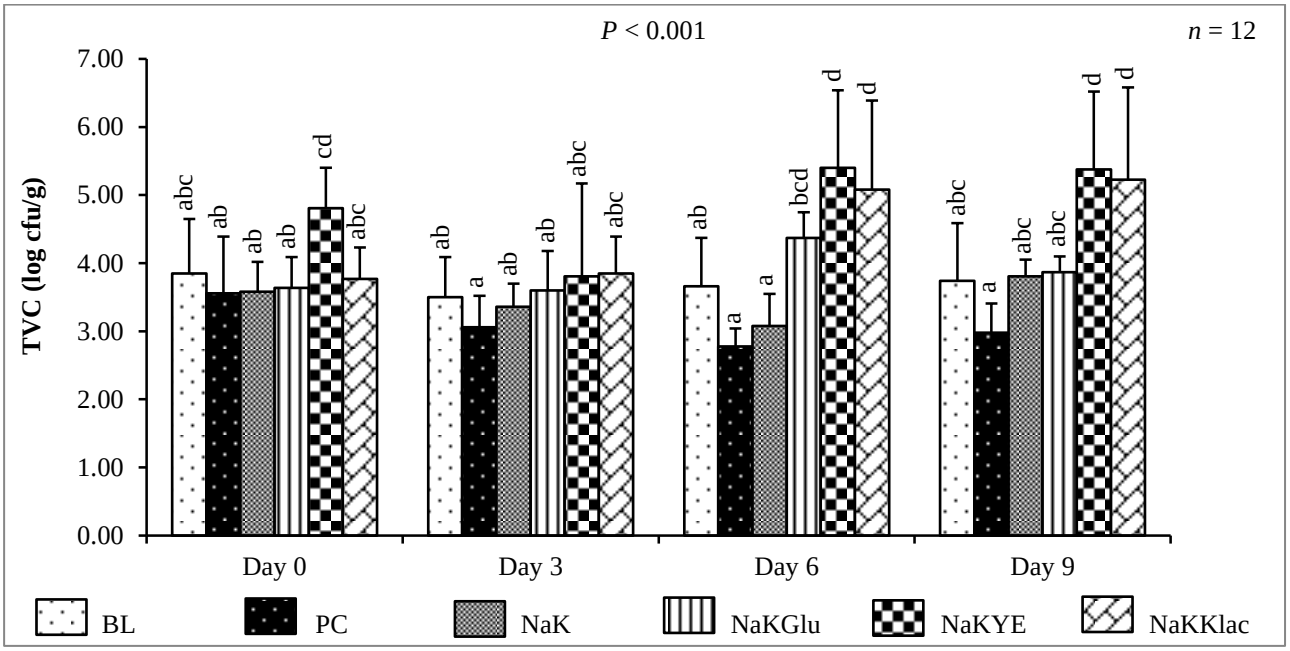

Figure 5.5. The effect of the interaction between added NaCl and/or replacer level and storage time on the TVC of bangers during storage at 4 °C for up to 9 days. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts for the same treatment differ significantly. Error bars represent standard deviations of means.

The growth of *E. coli* was not significantly affected by added NaCl/replacer formulations at any time interval during the 9 day shelf-life (Table 5.8). The counts of the NaK and NaKKlac groups did appear to be marginally lower than that of the other four groups after day 0. After day 3, the counts of the BL, PC, and NaKGlu groups decreased, that of the NaK increased and *E. coli* was no longer detected in the NaKYE and NaKKlac groups. From day 6 to day 9, no *E. coli* was detected in any of the six treatment groups. Due to the very low and inconsistent counts for *E. coli* that became undetectable after day 0 (NaKYE and NaKKlac) or after day 3 (BL, PC, NaK and NaKGlu), no storage time effect could be found (Table 5.9). *Staphylococcus aureus* was not detected in any of the treatment groups after any of the four sampling intervals (Table 5.8).

No treatment effect on yeast counts was found, nor were there any significant differences between treatment groups at any time interval (Table 5.8). The results of all six treatments were closely grouped together between the minimum of 3.48 ± 0.59 log cfu/g of the PC group and the maximum of 3.66 ± 0.79 log cfu/g of the NaKGlu group. If the YE extract contributed viable microorganisms to the TVC counts of the NaKYE treatment, it would have been expected to have been reflected in the yeast counts. The yeast counts increased to over 4 log cfu/g after day 3 except for that of the PC

group. Growth continued to over 5 log cfu/g after day 6 and to over 6 log cfu/g after day 9. Storage time significantly affected the yeast counts of all six treatment groups (Table 5.9). The yeast counts of the BL, NaK, NaKGlu, NaKYE and NaKKlac groups increased ($P < 0.001$ for the BL, NaK, NaKGlu, NaKKlac, and $P < 0.01$ for YE) from day 3 to day 6 and then again from day 6 to day 9. For the PC group, growth was slower and more limited. The yeast counts of this group were only higher ($P < 0.001$) after day 6 and remained constant up to day 9.

Similar to the results of the yeast counts, no significant effect on mould counts were observed (Table 5.8). Over time there was a total increase in mould counts to around 2 log cfu/g after 9 days. Storage time only significantly affected the mould counts of the NaKKlac group (Table 5.9). The low mould counts increased from day 3 to day 6 onwards and on day 9 these counts were higher ($P < 0.05$) than on day 3. As the YE had not contributed to yeast and mould counts, or even coliform counts, it seemed that the YE itself may have contributed to the higher overall growth of microorganisms already present in the batter of the NaKYE formulation. Changes and differences in various microbial parameters may have contributed to the previously discussed fluctuations and differences in pH of the various treatment groups. In addition to the higher ($P < 0.001$) overall TVC, the activities of the moderate mould counts and high yeast counts may have decreased or increased the pH of these groups depending on the dominating microorganism at the time of measurement. Lactic acid bacteria producing lactic acid may decrease the pH to outcompete other microorganisms (Gibson & Robert, 1986) while moulds are capable of metabolising lactic acid which may increase the pH as is the case with mould ripened fermented meat products (Spotti, Berni, & Cacchioli, 2008). Yeasts are also capable of depleting lactic acid levels thereby increasing the pH and possibly enhancing the survival of other microorganisms (Dalton, Board, & Davenport, 1984; Fleet, 1992; Samelis & Sofos, 2003).

5.3.6. Physical analyses: colour

Significant effects by formulation type on all five colour parameters were observed after every time interval (Table 5.10). Lightness (L^*) values generally decreased over the 9 day shelf-life, although they remained quite high due to the high added pork backfat content of 22.50% across all six treatment groups which is known to increase overall lightness in comminuted meat products (Chin et al., 2004). After day 0, the L^* of the NaK group was higher ($P < 0.001$) than that of the other five treatment groups. After day 3, the NaK group was lighter ($P < 0.001$) than both the NaKYE and NaKKlac treatment groups. After day 6, the NaK group was lighter ($P < 0.001$) than the BL, PC and NaKYE groups. At the end of shelf-life, the NaK group was lighter ($P < 0.001$) than all the

Table 5.10. Changes in the colour parameters of six banger formulations with different added NaCl and/or replacer levels at 4 °C over a 9 day shelf-life.

Day	Treatment	*L** n = 12	Sign. level	*a** n = 72	Sign. level	*b** n = 72	Sign. level	*C** n =72	Sign. level	*H** n = 72	Sign. level
0	**BL**	$75.62^{a} \pm 1.78$	$P < 0.001$	$7.50^{ab} \pm 0.82$	$P < 0.001$	$12.90^{a} \pm 0.90$	$P < 0.001$	$14.89^{a} \pm 0.91$	$P < 0.001$	$60.16^{ab} \pm 3.09$	$P < 0.001$
	PC	$75.85^{ab} \pm 1.75$		$7.12^{a} \pm 0.82$		$12.87^{a} \pm 1.01$		$14.82^{a} \pm 0.91$		$61.03^{b} \pm 3.42$	
	NaK	$76.69^{b} \pm 1.85$		$7.59^{bc} \pm 0.77$		$13.11^{ab} \pm 0.60$		$15.18^{ab} \pm 0.65$		$60.09^{ab} \pm 3.09$	
	NaKGlu	$75.69^{a} \pm 1.85$		$7.97^{c} \pm 0.77$		$13.31^{b} \pm 0.60$		$15.54^{b} \pm 0.55$		$59.11^{a} \pm 2.96$	
	NaKYE	$75.08^{a} \pm 1.69$		$7.71^{bc} \pm 0.90$		$14.79^{d} \pm 0.81$		$16.96^{d} \pm 0.84$		$63.14^{c} \pm 3.38$	
	NaKKlac	$75.35^{a} \pm 2.03$		$7.94^{c} \pm 1.07$		$13.73^{c} \pm 0.65$		$16.11^{c} \pm 1.04$		$59.10^{a} \pm 3.94$	
3	**BL**	$75.02^{bc} \pm 1.67$	$P < 0.001$	$7.19^{bc} \pm 0.75$	$P < 0.001$	$11.98^{a} \pm 0.95$	$P < 0.001$	$14.14^{a} \pm 0.74$	$P < 0.001$	$60.75^{c} \pm 3.81$	$P < 0.001$
	PC	$74.97^{abc} \pm 1.70$		$6.69^{a} \pm 0.77$		$12.15^{a} \pm 0.66$		$13.94^{a} \pm 0.96$		$59.32^{bc} \pm 2.69$	
	NaK	$75.69^{c} \pm 1.51$		$7.06^{ab} \pm 0.70$		$12.06^{a} \pm 0.56$		$13.99^{a} \pm 0.57$		$59.68^{c} \pm 2.86$	
	NaKGlu	$74.90^{abc} \pm 2.10$		$7.58^{cd} \pm 0.87$		$12.05^{a} \pm 0.54$		$14.26^{a} \pm 0.61$		$57.99^{ab} \pm 3.13$	
	NaKYE	$74.13^{a} \pm 1.88$		$7.19^{bc} \pm 0.83$		$13.69^{c} \pm 0.45$		$15.54^{c} \pm 0.68$		$62.34^{d} \pm 2.78$	
	NaKKlac	$74.68^{ab} \pm 2.22$		$7.70^{d} \pm 1.12$		$12.54^{b} \pm 0.54$		$15.02^{b} \pm 0.87$		$57.67^{a} \pm 4.38$	
6	**BL**	$72.96^{a} \pm 2.02$	$P < 0.001$	$7.20^{bc} \pm 0.69$	$P < 0.001$	$11.63^{b} \pm 0.61$	$P < 0.001$	$13.69^{bc} \pm 0.66$	$P < 0.001$	$58.26^{ab} \pm 2.71$	$P < 0.001$
	PC	$73.35^{a} \pm 2.39$		$6.66^{a} \pm 0.80$		$11.18^{a} \pm 0.58$		$13.04^{a} \pm 0.55$		$59.13^{b} \pm 3.56$	
	NaK	$74.44^{b} \pm 2.17$		$6.91^{ab} \pm 0.66$		$11.67^{b} \pm 0.68$		$13.58^{b} \pm 0.64$		$59.36^{b} \pm 2.96$	
	NaKGlu	$73.90^{ab} \pm 2.48$		$7.37^{c} \pm 0.74$		$11.91^{bc} \pm 0.86$		$14.01^{c} \pm 0.73$		$58.27^{ab} \pm 3.10$	
	NaKYE	$72.88^{a} \pm 2.11$		$6.99^{ab} \pm 0.83$		$13.24^{d} \pm 0.59$		$15.06^{e} \pm 0.58$		$62.01^{c} \pm 3.02$	
	NaKKlac	$73.86^{ab} \pm 2.12$		$7.39^{c} \pm 0.97$		$12.13^{c} \pm 0.64$		$14.43^{d} \pm 0.87$		$57.53^{a} \pm 4.46$	
9	**BL**	$72.37^{ab} \pm 1.63$	$P < 0.001$	$5.13^{a} \pm 1.21$	$P < 0.001$	$11.44^{b} \pm 0.80$	$P < 0.001$	$12.61^{b} \pm 0.53$	$P < 0.001$	$65.82^{cd} \pm 6.13$	$P < 0.001$
	PC	$72.63^{b} \pm 2.34$		$5.61^{b} \pm 0.80$		$10.92^{a} \pm 0.54$		$12.31^{a} \pm 0.54$		$62.84^{ab} \pm 3.74$	
	NaK	$74.21^{c} \pm 1.80$		$5.42^{ab} \pm 1.15$		$11.39^{b} \pm 0.46$		$12.69^{b} \pm 0.64$		$64.53^{bc} \pm 5.07$	
	NaKGlu	$72.58^{b} \pm 1.75$		$5.31^{ab} \pm 1.05$		$11.56^{b} \pm 0.77$		$12.79^{b} \pm 0.70$		$65.34^{c} \pm 5.03$	
	NaKYE	$71.62^{a} \pm 1.91$		$5.45^{ab} \pm 0.67$		$13.03^{c} \pm 0.57$		$14.19^{d} \pm 0.43$		$67.59^{d} \pm 3.32$	
	NaKKlac	$72.09^{ab} \pm 2.15$		$6.34^{c} \pm 1.00$		$11.38^{b} \pm 0.62$		$13.11^{c} \pm 0.67$		$60.65^{a} \pm 4.56$	

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

Means with different superscripts in the same column on the same day differ significantly

other treatment groups and the NaKYE group was darker ($P < 0.001$) than the PC, NaK and NaKGlu treatment groups having had the greatest overall decrease in L^* over time.

Storage time significantly affected the L^* of all six treatment groups (Table 5.11) with L^* decreasing from day 0 to day 9. The BL and PC groups shared an identical progression with stable L^*-values up to day 3, only lower ($P < 0.001$ for both) on day 6, and stable thereafter. Lightness decreased ($P < 0.001$) in the NaK group from day 0 to day 3 and then again by day 6. In the NaKGlu group, L^* only decreased ($P < 0.001$) by day 6 and then again by day 9. Lightness was most significantly affected in the NaKYE group where decreases were significant ($P < 0.001$) after every sampling time. The NaKKlac group had a gradual decrease in L^* up to day 6, with lower ($P < 0.001$) values only found after day 6 and then again after day 9. The effect of the interaction between added NaCl level and/or replacer level and storage time confirmed these observations (Table 5.12). From day 3 onwards, the NaK group was lighter ($P < 0.001$) in colour than the NaKYE group. By day 6, NaK was also lighter ($P < 0.001$) in colour than the BL and PC groups and by day 9, NaK was lighter ($P <0.001$) in colour than all five of the other treatment groups. It is possible that the KCl in the NaK and the YE in the NaKYE groups may have contributed to their overall greater L^* stability and instability, respectively. In Chapter 4 on p. 96 it was mentioned that L^* is apparently not affected by the oxidative status of myoglobin (McKenna et al., 2005), but closely related to muscle structure (MacDougall, 1982) which affects WHC (Ripoll et al., 2011). This may be further explained by the light scattering ability of the myofibrils due to their expandability and contractibility (Offer & Trinick, 1983). No changes in WHC were suspected in relation to the cooking and refrigeration losses discussed later on, therefore, no changes in WHC were suspected that could have affected the lightness of these two groups

After day 0, the NaKGlu and NaKKlac groups had higher ($P < 0.001$) a^* than that of the PC group (Table 5.10). Redness decreased across all six groups after day 3 with that of the PC group lower ($P < 0.001$) than that of the BL, NaKGlu, NaKYE, and NaKKlac groups. The a^* of the NaKGlu groups was in turn higher ($P < 0.001$) than that of the BL, PC, and NaK groups. Deterioration of redness continued to after day 6 with that of the PC group lower ($P < 0.001$) than that of the BL, NaKGlu, and NaKKlac groups. The NaKGlu group maintained the highest a^* that was also higher ($P < 0.001$) than that of the PC, NaK and NaKYE groups. The a^* of all six groups were at the lowest levels after day 9 with that of the BL group lower ($P < 0.001$) than that of the PC group. All the other groups also had lower ($P < 0.001$) a^* than that of the NaKKlac group.

Table 5.11. The storage time effect over a 9 day shelf-life at 4 °C on the colour parameters of six banger formulations with different added NaCl and/or replacer levels.

Treat.	Storage time	*L** n = 72	Sign. level	*a** n = 72	Sign. level	*b** n = 72	Sign. level	*C** n = 72	Sign. level	*H** n = 72	Sign. level
BL	**0**	$75.62^{b} \pm 1.78$	$P < 0.001$	$7.50^{b} \pm 0.82$	$P < 0.001$	$12.90^{c} \pm 0.90$	$P < 0.001$	$14.89^{d} \pm 0.91$	$P < 0.001$	$60.16^{b} \pm 3.42$	$P < 0.001$
	3	$75.02^{b} \pm 1.67$		$7.19^{b} \pm 0.75$		$12.15^{b} \pm 0.66$		$14.14^{c} \pm 0.74$		$59.32^{ab} \pm 2.69$	
	6	$72.96^{a} \pm 2.02$		$7.20^{b} \pm 0.69$		$11.63^{a} \pm 0.61$		$13.69^{b} \pm 0.66$		$58.26^{a} \pm 2.71$	
	9	$72.37^{a} \pm 1.63$		$5.13^{a} \pm 1.21$		$11.44^{a} \pm 0.80$		$12.61^{a} \pm 0.53$		$65.82^{c} \pm 6.13$	
PC	**0**	$75.85^{b} \pm 1.75$	$P < 0.001$	$7.12^{c} \pm 0.91$	$P < 0.001$	$12.87^{c} \pm 1.01$	$P < 0.001$	$14.82^{d} \pm 0.70$	$P < 0.001$	$61.03^{b} \pm 3.64$	$P < 0.001$
	3	$74.97^{b} \pm 1.70$		$6.69^{b} \pm 0.77$		$11.98^{b} \pm 0.95$		$13.94^{c} \pm 0.96$		$60.75^{b} \pm 3.81$	
	6	$73.35^{a} \pm 2.39$		$6.66^{b} \pm 0.80$		$11.18^{a} \pm 0.58$		$13.04^{b} \pm 0.55$		$59.13^{a} \pm 3.56$	
	9	$72.63^{a} \pm 2.34$		$5.61^{a} \pm 0.80$		$10.92^{a} \pm 0.54$		$12.31^{a} \pm 0.54$		$62.84^{c} \pm 3.74$	
NaK	**0**	$76.56^{c} \pm 1.45$	$P < 0.001$	$7.59^{c} \pm 0.87$	$P < 0.001$	$13.11^{d} \pm 0.65$	$P < 0.001$	$15.18^{d} \pm 0.65$	$P < 0.001$	$60.09^{a} \pm 3.09$	$P < 0.001$
	3	$75.69^{b} \pm 1.51$		$7.06^{b} \pm 0.70$		$12.06^{c} \pm 0.56$		$13.99^{c} \pm 0.57$		$59.68^{a} \pm 2.86$	
	6	$74.44^{a} \pm 2.17$		$6.91^{b} \pm 0.66$		$11.67^{b} \pm 0.68$		$13.58^{b} \pm 0.64$		$59.36^{a} \pm 2.96$	
	9	$74.21^{a} \pm 1.80$		$5.42^{a} \pm 1.15$		$11.39^{a} \pm 0.46$		$12.69^{a} \pm 0.64$		$64.53^{b} \pm 5.07$	
NaKGlu	**0**	$75.69^{c} \pm 1.85$	$P < 0.001$	$7.97^{c} \pm 0.77$	$P < 0.001$	$13.31^{c} \pm 0.60$	$P < 0.001$	$15.54^{c} \pm 0.55$	$P < 0.001$	$59.11^{b} \pm 2.96$	$P < 0.001$
	3	$74.90^{c} \pm 2.10$		$7.58^{b} \pm 0.87$		$12.05^{b} \pm 0.54$		$14.26^{b} \pm 0.61$		$57.99^{a} \pm 3.13$	
	6	$73.90^{b} \pm 2.48$		$7.37^{b} \pm 0.74$		$11.91^{b} \pm 0.86$		$14.01^{b} \pm 0.73$		$58.27^{a} \pm 3.10$	
	9	$72.58^{a} \pm 1.75$		$5.31^{a} \pm 1.05$		$11.56^{a} \pm 0.77$		$12.79^{a} \pm 0.70$		$65.34^{a} \pm 5.03$	
NaKYE	**0**	$75.08^{d} \pm 1.69$	$P < 0.001$	$7.71^{c} \pm 0.90$	$P < 0.001$	$14.79^{c} \pm 0.81$	$P < 0.001$	$16.96^{d} \pm 0.84$	$P < 0.001$	$63.14^{a} \pm 3.38$	$P < 0.001$
	3	$74.13^{c} \pm 1.88$		$7.19^{b} \pm 0.83$		$13.69^{b} \pm 0.45$		$15.54^{c} \pm 0.68$		$62.34^{a} \pm 2.78$	
	6	$72.88^{b} \pm 2.11$		$6.99^{b} \pm 0.83$		$13.24^{a} \pm 0.59$		$15.06^{b} \pm 0.58$		$62.01^{a} \pm 3.02$	
	9	$71.62^{a} \pm 1.91$		$5.45^{a} \pm 0.67$		$13.03^{a} \pm 0.57$		$14.19^{a} \pm 0.43$		$67.59^{b} \pm 3.32$	
NaKKlac	**0**	$75.35^{c} \pm 2.03$	$P < 0.001$	$7.94^{c} \pm 1.07$	$P < 0.001$	$13.73^{d} \pm 0.65$	$P < 0.001$	$16.11^{d} \pm 1.04$	$P < 0.001$	$59.10^{ab} \pm 3.94$	$P < 0.001$
	3	$74.68^{bc} \pm 2.22$		$7.70^{bc} \pm 1.12$		$12.54^{c} \pm 0.54$		$15.02^{c} \pm 0.87$		$57.67^{a} \pm 4.38$	
	6	$73.86^{b} \pm 2.12$		$7.39^{b} \pm 0.97$		$12.13^{b} \pm 0.64$		$14.43^{b} \pm 0.87$		$57.53^{a} \pm 4.46$	
	9	$72.09^{a} \pm 2.15$		$6.34^{a} \pm 1.00$		$11.38^{a} \pm 0.62$		$13.11^{a} \pm 0.67$		$60.65^{b} \pm 4.56$	

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and for the same treatment differ significantly

NS = Not significant

Storage time significantly affected the *a**-values of all six treatments (Table 5.11), resulting in decreased redness from day 0 to day 9. Redness of the NC group was most stable with a significant ($P < 0.002$) decrease only found on day 9. For the PC, NaK, NaKGlu and NaKYE groups, significant ($P < 0.001$ for all four) decreases were found on day 3 and only again on day 9. In the NaKKlac group, a significant ($P < 0.001$) decrease was found after day 6 and then again after day 9. The effect of the interaction between added NaCl and/or replacer level and storage time on *a**-values further illustrated the effect of added NaCl on redness stability (Figure 5.6). Up to day 6, the *a**-values of the PC group tended to be lower ($P < 0.001$) than that of the NaKGlu, NaKYE and NaKKlac groups. By day 9, however, there were no longer any significant difference between the *a**-values of the PC group and that of any of the other five treatments.

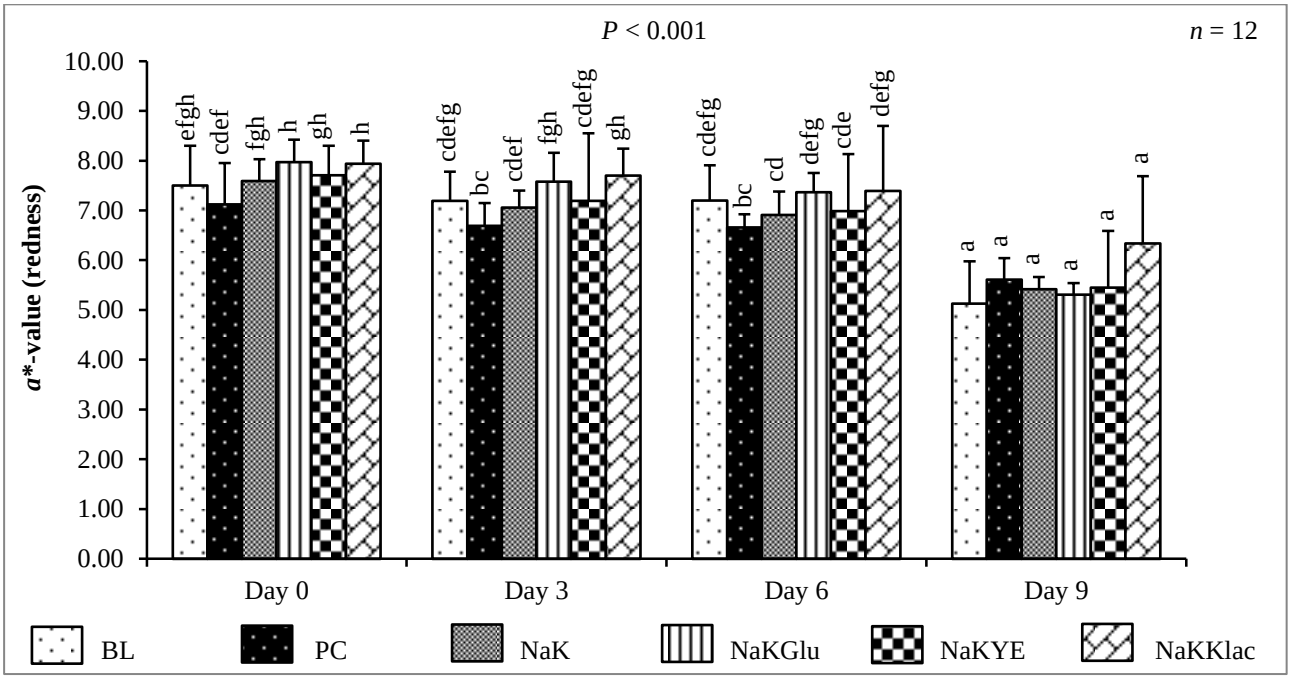

Figure 5.6. The effect of the interaction between added NaCl and/or replacer level and storage time on the a*-value (redness) of bangers during storage at 4 °C for up to 9 days. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts for the same treatment differ significantly. Error bars represent standard deviations of means.

The addition of only NaCl at 1.00% and 2.00% led to the greatest overall losses in *a** attributed to the pro-oxidative effect of NaCl on myoglobin pigment stability (Andersen et al., 1990; Trout, 1990) resulting in metmyoglobin formation. Potassium lactate at 2.50% w/w in fresh ground lamb patties stored for 3 days at 1°C has been shown to increase surface colour stability and reduce surface metmyoglobin formation. The effect was attributed to K-lactate increasing metmyoglobin reducing activity in the meat (Ramanathan, Mancini, Joseph, Yin, Tatiyaborworntham, Petersson, et al., 2011). It appeared as if the much lower K-lactate concentration of 0.20% w/w in the NaKKlac

treatment group exhibited a similar positive effect on *a** stability. In contrast to these and the previous authors' report, K-lactate added at a relatively high level of 2% w/w to fermented sausages and dry-cured pork loins, had no effect on colour (Gou, Guerrero, Gelabert, & Arnau, 1996). The anti-oxidant activities of the sodium ascorbate, sodium metabisulphite, sodium tripolyphosphate, and spices (Gould, 2000; Arneth, 2001; Honikel, 2008; Tangkanakul, et al., 2009; Li et al., 2010; Cheng et al., 2011; Mancini, 2013; Kiliç et al., 2014) may have contributed to such a low concentration of K-lactate having a positive effect on *a** stability. In general, redness stability under O_2 inclusive conditions deteriorated from day 0 for all six treatments in contrast to the reported stability of up to 7 days (Schivazappa et al., 2004).

Yellowness generally decreased over the 9 day shelf-life of all six treatment groups, although the effect was more severe for some treatments than others (Table 5.10). After day 0, the NaKYE group had higher ($P < 0.001$) *b** than all five other treatments and the BL and PC groups had lower ($P < 0.001$) *b** than the NaKGlu, NaKYE and NaKKlac groups. The much higher *b** of the NaKYE groups was easily attributed to the rich brown-yellow colour of the added YE. After day 3, the *b** of the BL, PC, NaK, and NaKGlu groups were lower ($P < 0.001$) than that of the NaKYE group which in turn had lower ($P < 0.001$) *b** compared to the NaKKlac group. After day 6, the *b** of the PC group was lower ($P < 0.001$) than that of the other five groups and the *b** of the NaKYE was higher ($P < 0.001$) than that of the other five groups. The *b** of the NaKKlac group was also higher ($P < 0.001$) than that of the BL and NaK group. At the end of the 9 day shelf-life, the NaKYE group maintained the highest *b** over that of the BL, NaK, NaKGlu, and NaKKlac groups, in turn higher ($P < 0.001$) than that of the PC group.

Storage time significantly affected the *b**-values of all six treatments (Table 5.11) with yellowness decreasing over time. For the BL, PC and NaKYE groups, *b** decreased ($P < 0.001$ for all three groups) from day 0 to day 3 and day 3 to day 6. Yellowness decreased ($P < 0.001$) after every sampling time in the NaK and NaKKlac treatment groups. For the NaKGlu treatment group, *b** decreased ($P < 0.001$) by day 3 and then only again by day 9. The interaction between added NaCl level and/or replacer level and storage time confirmed the general decrease in yellowness across all six treatment groups and the high *b**-values of the NaKYE group in particular (Table 5.12). For the PC group, *b** decreased ($P < 0.001$) after every time interval up to day 6 and by day 9 it had the lowest *b** of any treatment group. In contrast, the *b**-values of NaKYE also decreased ($P < 0.001$) from day 0 to day 6, although it was still significantly higher than any treatment group at any sampling time.

Table 5.12. The effects of the interaction between added NaCl and/or replacer level and storage time on various colour parameters of bangers during storage at 4°C for up to 9 days.

Day	Treatment	*L**	*b**	*C**	*H**
		n = 72	*n* = 72	*n* = 72	*n* = 72
0	**BL**	$75.62^{jkl} \pm 1.78$	$12.90^{hij} \pm 0.90$	$14.89^{h} \pm 0.91$	$60.16^{bcd} \pm 3.42$
	PC	$75.85^{kl} \pm 1.75$	$12.87^{hi} \pm 1.01$	$14.82^{gh} \pm 0.70$	$61.03^{cdef} \pm 3.64$
	NaK	$76.56^{l} \pm 1.45$	$13.11^{ij} \pm 0.65$	$15.18^{hi} \pm 0.65$	$60.09^{bcd} \pm 3.09$
	NaKGlu	$75.69^{kl} \pm 1.85$	$13.31^{jk} \pm 0.60$	$15.54^{i} \pm 0.55$	$59.11^{abc} \pm 2.96$
	NaKYE	$75.08^{hijk} \pm 1.69$	$14.79^{l} \pm 0.81$	$16.96^{k} \pm 0.84$	$63.14^{fgh} \pm 3.38$
	NaKKlac	$75.35^{ijkl} \pm 2.03$	$13.73^{k} \pm 0.65$	$16.11^{j} \pm 1.04$	$59.10^{abc} \pm 3.94$
3	**BL**	$75.02^{ghijk} \pm 1.67$	$12.15^{fg} \pm 0.66$	$14.14^{ef} \pm 0.74$	$59.32^{abc} \pm 2.69$
	PC	$74.97^{ghijk} \pm 1.70$	$11.98^{def} \pm 0.95$	$13.94^{de} \pm 0.96$	$60.75^{cde} \pm 3.81$
	NaK	$75.69^{kl} \pm 1.51$	$12.06^{ef} \pm 0.56$	$13.99^{de} \pm 0.57$	$59.68^{abc} \pm 2.86$
	NaKGlu	$74.90^{ghijk} \pm 2.10$	$12.05^{ef} \pm 0.54$	$14.26^{ef} \pm 0.61$	$57.99^{ab} \pm 3.13$
	NaKYE	$74.13^{efgh} \pm 1.88$	$13.69^{k} \pm 0.45$	$15.54^{i} \pm 0.68$	$62.34^{defg} \pm 2.78$
	NaKKlac	$74.68^{ghijk} \pm 2.22$	$12.54^{gh} \pm 0.54$	$15.02^{h} \pm 0.87$	$57.67^{a} \pm 4.38$
6	**BL**	$72.96^{bcde} \pm 2.02$	$11.63^{cd} \pm 0.61$	$13.69^{d} \pm 0.66$	$58.26^{ab} \pm 2.71$
	PC	$73.35^{cdef} \pm 2.39$	$11.18^{ab} \pm 0.58$	$13.04^{bc} \pm 0.55$	$59.13^{abc} \pm 3.56$
	NaK	$74.44^{ghijk} \pm 2.17$	$11.67^{cde} \pm 0.68$	$13.58^{d} \pm 0.64$	$59.36^{abc} \pm 2.96$
	NaKGlu	$73.90^{defgh} \pm 2.48$	$11.91^{def} \pm 0.86$	$14.01^{def} \pm 0.73$	$58.27^{ab} \pm 3.10$
	NaKYE	$72.88^{bcd} \pm 2.11$	$13.24^{ij} \pm 0.59$	$15.06^{h} \pm 0.58$	$62.01^{def} \pm 3.02$
	NaKKlac	$73.86^{defg} \pm 2.12$	$12.13^{fg} \pm 0.64$	$14.43^{fg} \pm 0.87$	$57.53^{a} \pm 4.46$
9	**BL**	$72.37^{abc} \pm 1.63$	$11.44^{bc} \pm 0.80$	$12.61^{ab} \pm 0.53$	$65.82^{ij} \pm 6.13$
	PC	$72.63^{abc} \pm 2.34$	$10.92^{a} \pm 0.54$	$12.31^{a} \pm 0.54$	$62.84^{efg} \pm 3.74$
	NaK	$74.21^{fghi} \pm 1.80$	$11.39^{bc} \pm 0.46$	$12.69^{abc} \pm 0.64$	$64.53^{ghi} \pm 5.07$
	NaKGlu	$72.58^{abc} \pm 1.75$	$11.56^{bcd} \pm 0.77$	$12.79^{bc} \pm 0.70$	$65.34^{hij} \pm 5.03$
	NaKYE	$71.62^{a} \pm 1.91$	$13.03^{ij} \pm 0.57$	$14.19^{ef} \pm 0.43$	$67.59^{j} \pm 3.32$
	NaKKlac	$72.09^{ab} \pm 2.15$	$11.38^{bc} \pm 0.62$	$13.11^{c} \pm 0.67$	$60.65^{cde} \pm 4.56$
Significance level		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate

*L** = lightness; *b** = yellowness; *C** = chroma; and *H** = hue angle

Means with different superscripts in the same column and on the same day differ significantly

The oxidation of oxymyoglobin to metmyoglobin has been reported to cause an increase in *b**-values (Fernández-López et al., 2000), although in these models an almost reversed progression was found. *Pseudomonas* spp., such as *Pseudomonas fragi*, commonly associated with raw meat, has been reported to have the ability to convert metmyoglobin (brown) to deoxymyoglobin (purple-red) thereby decreasing *b**, increasing *a**, and causing a reddening effect (Motoyama, Kobayashi, Sasaki, Nomura, & Mitsumoto, 2010). The presence of *Pseudomonas* spp. in this study were not confirmed and there were no subsequent increases in *a**-values over time that might have indicated a reddening effect by microbial activity. In all probability, the decreases in *L**, *a**, and *b** could be attributed to the deterioration caused by the display lighting used over the course of the 9 day shelf-life.

Discolouration was determined as the loss in colour brightness with a progression towards greying at lower *C**-values. Generally, the *C**-values of all six treatment groups decreased over the course of the 9 day shelf-life. After day 0, immediate differences between treatment groups were observed. Chroma was lower ($P < 0.001$) for the BL and PC groups, higher ($P < 0.001$) for the NaKKlac

group and the highest for the NaKYE group. The higher ($P < 0.001$) a^* and highest ($P < 0.001$) b^* contributed to an overall increase in colour brightness for this group. After day 3, the NaKYE and NaKKlac groups maintained higher ($P < 0.001$) C^* than the other four treatment groups. After day 6, differences between groups were more pronounced with greater significant differences between groups. At 13.04 ± 0.55, the C^* of the PC group was much lower than the highest C^* of 15.06 ± 0.58 of the NaKYE group. This indicated a greater loss of colour when only NaCl or NaCl with KCl was used over more complex formulations that included: NaCl and K-gluconate; NaCl, KCl, and YE; and NaCl, KCl, and K-lactate. After day 9, the C^* of the PC group was lower ($P < 0.001$) than that of the BL, NaK, and NaKGlu groups, which in turn was lower than that of the NaKGlu group and the C^* of the NaKYE group maintained a higher ($P < 0.001$) C^* over all five other groups. Storage time significantly affected the Chroma of all six treatment groups (Table 5.10) with an overall decrease in brightness. The C^*-values of the BL, PC, NaKYE and NaKKlac groups decreased ($P < 0.001$ for all five groups) over every time interval from day 0 to day 9. In the NaKGlu group, this effect was more gradual with C^* lower ($P < 0.001$) after day 3 and then only again after day 9. The effect of the interaction between added NaCl level and/or replacer level confirmed significant ($P < 0.001$) decreases in H^* for all six treatments over different time periods. For the BL, PC, NaKYE and NaKKlac groups, this was true for every consecutive sampling time. By day 9, the NaKGlu, NaKYE and NaKKlac groups still had higher ($P < 0.001$) C^*-values than the NaCl-only groups on day 0.

Hue angle, representing the purity of colour, with H^* nearer to 0° being closer to red and H^* nearer to 90° being closer to yellow, was also significantly influenced by formulation type over time. As a direct result of the colour of the YE, the H^* of the NaKYE treatment was larger ($P < 0.001$) than that of all five other treatment groups (Table 5.10). The H^* of both the NaKGlu and NaKKlac treatments were smaller ($P < 0.001$) and thus closer to red. After day 3, the BL and NaK groups had larger ($P < 0.001$) H^* than both the NaKGlu and NaKKlac groups. After day 6, the H^* of the six groups were the closest to each other, although NaKYE still had the largest and NaKKlac still had the smallest H^*. After day 9, the H^* of all six groups increased to the largest angles over the complete 9 day shelf-life, indicating that the colour of all six products had shifted to being more yellow. The NaKYE had the largest ($P < 0.001$) H^* and NaKKlac the smallest ($P < 0.001$) H^*. Storage time significantly affected the H^*-values of every treatment group (Table 5.11) with colour shifting more to the yellow end of the spectrum than the red by the end of shelf-life. The two NaCl-only groups, BL and PC, had smaller ($P < 0.001$ for both) H^* by day 6 and greater ($P < 0.001$) H^* by day 9 that were significantly greater than on day 0. In both the NaK and NaKYE groups, H^* was fairly stable up to day 6 after which it increased ($P < 0.001$ for both) to the values found on day 9.

In the NaKGlu group, *H** remained stable up to day 6 and was only greater ($P < 0.001$) on day 9. The *H** of the NaKKlac group was relatively stable up to day 6 and the *H** on day 9 was only significantly ($P < 0.001$) greater than that of day 3 and day 6. The effects of the interaction between added NaCl level and/or replacer level and storage time showed that the *H**-values of all six treatment groups generally decreased from day 0 to day 6 (Table 5.12). By day 9, the *H**-values of all six groups had increased and were higher ($P < 0.001$) than on day 6 for all six treatment groups; higher ($P < 0.001$) than on day 3 for the BL, NaK, NaKGlu, NaKYE and NaKKlac groups; and higher ($P < 0.001$) than on day 0 for the BL, NaK, NaKGlu and NaKYE groups.

The use of 1% KCl in combination with 1% NaCl resulted in a generally lighter banger. Similar to the report by Gou et al. (1996), where a 50% substitution of NaCl with KCl was used and found to have no effect on the colour of fermented sausages, the 50% substitution of NaCl with KCl in this study had limited and short-lived effects on the colour of fresh pork banger sausages. The addition of 1.00% YE led to a banger that became increasingly more yellow and darker over time, while the use of 0.80% KCl and 0.20% K-lactate with 1.00% NaCl, maintained redness better even compared to the use of 2.00% NaCl for up to 6 days after manufacturing. Meat colour affects the consumer purchasing decision (Mancini & Hunt, 2005) and redness is the most important aspect of colour in objective pork quality evaluation (Dvorak et al., 2001) therefore, an overly yellow pork banger sausage may be rejected by consumers.

5.3.7. Sensory analysis

A breakdown of the 75-member consumer panel revealed the following information. According to gender, the panel was split between 43 females and 32 males. According to age group, the panel was split as: 4% under the age of 19; 73% between the ages of 20-29, attributed to the large student demographic; 2% between the ages of 30-39; 7% between the ages of 40-49; 12% between the ages of 50-59; and 2% over the age of 60. The results of the consumer sensory panel revealed very similar rankings for all six treatments groups across all five selected sensory attributes (Figure 5.7). No significant differences between treatment groups could be identified for saltiness, texture or aroma as sensory attributes. There were, however, small differences between treatment groups for these rankings. In general, it could be determined that the PC group had the highest rankings and the NaKGlu group had the lowest rankings for these three attributes.

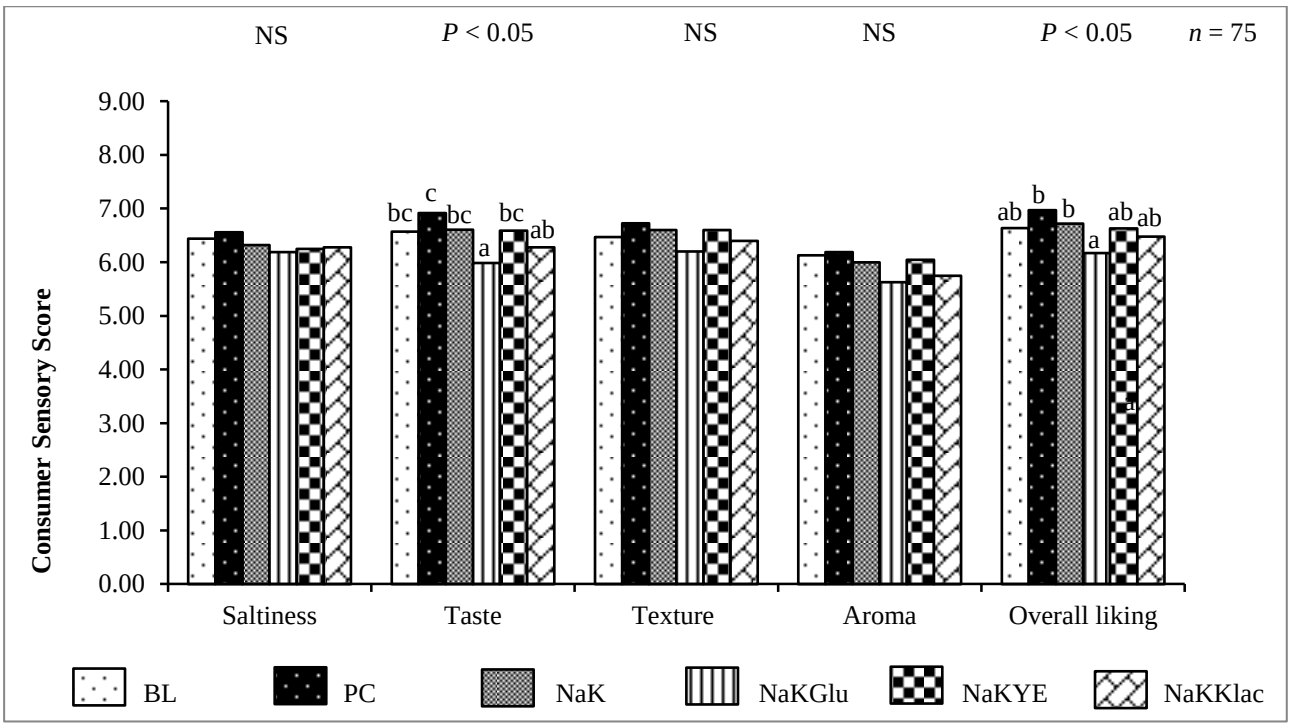

Figure 5.7. Consumer sensory rankings of six banger formulations based on different added NaCl and/or replacer levels.BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Means with different superscripts for the same ranking differ significantly.

In fermented sausages, the use of K-lactate at 20% of the NaCl content also resulted in small but insignificant changes in texture determined through sensory evaluation (Gelabert, Gou, Guerrero, & Arnau, 2003). Of the four replacer formulations, the NaK and NaKYE groups compared most favourably to the PC group on the basis of texture and aroma. Similar and significant results for aroma and texture has been reported for fermented sausages formulated with a 50% replacement of NaCl with KCl and for a 50% replacement of NaCl with KCl and 1.00% added YE (Campagnol, dos Santos, Wager, Terra, & Pollonio, 2011). Significant differences between treatment groups were found for taste and overall liking as sensory attributes. The PC group ranked higher ($P < 0.05$) for taste than both the NaKGlu group and the NaKKlac group. The flavour of K-lactate has reportedly been detected at levels of 10-20% of w/w substitution of NaCl in fermented sausages (Gelabert et al., 2003). The rankings for taste of the BL, NaK and NaKYE groups were also higher ($P < 0.001$) than that of the NaKGlu group. In terms of overall liking, both the PC group and the NaK group ranked higher ($P < 0.05$) than the NaKGlu group. No other treatment groups had significantly higher rankings compared to the NaKGlu group in terms of overall liking.

As reported in Chapter 4 on p. 132, consumers were unable to significantly differentiate the BL group from the PC group. Reducing the added NaCl to 1.00% and then employing various replacer combinations on a w/w basis (NaK) or slightly less (NaKYE), made the NaK and NaKYE

treatments indistinguishable from the PC group. The NaKKlac group only fared worse on the basis of taste. Some authors reported negative effects on the sensory properties of fermented sausages at 50% KCl (Gelabert et al., 2003; Campagnol et al., 2011) and suggested a limit of 40% KCl (Gelabert et al., 2003). Bacon manufactured with 40% KCl experienced no adverse effects in terms of sensory properties (Wu et al., 2014). On the other hand, replacing NaCl with up to 70% KCl has been reported for fresh pork sausages without any negative effects on sensory properties, although a modified form of MSG was included (Pasin et al., 1989). The results of this study showed that KCl could be used to replace 50% of the original NaCl content without any ill effects. It is possible that the product type with regard to processing (fermented or fresh) and complexity (number and volumes of spices and other additives) may affect how KCl at this level might be perceived. Similarly, the addition of 1.00% YE with a 50% replacement of NaCl with KCl resulted in sausages with sensory properties to that of the 2.00% NaCl control as reported by Campagnol et al. (2011).

With very limited information available on the use of K-gluconate as NaCl replacer, it was unclear as to how the use thereof could have such a significant negative effect on the taste of the NaKGlu group. It has been shown that in aqueous solutions at concentrations of 100 mM, the taste reaction time of Na-gluconate was roughly double that of NaCl. A linear relationship between taste reaction time and the square root of anionic weight in addition to the direct relationship between taste reaction time and molecular weight has been illustrated (Delwiche, Halpern, & Desimone, 1998). It is conceivable that K-gluconate has a similarly delayed taste reaction time to that of Na-gluconate. This may have resulted in consumers experiencing a delay in saltiness that they may not have associated with saltiness, but as a drawn-out salt taste that increased upon further mastication of the sample.

Frequency distribution was used to break down the rankings of the 75-member consumer panel to determine how likely a consumer was to choose a specific hedonic descriptive to describe taste (Figure 5.8) and overall liking (Figure 5.9). Descriptives such as “Like extremely” to “Like slightly” were regarded as positive descriptives with positive values. The “neither like nor dislike” descriptive was regarded as being neutral and indicated by absolute values on the x-axis. Descriptives such as “Dislike slightly” to “Dislike extremely” was regarded as negative descriptives with negative values. The PC group with the highest ($P < 0.05$) ranking for taste had a positive attribute total of 65 and a negative attribute total of -7. The NaKGlu group with the lowest ($P < 0.05$) ranking for taste had a positive attribute total of only 51 and a negative attribute total of -17. The PC group was never described with descriptives such as “Dislike very much” and “Dislike extremely”, in contrast to the NaKGlu group where these descriptives were used. The BL, NaK, NaKGlu, and NaKKlac groups were most positively described with the “Like moderately”

descriptive, the PC group with the "Like very much" descriptive and for the NaKYE group it was a tie between "Like very much" and "Like moderately". The BL group had the highest neutral ranking for "Neither like nor dislike" of 8 and the PC group the lowest neutral ranking of 4. The BL, PC, NaK, NaKYE, and NaKKlac groups were most negatively described with the "Dislike slightly" descriptive and the NaKYE and NaKKlac groups had the greatest frequency of use for this descriptive. The NaKGlu group was most negatively described with the "Dislike moderately" descriptive.

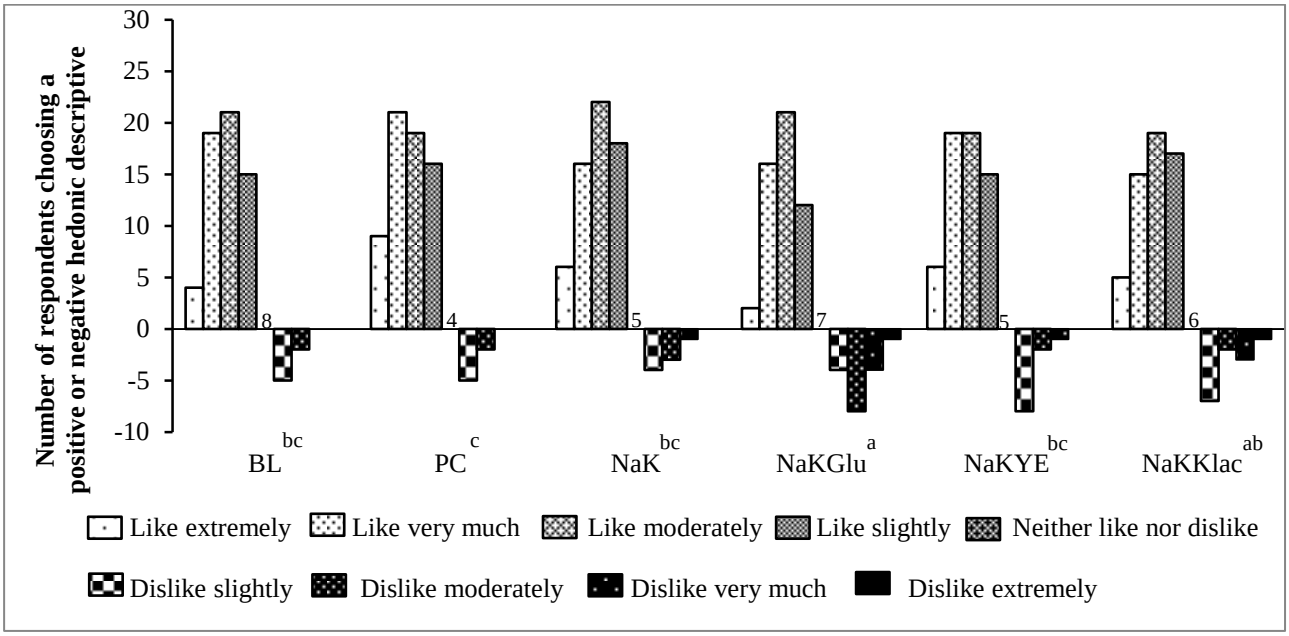

Figure 5.8. Frequency distribution of respondents having chosen either a positive (like), negative (dislike), or neutral (neither like nor dislike) descriptive to describe "taste" as single sensory attribute. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Zero value rankings appear as blank spaces on the x-axis. Title superscripts indicate significant differences between treatment groups for this specific sensory attribute.

The PC and NaK groups tied for the highest ($P < 0.05$) rankings for overall liking, had positive attribute totals of 65 and 63, respectively, and negative attribute totals of -5 and -7, respectively. The NaKGlu group with the lowest ($P < 0.05$) ranking for overall liking, had a positive attribute total of only 54 and a negative attribute total of -12. Both the PC and NaK groups were never described with descriptives such as "Dislike very much" and "Dislike extremely". The PC, NaK, NaKGlu, NaKYE and NaKKlac groups were most positively described with the "Like moderately" descriptive, while for the NC group it was a tie between "Like very much" and "Like moderately". All six treatment groups were most negatively described with "Dislike slightly" with the NaKYE group having the highest frequency of use for this descriptive. The NaKYE and NaKKlac groups had similar frequencies of use of the "Dislike very much" descriptive to that of the NaKGlu group.

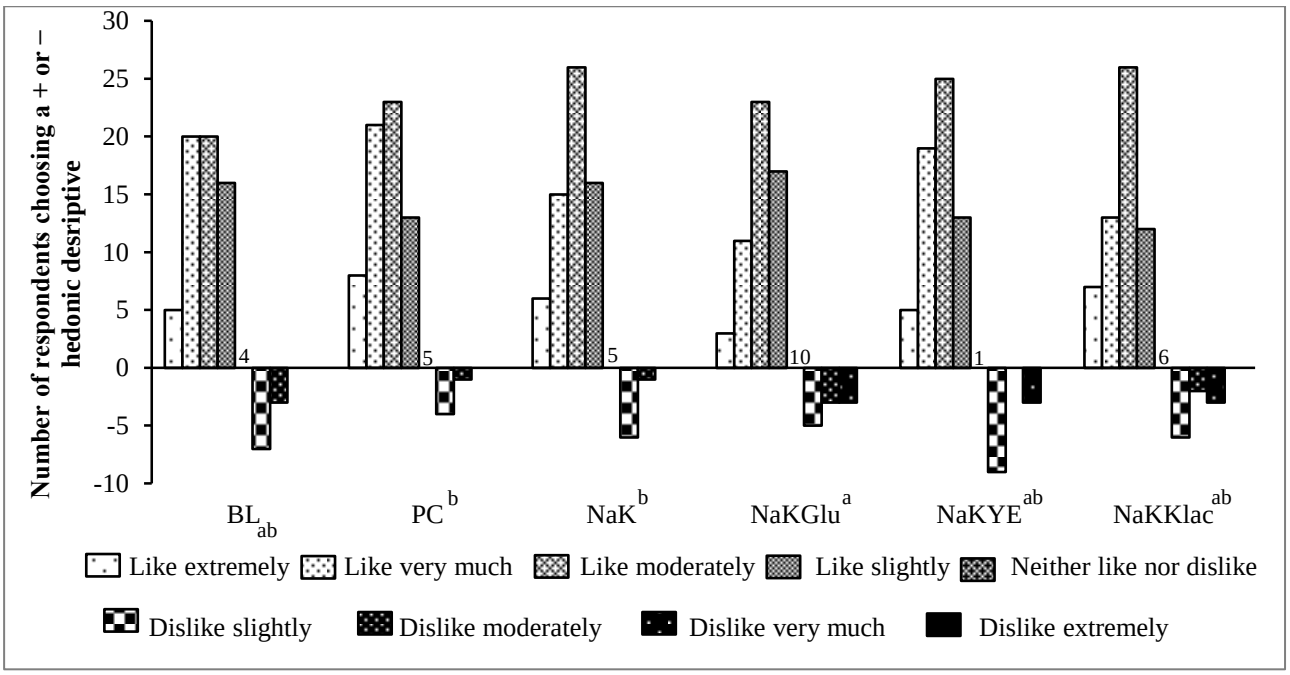

Figure 5.9. Frequency distribution of respondents having chosen either a positive (like), negative (dislike), or neutral (neither like nor dislike) descriptive to describe "overall liking" as single sensory attribute. BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate. Title superscripts indicate significant differences between treatment groups for this specific sensory attribute. Zero value rankings appear as blank spaces on the x-axis.

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

To simplify and summarize the multitude of quality and stability parameters evaluated during this study, a multivariate, 2-dimensional PCA plot was drawn. Figure 5.10 illustrates all the parameters significantly (minimum of $P < 0.05$) affected in relation to the six different treatment groups. The two dimensions accounted for a combined 72.07% of the total variation in the data set. The first dimension (F1) explained 44.80% and the second dimension (F2) 22.28% of the variance in the data. Therefore F1 was regarded as the main dimension that divided the six treatments into two groups: PC, BL, NaK and NaKGlu on the left; and NaKKlac and NaKYE on the right.

High NaCl and Na content, sensory attributes such as overall acceptability and taste, and higher percentages of total, cooking and refrigeration losses were strongly associated with the PC group. The BL and NaK groups were closely clustered together more than any other groups. Effects on lightness over multiple days and on the coliform counts were closely associated with these two groups. In terms of both dimensions, the BL and NaK groups were more closely clustered with the PC group, summarising their similar results to most of that of the PC group. The NaKGlu group was also somewhat associated with these parameters, although it also shared some association in a_w and redness over multiple days with the NaKKlac group on the basis of the second dimension (F2).

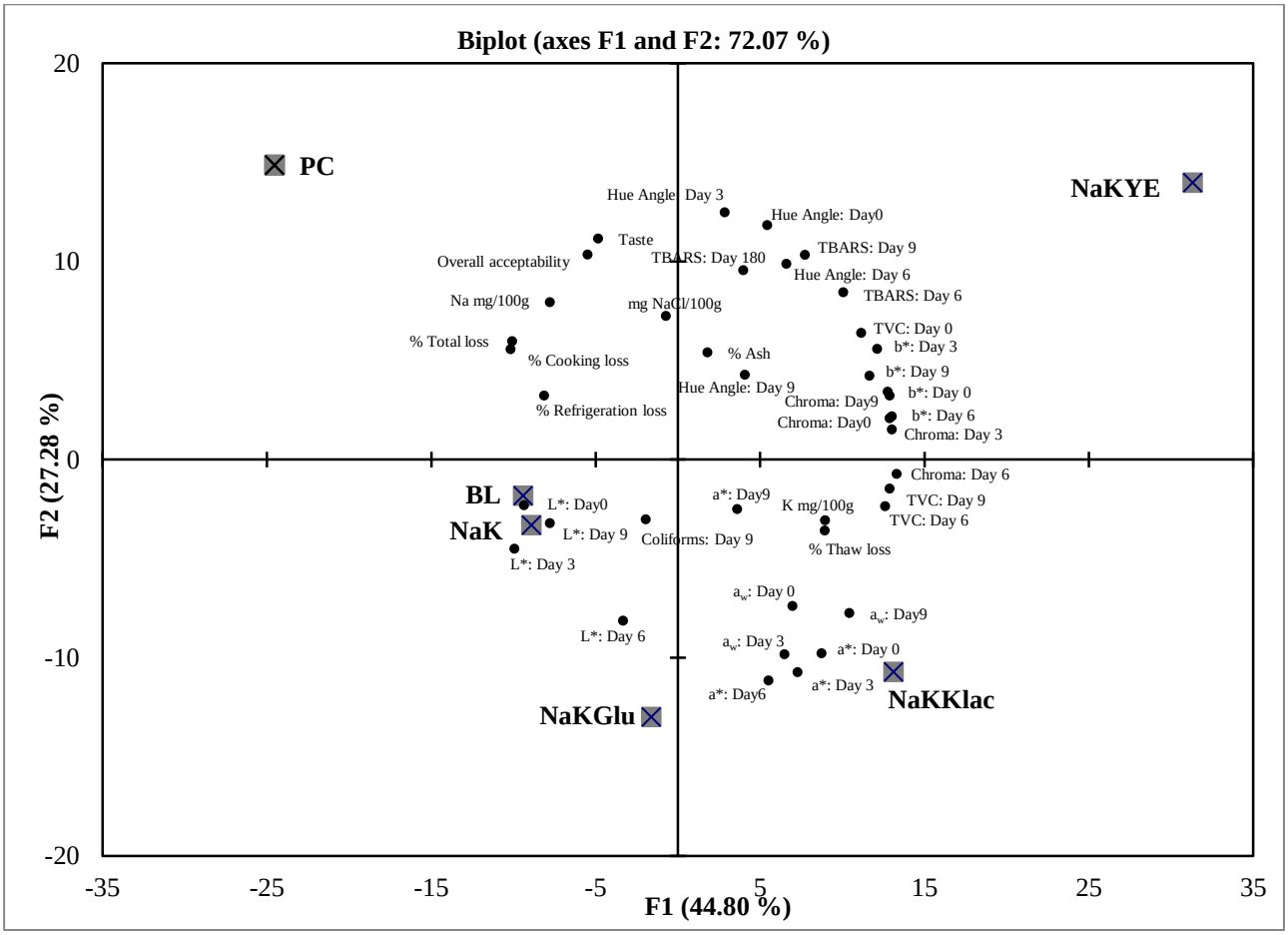

Figure 5.10. Principle Component Analysis of 40 quality and stability parameters of pork bangers significantly affected by different added NaCl and/or replacer combinations.

By far the greatest number of significant effects on parameters was associated with both the NaKYE and NaKKlac treatment groups in F1. For the NaKYE group this included: the largest Hue angles, highest yellowness and Chroma values over multiple days; the highest TBARS on days 6, 9 and 180; the highest percentage ash; and the highest TVC on day 0. For the NaKKlac group, this included much greater association with: higher Chroma values on day 6; high TVC on days 6 and 9; the highest K content; high a_w over multiple days; and the highest redness values over multiple sampling times. The NaKKlac group also had greater association with significant effects on multiple parameters across F2 than any of the other five treatment groups although this explained much less of the variations than for F1. In general, the PC, NaKGlu, NaKYE and NaKKlac groups were distributed far from each other, showing little commonality between significant effects on parameters. In contrast, the close proximity of the NaK to the BL group showed a much greater commonality as well as that the NaK treatment as a replacer option shared greater similarity to a 1.00% added NaCl level (BL) than to a 2.00% added NaCl level (PC). The second best similarity in effects was found for the NaKGlu treatment group and the lowest level in similarity was observed in the NaKYE treatment group.

5.4. Conclusions

One of the main focus areas regarding Na reduction in processed meat products is the evaluation of the application of various salt or Na replacers in an effort to reduce the dependency on salt alone in maintaining product stability, safety and quality. The end goal of this is to improve the overall nutritional composition of a group of products debatably associated with not being very good for overall human health. In this study, most of the replacers evaluated, using one processed meat model (pork banger sausages), acted as partial Na replacers with the exception of YE which contains salt.

Thaw losses of the four replacer formulations were higher than that of the positive control and baseline added NaCl level. Refrigeration losses were lower for partial replacement with only KCl, KCl with YE, and KCl with K-lactate, than for the baseline NaCl level with no replacement. Neither thaw nor refrigeration losses of the replacer formulations were visually discernable from that of the positive control NaCl level. Cooking losses of the four replacer groups were all lower than that of the positive control group and baseline group. The KCl and K-lactate partial replacement treatment led to the lowest cooking losses compared to the partial replacement with K-gluconate.

The ash, NaCl, Na, and K contents differed according to the quantities and types of replacers used. Three of the four replacer formulations had ash contents similar to the positive control and the K-gluconate replacer had an ash content midway between the positive control and baseline NaCl inclusion levels. Where KCl was present, NaCl content was overestimated due to the Volhard titration being an indirect method relying on the chloride content. Atomic absorption spectroscopy was again shown to be the more accurate method by directly determining Na content, although it is a more costly and complicated method requiring specialised equipment. Formulated Na content closely matched actual Na content when the Na contributed by each component was carefully estimated. With fine tuning, precise Na content limits can be matched precisely over only a couple of batches of a particular formulation. A 1:1 ratio of NaCl to KCl as well as the use of multiple K-contributing replacers in one formulation was shown to double the K content of the bangers to levels that may positively influence the Dietary Reference Intake (DRI) of K. Not only may Na consumption be reduced, but K consumption may be meaningfully increased by processed meat products heavily criticised for their effects on human health.

The replacer formulations had very limited effects on basic chemical parameters. No significant effects on pH were found that could ultimately have affected any number of other parameters. Initially, all of the replacer formulations had substantially higher a_w equal to a 50% reduction in

NaCl content. The KCl and K-lactate had the most consistently high a_w level while the other replacer treatments only had periodically higher a_w. No effects on moisture content were observed in part due to the small differences in meat content as opposed to the banger formulations in Chapter 4. This presented an advantage over Na reduction without replacement where the difference in added NaCl content had to be made up for by adding more meat. Even the addition of YE at 1.00% w/w had no effect on moisture content.

No replacer formulation significantly improved lipid oxidative stability of bangers as fresh or frozen meat products compared to high and low added NaCl levels. There were, however, significant differences between replacer formulations. In both fresh and frozen bangers, partial replacement of NaCl with only KCl or only K-gluconate led to increased lipid oxidative stability compared to partial replacement with KCl together with YE. The KCl and YE formulation significantly deteriorated lipid oxidative stability. Levels of secondary lipid oxidation products were far below detection thresholds for rancidity regardless of formulation type.

The use of YE immediately increased TVC after manufacturing, the combination of kCl with YE and the combination of KCl with K-lactate significantly reduced the overall shelf-life. The inclusion of K-lactate, known for its antimicrobial activity, also appeared to have negatively affected shelf-life at a low inclusion level of 0.20% w/w. Potassium gluconate fared better and only inconsistently led to greater microbial growth. Most promising was that the KCl-only treatment gave results indistinguishable from the positive control NaCl level. As previously established, the baseline NaCl treatment without replacers gave TVC in agreement with the positive control treatment. None of the replacer treatments had any significant effects on any of the other microbial parameters and the baseline NaCl treatment only led to higher coliform counts at the end of shelf-life. The few negative effects of some replacers on microbial stability will be greatly limited commercially since most processors sell banger products as frozen products to be cooked from frozen.

Of all the various parameters, the colour parameters appeared to have been affected the most by added NaCl level and replacer formulation. Partial replacement with only KCl maintained the highest lightness values and partial replacement with KCl and YE maintained the lowest lightness. For the most part, the highest NaCl level resulted in the lowest redness values, although it was overtook by the lowest NaCl level without replacement at the end of shelf-life. Partial replacement with KCl and K-lactate maintained the highest overall redness. Yellowness was higher when YE was used in the formulation while high NaCl without replacement consistently maintained the lowest yellowness. Partial replacement with KCl and YE best maintained colour brightness followed by partial replacement with KCl and K-lactate. In contrast, the colour brightness decreased

over time at the highest NaCl level without replacement. Colour purity was the lowest and most stable with partial replacement with KCl and K-lactate. Partial replacement with KCl and YE resulted in consistently the highest colour purity. This was attributed to the significant contribution made to yellowness by the addition of YE. 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 certain replacers directly affected the meat product colour. Subjective colour evaluation by means of sensory evaluation is needed to determine if consumers will be able to detect effects in the product colour and how this will affect their liking of the reformulated products.

Differences between treatments for the consumer sensory analyses were mostly limited. Significant differences were only found for taste and overall liking as sensory attributes. Consumers ranked the highest added NaCl group the highest, and the replacer group with a partial replacement with K-gluconate, the lowest. For overall liking of the bangers, consumers ranked both the highest added NaCl group and the partial replacement with KCl group higher than the group with a partial replacement with K-gluconate. It was confirmed that consumers preferred high salt levels although they did not indicate this preference directly as saltiness, but rather taste. Partial replacement combinations involving the use of KCl, YE and K-lactate provided suitable replacers for NaCl in terms of sensory evaluation.

The partial replacement of NaCl with KCl, K-gluconate, K-lactate, and YE gave mostly favourable results in terms of the chemical, microbial and sensory quality of pork bangers. Only singular and mostly limited discrepancies were identified in terms of a specific replacer combination. Some of these discrepancies may be addressed by making changes in how the product is processed, e.g. frozen bangers rather than fresh bangers. The very limited effects on basic chemical parameters, lipid oxidative stability and the major improvement in cooking losses provided further evidence for the suitability of these replacers for use as NaCl replacers. Consumers were again shown to prefer higher added NaCl levels, although it was found to be directly linked to taste and only indirectly to salty taste. Only K-gluconate was found to be disliked by consumers, indicating that lower concentrations may be needed. Effect on meat colour need to be evaluated quantitatively as well as qualitatively to determine if consumers can establish if differences exist and to indicate their preference. The replacement of Na with K gave the bangers nutraceutical potential with meaningful increases in total K content. Cost is a final consideration of the suitability of these replacers that will play a major part in the selection of replacers for commercial products. Taking into account the results of this study in addition to cost, it is believed that KCl remains the cheapest, simplest and most elegant replacer to be considered for the partial replacement of NaCl.

CHAPTER 6

THE GROWTH AND SURVIVAL OF *Escherichia coli* AND *Staphylococcus aureus* REFERENCE STRAINS IN BANGER BATTERS FORMULATED WITH REDUCED OR PARTIALLY REPLACED NaCl

ABSTRACT

In this study, salt reduction at 0.00%, 25.00%, 50.00% and 100.00% without salt replacers and partial salt replacement at ~ 50.00% with combinations of KCl, K-gluconate, yeast extract and K-lactate were evaluated for effects on the growth or survival of Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) reference strains. The two bacterial strains were separately inoculated to final levels of 10^3 cfu/g into pork banger batters that were then stored at 4 °C or 10 °C for up to 9 days. The purpose of this study was to determine if these potential pathogens could use reduced or partially replaced NaCl content to their advantage under ideal or slightly abusive storage temperatures in banger formulations with otherwise unchanged levels of substances with primary or secondary antimicrobial properties. The inoculated E. coli strain survived the different added NaCl levels and partial replacement of NaCl by various replacers without any significant differences between treatment groups. Survival decreased very little from days 0 to 9 and there were no significant differences due to storage temperature. The inoculated S. aureus strain was affected by different added NaCl levels, partial replacement of NaCl with various replacers, storage time and storage temperature. Control salt levels most significantly decreased survival at 4 °C. Very limited growth was observed depending on treatment type, storage time and storage temperature. Reduced or partially replaced NaCl did not significantly increase the risk of these two bacterial strains beyond the initial inoculation levels. For the most part, other formulation components and storage conditions exhibited the greatest bacteriostatic and anti-microbial potential.

Keywords: Escherichia coli; Staphylococcus aureus; pathogens; salt reduction, salt replacers

6.1. Introduction

Meat and processed meat products are known to be highly susceptible to the exploitation of microbiota. This is mainly attributed to the generally favourable pH of meat being in the range of 5.5-6.5, high moisture content and the complex nutrient composition of meat (Nychas et al., 2008;

Doulgeraki et al., 2012). Spoilage of affected products is the result of microbial activities over time, usually as a result of storage of the products (Doulgeraki et al., 2012). Spoilage of raw meat can be defined as a situation where microorganisms are present in large enough numbers so as to cause changes in the product making it unappealing and unsuitable for consumption (Gram et al., 2002: Fung, 2010). The initial microbial load is dependent on the physiological status of the animal *ante-mortem*, the spread of microorganisms in the abattoir and during subsequent processing. The rate of spoilage is determined amongst others, by the temperature and storage conditions during distribution (Nychas et al., 2008). The composition of the microbial population that may potentially lead to spoilage of the product is dependent on: the storage conditions of the product and the initial microbial population composition and competitiveness of the types present.

Reportedly two distinct environmental conditions are possible that may determine the population composition. The first is where facultative anaerobic microbes or anaerobic Gram-positive bacteria dominate under conditions of low oxygen availability e.g. when meat is stored under low oxygen availability naturally, or in the presence of purposefully added antimicrobial gases. The second situation is where aerobic or facultative anaerobic Gram-negative bacteria or other microbes dominate under high oxygen conditions (Doulgeraki et al., 2012). A third situation is a combination of the previous two where one group of microorganisms eventually displaces a group of different predecessors due to changes in substrates available to metabolise (Jones, 2004). This is probably the most applicable scenario in the storage of a non-vacuum packaged meat product such as fresh sausages. Bacteria capable of growth at refrigeration temperatures are known as psychrotrophic bacteria and this includes a diverse group of bacteria such as *Acinetobacter*, *Pseudomonas*, *Brochothrix*, *Flavobacterium*, *Psychrobacter*, *Moraxella*, *Staphylococcus*, *Micrococcus*, *Clostridium*, the LAB, and genera of the *Enterobacteriaceae* family (Dainty, Shaw, & Roberts, 1983; Dainty & Mackey, 1992; Labadie, 1999; Doulgeraki et al., 2012).

Another concern in the spoilage of meat products is the growth of potential pathogens that may affect meat product safety. Species such as *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus* (Smith-Palmer et al., 1998), the *Acinetobacter calcoaceticus−Acinetobacter baumanii* (Acb) complex (Peleg & Hooper, 2010), and opportunistic species such as *Pseudomonas aeruginosa* (Yi, Zhu, Fu, & Li, 2010) are just a few familiar examples.

During the processing of food various steps act as hurdles that selectively limit some microorganisms while other manage to slip through to the next step. Primarily absent ones may even be introduced during various subsequent processing steps. A change or failure in the control system may allow the emergence or re-emergence of a pathogen (Miller et al., 1998). A prime

example of this may be a decrease in the normal NaCl content of a meat product and possibly the partial replacement of a proven NaCl content with other compounds with known lower or undetermined efficacy. The concentration of NaCl required to limit pathogens vary, depending on the microbial species, pH, temperature, oxygen levels and other components in food such as moisture, fat, and additives (Doyle & Glass, 2010). A study by Gibson and Roberts (1986) revealed that various strains of *Salmonella* spp. and enteropathogenic *E. coli* could tolerate higher NaCl concentrations better at higher temperatures together with higher pH values. This may lead to food safety problems for food products stored at higher temperatures or experiencing temperature abuse and low acidity.

A number of models have been created to describe the effects of combinations of environmental factors on the growth of pathogenic bacteria. ComBase as a modelling toolbox provides a quantitative method with which to predict microbial responses to changes in three or more environmental factors. Its usefulness is, however, limited due to the fact that some of the basic data is based on the growth of microbes in laboratory media or in specific foods. These models can, therefore, not predict exact results for particular food stuffs; they can, however, provide estimates of interactions amongst various factors that should be tested for in actual foods (Doyle & Glass, 2010). This is further complicated by the significant differences in the behaviour of bacteria studied *in vitro* compared to those studied in actual food matrices. An example of this is the *sea* gene responsible for the expression of the staphylococcal enterotoxin A (SEA), the most reported cause of staphylococcal food poisoning. In meat products as diverse as boiled ham, hot-smoked ham, Serrano ham and black pepper salami, significant differences in expression rates and time of expression were reported. This was in addition to the differences in expression rates and time of expression between the meat products as media or brain heart infusion (BHI) broth as defined medium (Wallin-Carlquist, Márta, Borch, & Rådström, 2010). The purpose of this part of the project was to investigate the growth and survival of two reference bacteria strains: *E. coli* as an example of Gram-negative bacteria, and *S. aureus* as an example of Gram-positive bacteria in closely simulated raw sausage models formulated either with reduced NaCl content or reduced NaCl content with NaCl replacers. Specific aims for this chapter included:

1. Determining if a reduction or partial replacement of NaCl affected microbial survivability and growth,
2. Determining whether a Gram-negative or Gram-positive bacterial strain proved to be more opportunistic at a reduced or partially replaced NaCl content, and
3. Determining the effect of two storage temperatures (4 and 10 °C) on bacterial growth at reduced or partially replaced NaCl content.

6.2. Materials and methods

6.2.1. Bacterial strain selection and sourcing

Escherichia coli and *S. aureus* were selected as Gram-negative and Gram-positive representatives of commonly associated food spoilers and potential pathogens. Two specific strains were sought with very low pathogenic potential mostly due to safety constraints. The first strain selected was *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) of Serotype O6 and Biotype hereon only referred to as *E. coli* and the second strain was *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 6538™) hereon only referred to as *S. aureus*. Both of these strains are reference strains, each used in a large number of studies and were originally sourced from the American Type Culture Collection (Manassas, USA). The *E. coli* strain was originally a clinical isolate, does not produce any verotoxin and is commonly used for media testing and susceptibility testing of anti-microbial activity (ATCC, 2015a). The *S. aureus* strain was originally isolated from a human lesion and is commonly used in media testing, inhibition testing and the testing of antimicrobial agents and preservatives (ATCC, 2015b).

Two temperatures were selected to each represent a temperature where meat products should ideally be kept or to represent an abusively high temperature that might mistakenly be regarded as acceptable. For the former, a temperature of 4 °C was selected as a temperature shown to extend the shelf-life of meat products and for the latter, a temperature of 10 °C was selected as a temperature where definite spoilage occurs at a faster rate (McMullen & Stiles, 1993) and an example of an abusive temperature that may be the result of refrigeration equipment that is not properly maintained.

6.2.2. Experimental design

Two different experimental designs were followed: one with a salt reduction and the second, with a salt replacement.

6.2.2.1. Salt reduction

The experimental design consisted of a 4x2x4x2 (n = 64) factorial design. This was made up out of four formulations (NC = 0.00%, LI = 1.00%, HI = 1.50%, and PC = 2.00% added NaCl), two temperatures (4 and 10 °C), four sampling intervals (days 0, 3, 6, and 9) and two replicates per treatment group per sampling interval. All four treatment groups were only spiked with one bacterial strain at a time and incubation of the spiked samples took place at one temperature at a time.

6.2.2.2. Salt replacement

The experimental design consisted of a 6x2x4x2 (n = 96) factorial design. This was made up out of six formulations (BL = 1.00% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl and 1.00% KCl; NaKGlu = 1.00% NaCl and 1.00% K-gluconate; NaKYE = 0.80% NaCl, 0.80% KCl, and 1.00% YE; and NaKKlac = 1.00% NaCl, 0.80% KCl, and 0.20% K-lactate), two temperatures (4 and 10 °C), four sampling intervals (days 0, 3, 6, and 9) and two replicates per treatment group per sampling interval. All six treatment groups were only spiked with one bacterial strain at a time and incubation of the spiked samples took place at one temperature at a time.

6.2.3. Sourcing of lean meat, backfat, additives and spices

Sourcing of all lean meat, pork backfat, additives and spices was conducted as described in Chapter 4, p. 66, 70−71 of this thesis.

6.2.4. Formulation of the banger batters

Two distinct groups of banger formulations were used based on whether NaCl content was reduced or replaced. The banger batters based on the reduction of NaCl were formulated following the formulations described in Chapter 4 on pp. 70−71. The bangers based on the partial replacement of NaCl were formulated following the formulations described in Chapter 5 on pp. 143−144. Larger batches of the formulations were needed for sample preparation therefore all the formulations were scaled-up from 2.50 kg to 4.00 kg with all of the formulation components used in identical proportions as in Table 4.3 in Chapter 4 and Table 5.2 in Chapter 5.

6.2.5. Manufacturing of the banger batters

Two separate replicates were manufactured at least two months apart for the NaCl reduction batters consisting of four formulations, and two separate replicates were manufactured at least two months apart for the NaCl replacement batters consisting of six formulations. This was done to negate variations in raw materials and processing and environmental conditions. The manufacturing of the batters proceeded as described in Chapter 4 on p. 73 up to where casings were to be filled. For this part of the study, the batters were not filled into natural hog casings but rather weighed off aseptically as 99 g portions that were placed in sterile 207 mL WhirlPak™ bags (Lasec, Bloemfontein, South Africa). There were multiple reasons for following this approach. It would have been very difficult to add the one of the two bacterial strains at a specific cell concentration in a known volume to a specific mass of sausage batter. This should then have been attempted for the

whole batter batch meaning that the entire 4.00 kg batch should then have been mixed very well for a completely homogenous distribution of the bacterial strain. The main concern was introducing these two bacterial strains into the meat processing facility where meat products are manufactured that are often consumed by people. It was, therefore, decided to take the finished batters to a microbiology lab where the bacteria could be added at a later stage. After batter preparation, a 99 g portion of batter was pushed into a flat slab of ~ 60 mm diameter at the bottom of the bag, the bag was flattened and all air was pressed out. The bag was then rolled up, the wired tabs folded inwards and secured with a strip of masking tape (18 mm diameter) and the bag was marked with the treatment group name.

6.2.6. Sample preparation

For a single replicate of the NaCl reduction part of the study, 128 of the 99 g quantities of batter was weighed off and kept frozen at -18 °C until needed. Similarly, for a single replicate of the NaCl replacement part of the study, 192 of the 99 g quantities of batter was weighed off and kept frozen at -18 °C until needed. An additional 100 g of each batter was collected aseptically and stored at 4 °C for same-day microbial load determination. For a given round of spiking, eight samples per treatment group, thus 32 samples in total, were used for the NaCl reduction formulations. For the spiking of the NaCl replacement formulations, eight samples per treatment group, thus 48 samples in total were used.

6.2.7. Microbial load analyses

Ten gram samples were collected in duplicate from the 100 g batter previously collected from each treatment group. Before sampling, a batter sample was mixed again by hand according to a standardized method described in the following subsection. This hand mixing method was developed since the sticky and firm texture of the batters made mixing by the previously used mechanical method impractical and inconsistent. Further microbial analyses procedures were consistent with those described in Chapter 4 on p. 78 with the addition of a procedure for the detection and enumeration of LAB. For this, a further 1 mL of each dilution was pour plated using MRS agar consisting of De Man, Rogosa & Sharpe broth (MRS broth; CM0359, Oxoid) with 1.50% w/v agar bacteriological (Agar No. 1, LP0011; Oxoid) for the enumeration of LAB. The MRS plates were then incubated at 32 °C for 48 h (Harrigan, 1998).

6.2.8. Hand mixing procedure

The homogenization of 10 g samples with phosphate buffer or nutrient broth proceeded using the mechanical method as described in Chapter 4 on p. 77. All the 99 g quantities of batter were hand mixed using the following procedure and as illustrated in Fig. 6.1. Before mixing, the masking tape securing the wired tabs was removed and the entire length of the bag was rolled open. On the flat bench top, with one open hand firmly placed on top of the empty part of the bag (iv), the index, middle and ring fingers of the other hand was placed on top of the bag and used to flatten and spread the sample (i) in a circular fashion for precisely one minute. After the first thirty seconds and after one minute, the sample was firmly pushed toward the bottom of the bag (i) from where it had invariably spread to a larger area (iii). The sample was not allowed to spread to a larger area (iv) of the bag as this would have limited how much sample could be mixed at a given moment.

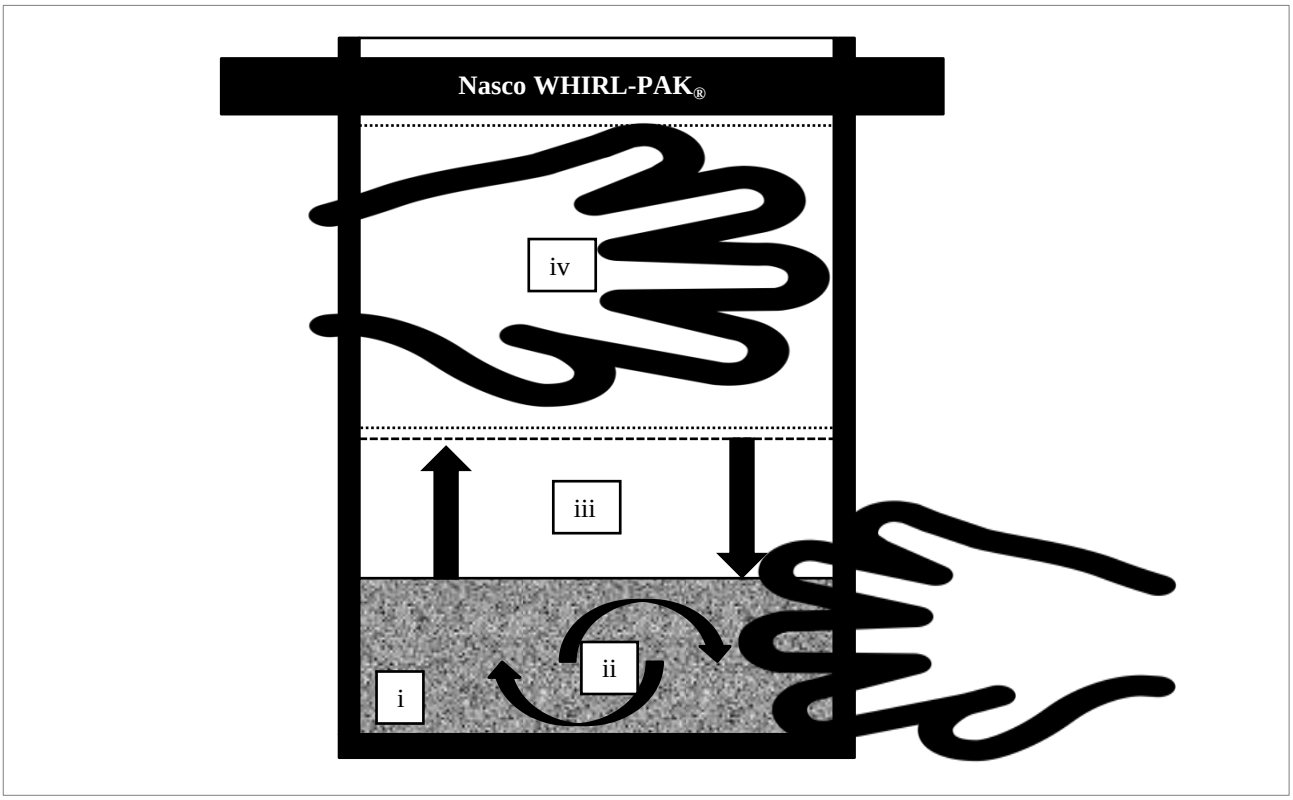

Figure 6.1. Illustration of the hand mixing procedure used to overcome the difficulty in homogenising the 99 g batter samples.

6.2.9. Preparation of bacterial inocula

The initial inoculation level chosen for both bacterial strains was ± 3.5 log cfu/g of a final 100 g batter sample consisting of 99 g sausage batter and 1 mL of a ± 5-6 log inoculum. This inoculation level was theoretically high enough to determine any immediate minimum bactericidal concentration (MBC) effect on day 0 or minimum inhibitory concentration (MIC) effect from day 0 onwards. It was also low enough to allow for a theoretical increase in inoculum strain numbers over

time without bacterial cells from the inoculum immediately facing competition from microorganisms inherent to the batter.

6.2.9.1 Escherichia coli

The *E. coli* (ATCC® 25922™) previously maintained on a nutrient agar slant (CM0003; Oxoid) at 4 °C was streaked out on pre-poured agar plates of Brilliance *E. coli*/coliform selective agar (BEC/C; CM1046; Oxoid) and incubated at 37 °C for 24 h. Growth as purple, circular colonies with a diameter of ≤ 5 mm was confirmed by visual evaluation only as no further confirmation is needed (Wohlsen, 2001). A loopful of a single representative colony was suspended in 10 mL MacConkey (purple) broth (CM505; Oxoid) and incubated at 37 °C for 18 h. Change in colour from dark purple to turbid yellow confirmed growth. A 3 mL aliquot of the broth was then added to 300 mL MacConkey broth in a pre-sterilised 500 mL shake flask and incubated in a shaking incubator (New Brunswick Scientific, Edison, USA) at 120 rpm at 37 °C for 18 h. Four 25 mL volumes of the broth were poured into four 40 mL centrifuge tubes (Beckman Coulter, Johannesburg, South Africa), and centrifuged at 3000 rpm (1089 x g) for 10 min at 4 °C using a Avanti J-26 XPI centrifuge with fixed-angle rotor head JA25-50 (Beckman Coulter, Johannesburg, South Africa). The supernatant was decanted from each tube and each pellet was re-suspended in 20 mL of 0.10% buffered peptone water (BPW; CM0509 Oxoid).

After another round of centrifugation the pellets were re-suspended in 20 mL of 0.10% BPW. This was done to remove any remaining purple pigments that could influence the transmittance measurements. The cell suspensions were then adjusted to 45% transmittance using McFarland Standards (barium chloride suspensions) with a turbidimeter (Biolog, Anatech Instruments, Johannesburg, South Africa). A blank reference test tube was used to adjust the turbidimeter to 100% transmittance before cell suspensions were adjusted. For each of two successive suspensions, a clean sterile test tube with 20 mm by 150 mm dimensions was filled with 20 mL 0.10% BPW to which the concentrated cell suspension was added drop wise. The test tube was vortexed after each addition and allowed to stand for 30 seconds to allow any air bubbles to rise to the surface. The two cell suspensions were then pooled into a sterile 250 mL Schott bottle for a final volume of ~ 45 mL. Serial dilutions were prepared in duplicate up to 10^{-7} using 0.10% BPW and 1 mL of each was plated out on BEC/C agar plates. The plates were incubated at 37 °C for 24 h and colonies were counted. Average counts were reported as ≥ 8 log cfu/mL. Further three-fold dilutions to 5-6 log cfu/mL were prepared to reach a final spiking concentration of ~ 3.5 log cfu/g in the 99 g batter samples after 1 mL was added to the samples. The final cell suspension was plated out in duplicate

the day before spiking and on the day of spiking to confirm that the cell suspensions were at the correct final dilutions.

6.2.9.2. Staphylococcus aureus

The *S. aureus* (ATCC® 6538™) maintained on a nutrient agar slant (CM0003; Oxoid) at 4 °C was streaked out on pre-poured agar plates of BP and then incubated as described in Chapter 4 on p. 77. Positive growth as shiny, black, convex colonies with a diameter of 1-5 mm with a thin white edge and surrounded by a clear zone in the normally turbid media was confirmed with the Pastorex™ Staph-Plus latex agglutination test (Bio-Rad, Johannesburg, South Africa). A loopful of a single representative colony was suspended in 10 mL BHI broth (CM1135, Oxoid) and incubated at 37 °C for 18 h. Growth was confirmed with an increase in turbidity of the broth. A 3 mL aliquot of the broth was then added to 300 mL BHI broth in a pre-sterilised 500 mL shake flask and incubated in a shaking incubator at 120 rpm at 37 °C for 18 h. Four 25 mL volumes of the broth were poured into four 40 mL centrifuge tubes, and centrifuged at 3000 rpm (1089 x g) for 10 min at 4 °C. The supernatant was decanted from each tube and the pellets were re-suspended in 20 mL of 0.10% BPW. The same procedures as for the *E. coli* were then followed.

6.2.10. Inoculation of the batters

Twenty four hours before spiking of the batters were carried out, eight previously frozen batter samples per treatment group were defrosted at 4 °C and allowed to adjust to either 4 °C or 10 °C. A Whirl-Pak™ bag was rolled off all the way to the bottom and 1 mL of the final cell suspension was added to the batter. The bag was then flattened to remove all the air, closed by folding over the bag opening four times and the batter and cell suspension was mixed using the hand mixing procedure and stored momentarily at 4 °C until all eight batter samples of one treatment group were spiked and mixed. The day 0 samples were analysed within 30 minutes of inoculation. The samples for days 3, 6, and 9 were closed as described under section 6.2.5 and placed in a low temperature incubator (Labcon; Vacuum Technologies, Johannesburg, South Africa) at the following temperatures: *E. coli* at 4°C, *S. aureus* at 4 °C, *E. coli* at 10°C, and *S. aureus* at 10 °C. Only one bacterial strain was spiked and incubated at a time. Ten grams of a sample was then aseptically transferred to a 100 mL Whirl-Pak™ bag, 90 mL of 0.10% BPW was added and the sample was mechanically homogenised as described in Chapter 4 on p. 77. A dilution series up to 10^{-3} was prepared using 9 mL 0.10% BPW and 1 mL was spread-plated in duplicate on BEC/C plates in the case of the *E. coli* strain and 1 mL divided into 0.50 mL volumes were spread-plated in duplicate on BP plates in the case of the *S. aureus* strain. The 1 mL samples were divided into smaller volumes

due to the BP plates not absorbing all of the liquid, resulting in smudging and spreading of the *S. aureus* colonies making enumeration impractical. All plates were incubated at 37 °C for 24 h after which enumeration was performed using a manual colony counter.

6.2.11. Statistical analyses

A one-way ANOVA procedure (NCSS, 2007) was used to determine the effect of different added NaCl and/or replacer levels on the initial bacterial load parameters of the batters before inoculation. A two-way ANOVA procedure (NCSS, 2007) was used to determine the effect of different added NaCl and/or replacer levels and storage temperature on the growth and survival of the two bacterial reference strains. Differences were considered statistically significant at the $P < 0.05$ level or lower. Treatment means were then compared by means of the Tukey-Kramer multiple comparison test at $\alpha = 0.05$.

6.3. Results and discussion

Escherichia coli and *S. aureus* are transient and resident bacteria, respectively, on the hands and are associated with poor hygiene practices leading to cross-contamination of food stuffs (Taulo, Wetlesen, Abrahamsen, Narvhus, & Mkakosya, 2009).

6.3.1. Salt reduction

6.3.1.1. Microbial load analysis of pre-inoculated batters

The different added NaCl levels had no significant effect on the TVC of the four treatments after manufacturing (Table 6.1). The TVC of the LI, HI, and PC groups were however slightly higher than that of the NC group, similar to the day 0 TVC results in Chapter 4 in Table 4.25 on p. 128. This may be construed as a very limited bactericidal effect at added NaCl levels of 1.00% (w/w) and higher. Generally, the TVC of these four batters were substantially lower than that of the bangers in Chapter 4. This could be attributed to raw material variation e.g., meat from different animals and different seasons as well as the fact that the batters in Chapter 4 were handled to a greater extent. This included filling the batters into hog casings that possibly contained inherent microbes and this took a greater amount of time, possibly allowing some microbial growth as the batter temperatures increased to temperatures above 4°C for short time periods.

Coliform counts were also much lower than those reported in Chapter 4 and in contrast, added NaCl levels had no significant effect. No *E. coli* or *S. aureus* colonies were detected in any of the four batters of the two replicates. No significant differences in LAB counts were found and these counts

were very low. The competitive potential of the LAB cannot be underestimated as they are psychrotrophic and micro-aerophilic and resistant to both NaCl and nitrite (Egan, 1983; Dodds & Collins-Thompson, 1984; Franz et al., 1991). Furthermore, there were no significant differences between treatment groups for yeast or mould counts as reported in Chapter 4.

Table 6.1. The results of the microbial load analyses performed on four banger batter formulations with varying amounts of added NaCl before inoculation with either one of two reference strains of bacteria (n = 8).

Treatment	**NC**	**LI**	**HI**	**PC**	**Sign. level**
TVC (log cfu/g)	3.72 ± 0.28	3.27 ± 0.29	3.35 ± 0.33	3.33 ± 0.45	NS
Coliforms (log cfu/g)	0.88 ± 0.37	0.60 ± 0.58	0.97 ± 0.48	0.84 ± 0.68	NS
E. coli **(log cfu/g)**	ND	ND	ND	ND	NSA
S. aureus **(log cfu/g)**	ND	ND	ND	ND	NSA
LAB (log cfu/g)	0.24 ± 0.25	0.19 ± 0.22	0.23 ± 0.33	0.36 ± 0.39	NS
Yeasts (log cfu/g)	2.35 ± 0.58	2.28 ± 0.59	2.00 ± 0.51	2.37 ± 0.44	NS
Moulds (log cfu/g)	2.08 ± 0.33	2.12 ± 0.31	2.15 ± 0.22	2.32 ± 0.22	NS

NC = 0% added NaCl; LI = 1.00% added NaCl; HI = 1.50% added NaCl; and PC = 2.00% added NaCl
TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; LAB = lactic acid bacteria
ND = Not detected
NS = Not significant
NSA = Not statistically analysed

In terms of the TVC limit of < 6 log cfu/g for a non-heat treated meat product (SANS 885, 2011), the batters were deemed suitable for inoculation with either one of the reference strains without the inherent microorganisms causing any immediate competition. Freezing, storage at -18 °C and slow thawing at 4 °C was also expected to somewhat decrease mainly bacterial numbers. Generally not regarded as an important decontamination technique, freezing leads to ice crystal growth inside and outside the cell that may damage cell integrity resulting in leakage of the intracellular solutes. Freezing also concentrates extracellular solutes around the cell possibly leading to further damage. After thawing, the greatest number of cells will eventually recover (Hagen, 1971). It was expected that the inherent bacteria would initially be at a disadvantage versus the freshly grown reference strains, providing a further possible buffer against competition from these organisms.

6.3.1.2. Main effects and interactions for E. coli in inoculated batters

Table 6.2 indicates that NaCl level, storage time and storage temperature as main effects had no effect on the growth or survivability of the inoculated *E. coli* strain. The interactions between main effects of: NaCl level with storage time, NaCl level with storage temperature, storage time with storage temperature, and NaCl level with storage time with storage temperature also had no significant effects on the growth or survivability of the inoculated *E. coli* strain.

Table 6.2. Analysis of variance (ANOVA) of the effects of NaCl level, storage time and storage temperature on the survival of *E. coli* in pork banger batters.

NaCl level	Storage time	Storage temperature	NaCl level X Storage time	NaCl level X Storage temperature	Storage time X Storage temperature	NaCl level X Storage time X Storage temperature
NS	NS	NS	NS	NS	NS	NS

NS = Not significant

6.3.1.3. The growth and survival of E. coli in inoculated batters

On day 0, the *E. coli* counts at different added NaCl levels at 4 °C and 10 °C varied very little from a minimum of 3.43 ± 0.64 log cfu/g at 10 °C for the LI group to a maximum of 3.65 ± 0.46 log cfu/g at 4 °C for the NC group (Table 6.3). This may be attributed to a low metabolic activity level of the inoculum that was stored in 0.10% BPW overnight at 4 °C before being added to the batters, also preventing any cold or osmotic shock responses that could have negatively affected cell viability, leading to reductions in counts. The time lapse between inoculation and further microbial analysis where a 10 g sample was suspended in 0.10 % BPW was also limited to within 30 minutes thereby ensuring that any other possible bactericidal effects were limited.

Table 6.3. The effect of the interaction between added NaCl level and storage temperature on the growth and survival of *E. coli* in pork banger batters (n = 8).

Treatment	Temperature (°C)	Day 0	Day 3	Day 6	Day 9
NC	**4**	3.65 ± 0.46	3.59 ± 0.63	3.57 ± 0.60	3.56 ± 0.52
LI		3.65 ± 0.53	3.54 ± 0.55	3.55 ± 0.57	3.52 ± 0.57
HI		3.52 ± 0.45	3.58 ± 0.52	3.55 ± 0.56	3.51 ± 0.58
PC		3.58 ± 0.46	3.54 ± 0.45	3.31 ± 0.43	3.48 ± 0.61
NC	**10**	3.65 ± 0.51	3.66 ± 0.48	3.61 ± 0.47	3.63 ± 0.40
LI		3.43 ± 0.64	3.67 ± 0.60	3.52 ± 0.51	3.53 ± 0.51
HI		3.60 ± 0.53	3.64 ± 0.54	3.54 ± 0.50	3.57 ± 0.55
PC		3.62 ± 0.43	3.55 ± 0.47	3.45 ± 0.54	3.47 ± 0.46
Significance level		NS	NS	NS	NS

NC = 0% added NaCl; LI = 1.00% added NaCl; HI = 1.50% added NaCl; and PC = 2.00% added NaCl
Means with different superscripts in the same row differ significantly
NS = Not significant

After day 3, the counts were consistent with that of day 0 and added NaCl level at any temperature had no effect. Similar results without significant effects were observed after day 6 and day 9. In agreement with these results, it has been reported that bacteria such as *E. coli* can enter a stationary phase with significant physiological changes that allows the cell to survive, amongst others, high NaCl levels (Hengge-Aronis, 1996; Abee & Wouters, 1999). With no increase in counts, that would have been indicative of growth, or decrease in counts, that would have been indicative of decreased survival rates, the treatments had a bacteriostatic effect. Microorganisms undergo physiological and morphological changes at sub-optimum temperatures which may include shortening of fatty acid chain lengths and increasing the ratio of unsaturation of cell membrane lipids (Russell et al., 1995).

The cold shock response was initially discovered in *E. coli* and found to lead to the activation of cold shock proteins by induction of the *cspA* gene, allowing protein synthesis to continue although at a reduced rate (Jones, Vanbogelen, & Neidhart, 1987).

Enteropathogenic (EPEC) and verocytotoxigenic (VTEC) strains of *E. coli* are also capable of growth at a temperature range of 5−10 °C (Gould, 1998). Both classes have been isolated from commercial beef mince and boerewors (Charimba, Hugo, & Hugo, 2012). *Escherichia coli* O157:H7 at a similar inoculation level of 3.5 log cfu/g, had significantly lower survivability at 4 °C and 10 °C in boerewors when 450 mg/kg sodium metabisulphite was used in conjunction with 1.26% added NaCl than when sodium metabisulphite was omitted (Charimba, Hugo, & Hugo, 2010). Two of the banger batters in this study contained sodium metabisulphite at only 166 mg/kg with added NaCl levels upwards of 1.26% (HI and PC). It is possible that preservatives such as sodium metabisulphite play a more important role against *E. coli* than NaCl, even though it is known to cause allergic reactions in sensitive individuals (Taylor, Higle, & Bush, 1986).

6.3.1.4. Main effects and interactions for S. aureus in inoculated batters

Table 6.4 indicates that NaCl level ($P < 0.001$), storage time ($P < 0.001$) and storage temperature ($P < 0.001$) as main effects had significant effects on the growth or survivability of the inoculated *S. aureus* strain. Of all the possible interactions between the main effects, the interaction of NaCl level with storage temperature had the only significant ($P < 0.05$) effect on the growth or survivability of the inoculated *S. aureus* strain.

Table 6.4. Analysis of variance (ANOVA) of the effects of NaCl level, storage time and storage temperature on the survival of *S. aureus* in pork banger batters.

NaCl level	Storage time	Storage temperature	NaCl level X Storage time	NaCl level X Storage temperature	Storage time X Storage temperature	NaCl level X Storage time X Storage temperature
$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$	NS	NS

NS = Not significant

6.3.1.5. The growth and survival of S. aureus in inoculated batters

On day 0, there was greater variation in counts between treatment groups at 10 °C than at 4 °C with a minimum of 3.68 ± 0.28 log cfu/g for the PC group and a maximum of 4.20 ± 0.09 log cfu/g for the NC group (Table 6.5). Regardless, neither storage temperature nor added NaCl level had any significant effect on day 0. The counts were relatively stable after day 3 with no significant differences between any treatment groups at any storage temperature. After day 6, the counts of the

NC group at 10 °C was higher ($P < 0.05$) than that of the HI group at 4 °C and that of the PC group at 10 °C (Table 6.5 and Figure 6.2). After day 9, the counts of the NC group at 4 °C was still the highest and higher ($P < 0.001$) than that of the HI group at 10 °C and that of the PC group at 4 °C (Table 6.5 and Figure 6.2). As seen from the significance levels of the main effects in Table 6.4, storage time also affected ($P < 0.001$) the *S. aureus* counts. This was reflected in the general decrease in counts from day 0 up to day 9.

Table 6.5. The effect of the interaction between added NaCl level and storage temperature on the growth and survival of *S. aureus* in pork banger batters ($n = 8$).

Treatment	Temperature (°C)	Day 0	Day 3	Day 6	Day 9
NC	**4**	3.78 ± 0.36	3.80 ± 0.51	3.44^{ab} ± 0.69	3.51^{ab} ± 0.60
LI		3.72 ± 0.49	3.61 ± 0.51	3.47^{ab} ± 0.65	3.25^{ab} ± 0.73
HI		3.75 ± 0.41	3.52 ± 0.47	3.33^{a} ± 0.63	3.25^{ab} ± 0.69
PC		3.74 ± 0.48	3.47 ± 0.68	3.55^{ab} ± 0.47	2.81^{a} ± 0.65
NC	**10**	4.20 ± 0.09	4.18 ± 0.58	4.06^{b} ± 0.13	3.98^{b} ± 0.39
LI		4.15 ± 0.11	3.94 ± 0.24	3.81^{ab} ± 0.26	3.52^{ab} ± 0.45
HI		3.74 ± 0.31	3.61 ± 0.27	3.33^{ab} ± 0.24	2.97^{a} ± 0.21
PC		3.68 ± 0.28	3.69 ± 0.43	3.32^{a} ± 0.30	3.09^{ab} ± 0.15
Significance level		NS	NS	$P < 0.05$	$P < 0.001$

NC = 0% added NaCl; LI = 1.00% added NaCl; HI = 1.50% added NaCl; and PC = 2.00% added NaCl
Means with different superscripts in the same row differ significantly
NS = Not significant

The results indicate that the inoculated *S. aureus* strain was unable to grow under these parameters and survival decreased over time regardless of added NaCl level or storage temperature. The decreases in counts were, however, more limited at 0.00% added NaCl as well as at 1.00% added NaCl with limited increased growth found for these two groups after day 6 and day 9 (Figure 6.2). The limit for *S. aureus* in a non-heat treated meat product is < 20 cfu/g due to the associated enterotoxin risk (SANS 885, 2011). A high contamination level as applied to these batters is unlikely to be found in real products, although it was shown that at a level of ~ 3.5 log cfu/g, the effect of even 2.00% added NaCl and a low storage temperature of 4 °C did not decrease the overall risk of survival of this bacterial species. An advantageous effect of lower cell concentration is that staphylococcal intoxication occurs at an enterotoxin dose of ≥ 1.00 µg, usually only reached when the cell numbers exceeds 5 log cfu/g (Notermans & Heuvelman, 1983; USDA, 2014). A temperature range of 7 °C to 48.5 °C with an optimum range of 30 °C to 37 °C (Schmitt, Schuler-Schmid, & Schmidt-Lorenz, 1990), a pH range of 4.0 to 10.0 with an optimum of 6.0 to 7.0 (Table 4.22 in Chapter 4 p. 122 the average was from 5.62 to 5.98) and NaCl concentration of up to 7.50% in food (USDA, 2014) allows the growth of *S. aureus*. In this study, survival was most limited at 4 °C and 10 °C at 1.50% and 2.00% added NaCl.

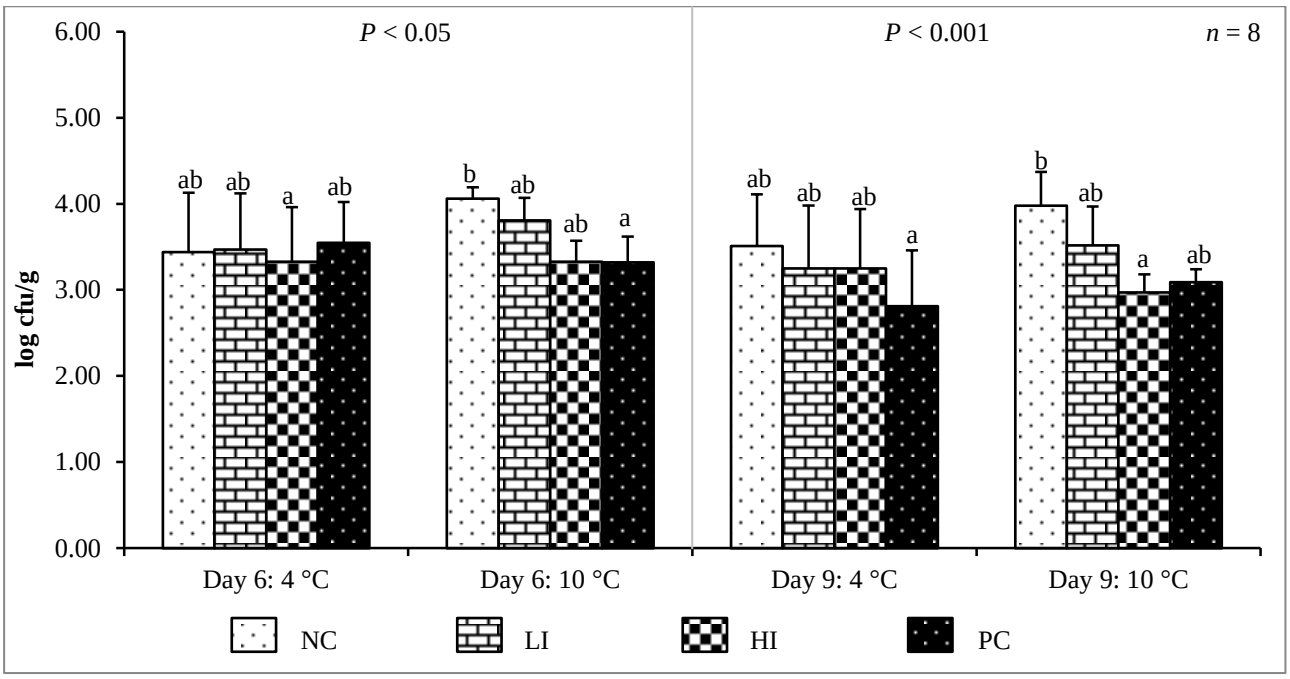

Figure 6.2. The effect of the interaction between added NaCl level and storage temperature on the growth and survival of *S. aureus* on days 6 and 9.
NC = 0% added NaCl; LI = 1.00% added NaCl; HI = 1.50% added NaCl; and PC = 2.00% added NaCl
Means with different superscripts on the same day differ significantly

6.3.2. Salt replacement

6.3.2.1. Microbial load analyses of pre-inoculated batters

Different added NaCl and/or replacer levels had a significant effect on the TVC of the six formulations before inoculation with either one of the two reference strains (Table 6.6). The TVC of the PC group was lower ($P < 0.001$) than those of the other five formulations. This indicated a bactericidal effect at 2% NaCl (PC), either directly by the added NaCl level or indirectly by decreased survivability during the fast transitions between media. The TVC of the NaKYE and NaKKlac groups were slightly higher than that of the baseline added NaCl and other NaCl replacer groups. This was similar to the day 0 TVC of the NaKYE group in Chapter 5 on p. 161. The lower NaCl level and replacer combination possibly allowed for greater survival during the relatively fast environmental changes from the 0.10% BPW to the sausage batter back to the 0.10% BPW used for serial dilutions and finally the media. Generally, the TVC counts were higher than that of the previous batters (Table 6.1) and closer to the TVC of the batter in Chapter 5 on p. 161. This was again attributed to raw material variation of mainly the meat and backfat. No immediate competition was expected from the inherent microorganisms.

No significant differences were found between coliform counts and these counts were substantially higher than of the batters in Table 6.1, although still below the limit of 2 log cfu/g for this product class (SANS 885, 2011). Low levels of *E. coli* were observed in all six formulations of up to 1.02 ±

Table 6.6. The results of the microbial load analyses performed on six banger batter formulations with different added NaCl and/or replacer combinations before inoculation with either one of two reference strains (n = 8).

Treatment	BL	PC	NaK	NaKGlu	NaKYE	NaKKlac	Sign. level
TVC (log cfu/g)	$4.04^{b} \pm 0.13$	$3.78^{a} \pm 0.19$	$4.05^{b} \pm 0.20$	4.04^{b} 0.07	$4.11^{b} \pm 0.17$	$4.15^{b} \pm 0.18$	$P < 0.001$
Coliforms (log cfu/g)	2.95 ± 0.28	2.72 ± 0.11	2.81 ± 0.26	2.90 ± 0.28	2.82 ± 0.29	2.81 ± 0.06	NS
***E. coli* (log cfu/g)**	0.83 ± 0.89	0.70 ± 0.81	0.69 ± 0.80	1.02 ± 1.13	0.68 ± 0.77	0.56 ± 0.62	NS
***S. aureus* (log cfu/g)**	ND	ND	ND	ND	ND	ND	NSA
LAB (log cfu/g)	1.62 ± 1.52	1.24 ± 1.35	1.60 ± 1.71	2.25 ± 0.97	2.27 ± 0.98	2.73 ± 0.56	NS
Yeasts (log cfu/g)	3.46 ± 0.14	3.36 ± 0.23	3.52 ± 0.23	3.42 ± 0.11	3.29 ± 0.06	3.46 ± 0.26	NS
Moulds (log cfu/g)	2.47 ± 0.52	2.46 ± 0.46	2.52 ± 0.53	2.44 ± 0.49	2.40 ± 0.45	2.50 ± 0.48	NS

BL = 1% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl + 1.00% KCl; NaKGlu = 1.00% NaCl + 1.00% K-gluconate; NaKYE = 0.80% NaCl 0.80% KCl + 1% YE; NaKKlac = 1.00% NaCl + 0.80% KCl + 0.20% K-lactate
TVC = total viable count; *E. coli* = *Escherichia coli*; *S. aureus* = *Staphylococcus aureus*; LAB = lactic acid bacteria
Means with different superscripts in the same column and on the same day differ significantly
ND = Not detected
NSA = Not statistically analysed
NS = Not significant

1.13 cfu/g as for the NaKGlu group. These levels were regarded to be too low to significantly affect the *E. coli* counts after inoculation with 3.5 log cfu/g of the *E. coli* reference strain.

No significant differences in LAB counts were observed and these counts were substantially higher than those of the previous batters (Table 6.1). The LAB counts of the BL, PC, and NaK groups were lower than that of the NaKGlu, NaKYE, and NaKKlac groups. Incidentally the LAB counts of the NaKKlac group which contained K-lactate were the highest. This was attributed to lactic acid being a metabolic product of LAB and these microorganisms being tolerant of its presence. No significant effects on yeast or mould counts were observed. Of all the microbial parameters, the results of the yeast and mould counts had the lowest variation between treatment groups. *Staphylococcus aureus* was not detected in any of the six batters.

6.3.2.2. Main effects and interactions for E. coli in inoculated batters

Table 6.7 indicates that NaCl and/or replacer level, storage time and storage temperature as main effects had no effect on the growth or survivability of the added *E. coli* strain. The interactions between main effects of: NaCl and/or replacer level with storage time, NaCl and/or replacer level with storage temperature, storage time with storage temperature, and NaCl and/or replacer level with storage time with storage temperature also had no significant effects on the growth of survivability of the inoculated *E. coli* strain.

Table 6.7. Analysis of variance (ANOVA) of the effects of NaCl and/or replacer combination, storage time and storage temperature on the survival of *E. coli* in pork banger batters.

NaCl / replacer level	**Storage time**	**Storage temperature**	**NaCl / replacer level X Storage time**	**NaCl / replacer level X Storage temperature**	**Storage time X Storage temperature**	**NaCl / replacer level X Storage time X Storage temperature**
NS	NS	NS	NS	NS	NS	NS

NS = Not significant

6.3.2.3. The growth and survival of E. coli in inoculated batters

The *E. coli* counts on day 0 varied very little with no significant differences between the six treatment groups (Table 6.8). The counts were all higher than the ~ 3.5 log cfu/g concentration they were inoculated into the batters and were also higher than the levels found in Table 6.3. This result was attributed to the level of *E. coli* already present in the batters contributing to an overall higher cell concentration after inoculation. No significant differences were found between those levels, therefore they had no effect on the *E. coli* levels. Again the short contact period between the added

E. coli strain and the various added NaCl and/or replacer levels and storage temperature appeared to have no significant effects. This was again attributed to the cold shock adaptability of *E. coli* and the low metabolic activity of the cells limiting metabolic processes.

No significant differences in *E. coli* counts were observed on days 3, 6 or 9. At most, added NaCl and/or replacer level, storage temperature and storage temperature and storage time had a bacteriostatic effect on the inoculated *E. coli* strain. The results of this part of the study support the evidence that the *E. coli* cell enter a stationary growth phase with significant physiological changes that allows the cell to survive osmotic and temperature conditions outside the optimal ranges (Jones et al., 1987; Hengge-Aronis, 1996; Abee & Wouters, 1999). *Escherichia coli* also exhibit "cross-stress protection" whereby application of one type of stressor, such as osmotic stress, may offer protection from another stressor, such as sub-optimal low temperatures

Table 6.8. The effect of the interaction between added NaCl and/or replacer combination and storage temperature on the growth and survival of *E. coli* in pork banger batters (n = 8).

Treatment	Temperature (°C)	Day 0	Day 3	Day 6	Day 9
BL	**4**	3.81 ± 0.58	3.72 ± 0.60	3.64 ± 0.58	3.63 ± 0.55
PC		3.68 ± 0.52	3.71 ± 0.55	3.59 ± 0.65	3.55 ± 0.49
NaK		3.78 ± 0.57	3.67 ± 0.52	3.61 ± 0.47	3.63 ± 0.51
NakGlu		3.76 ± 0.57	3.75 ± 0.56	3.66 ± 0.54	3.76 ± 0.52
NaKYE		3.81 ± 0.53	3.67 ± 0.52	3.64 ± 0.51	3.63 ± 0.53
NaKKlac		3.75 ± 0.58	3.68 ± 0.50	3.62 ± 0.58	3.68 ± 0.53
BL	**10**	3.75 ± 0.54	3.70 ± 0.48	3.78 ± 0.51	3.65 ± 0.47
PC		3.70 ± 0.53	3.61 ± 0.45	3.58 ± 0.43	3.57 ± 0.43
NaK		3.80 ± 0.50	3.67 ± 0.49	3.75 ± 0.49	3.79 ± 0.45
NaKGlu		3.76 ± 0.48	3.72 ± 0.52	3.77 ± 0.45	3.72 ± 0.44
NaKYE		3.76 ± 0.50	3.71 ± 0.48	3.78 ± 0.47	3.70 ± 0.43
NaKKlac		3.72 ± 0.52	3.69 ± 0.48	3.78 ± 0.48	3.70 ± 0.45
Significance level		NS	NS	NS	NS

BL = 1% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl + 1.00% KCl; NaKGlu = 1.00% NaCl + 1.00% K-gluconate; NaKYE = 0.80% NaCl + 0.80% KCl + 1% YE; NaKKlac = 1.00% NaCl + 0.80% KCl + 0.20% K-lactate
Means with different superscripts in the same column differ significantly
NS = Not significant

(Campbell et al., 2004). Therefore, even with numerous hurdles in place, *E. coli* in a reduced-NaCl product may have the capability to survive less than ideal conditions. From the results of this and the previous part of the study, it appeared that the *E. coli* strain was not osmotically stressed to the point that survival had been negatively affected. It has been reported that under stress conditions, *E.*

coli is capable of accumulating potassium inside the cell to equilibrate the osmotic pressure (Epstein, 1982; Sutherland, Cairney, Elmore, Booth, & Higgins, 1986). It is possible that potassium present in sufficiently high levels, as with the use of potassium-containing NaCl replacers, may actually improve the survival of *E. coli* under osmotic stress. This places an emphasis on the use of other antimicrobials, such as sodium metabisulphite, that can control *E. coli* and possible other bacteria, regardless of the added NaCl content.

6.*3.2.4. Main effects and interactions for S. aureus in inoculated batters*

Table 6.9 indicates that added NaCl and/or replacer level, storage time and storage temperature as the three main effects, affected ($P < 0.001$) the growth or survivability of the inoculated *S. aureus* strain. The interaction of the added NaCl and/or replacer level with storage temperature had a significant ($P < 0.001$) effect, while interactions of added NaCl and/or replacer level with storage time, and storage time with storage temperature, had no significant effects. The interaction of added NaCl and/or replacer level with storage time with storage temperature, had no significant effect on the growth or survival of the inoculated *S. aureus* strain.

Table 6.9. Analysis of variance (ANOVA) on the effect of NaCl/replacer level, storage time and storage temperature on the survival of *S. aureus* in pork banger batters.

NaCl / replacer level	Storage time	Storage temperature	NaCl / replacer level X Storage time	NaCl / replacer level X Storage temperature	Storage time X Storage temperature	NaCl / replacer level X Storage time X Storage temperature
$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	NS	NS

NS = Not significant

6.3.2.5. The growth and survival of S. aureus in inoculated batters

On day 0, there were no significant differences between the *S. aureus* counts of different treatment groups at two storage temperatures (Table 6.10). Most of the counts were close to the inoculation level of ~ 3.5 log cfu/g except those of the NaKGlu, NaKYE, and NaKKlac groups at 10 °C which were slightly higher at 3.73 ± 0.02 log cfu/g, 3.81 ± 0.04 log cfu/g and 3.74 ± 0.05 log cfu/g. Significant ($P < 0.001$) effects of added NaCl and/or replacer level and storage temperature were observed only after day 3 as illustrated in Figure 6.3. At 4 °C, the PC group had significantly lower counts compared to that of both the NaKGlu and NaKYE treatment groups. At 10 °C, the PC group had significantly lower counts than all but the NaK treatment group. The highest counts were observed for the NaKKlac group which were significantly higher than that of the BL, PC and NaK groups at 10 °C. After day 6, the PC group only had lower ($P < 0.001$) counts than the NaKYE

group at 4 °C and lower ($P < 0.001$) counts than both the NaKYE and NaKKlac groups at 10 °C. In agreement with the PC group, the NaK group also had lower ($P < 0.001$) counts compared to the NaKYE and NaKKlac groups after day 6 at 10 °C. From this it was deduced that, across both storage temperatures up to day 6, the 2% added NaCl of the PC group resulted in consistently lower counts than the NaKGlu and NaKKlac groups, while the BL and NaK groups had similar but more limited effects.

Table 6.10. The effect of the interaction between added NaCl and/or replacer combination and storage temperature on the growth and survival of *S. aureus* in pork banger batters ($n = 8$).

Treatment	Temperature (°C)	Day 0	Day 3	Day 6	Day 9
BL	**4**	3.61 ± 0.03	3.53^{abc} ± 0.05	3.45^{ab} ± 0.03	3.36^{bc} ± 0.05
PC		3.61 ± 0.03	3.42^{ab} ± 0.05	3.42^{a} ± 0.10	3.17^{a} ± 0.01
NaK		3.55 ± 0.07	3.57^{bcd} ± 0.08	3.45^{ab} ± 0.03	3.27^{ab} ± 0.04
NakGlu		3.63 ± 0.05	3.61^{cd} ± 0.09	3.54^{abc} ± 0.04	3.53^{def} ± 0.07
NaKYE		3.57 ± 0.11	3.62^{cd} ± 0.04	3.59^{bcd} ± 0.06	3.44^{cde} ± 0.07
NaKKlac		3.56 ± 0.07	3.55^{abc} ± 0.09	3.54^{abcd} ± 0.07	3.60^{ef} ± 0.10
BL	**10**	3.64 ± 0.05	3.46^{bc} ± 0.05	3.49^{abc} ± 0.06	3.39^{bcd} ± 0.01
PC		3.64 ± 0.05	3.39^{a} ± 0.09	3.42^{a} ± 0.07	3.35^{bc} ± 0.09
NaK		3.69 ± 0.03	3.46^{abc} ± 0.05	3.43^{ab} ± 0.06	3.48^{cdef} ± 0.08
NaKGlu		3.73 ± 0.02	3.60^{cd} ± 0.06	3.54^{abcd} ± 0.06	3.57^{ef} ± 0.03
NaKYE		3.81 ± 0.04	3.63^{cd} ± 0.09	3.64^{cd} ± 0.09	3.62^{f} ± 0.05
NaKKlac		3.74 ± 0.05	3.73^{d} ± 0.08	3.70^{d} ± 0.08	3.62^{f} ± 0.10
Significance level		NS	$P < 0.001$	$P < 0.001$	$P < 0.001$

BL = 1% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl + 1.00% KCl; NaKGlu = 1.00% NaCl + 1.00% K-gluconate; NaKYE = 0.80% NaCl + 0.80% KCl + 1% YE; NaKKlac = 1.00% NaCl + 0.80% KCl + 0.20% K-lactate
Means with different superscripts in the same column differ significantly
NS = Not significant

After day 9, the survival of the *S. aureus* strain was most reduced for the PC group at 4 °C. These counts were lower ($P < 0.001$) than that of the BL, NaKGlu, NaKYE and NaKKlac groups at 4 °C and significantly lower than the counts of all six groups at 10 °C. It was important to note that the most significant difference between the ideal storage temperature of 4 °C and a slightly abusive temperature of 10 °C became apparent with the significantly lower counts of the PC group at 4 °C than that of the PC group at 10 °C. The NaKYE and NaKKlac groups at 10 °C had the highest overall *S. aureus* counts after day 9. Generally the counts of all six treatment groups decreased from day 0 to day 9 (Table 6.10) and where growth was found, it was limited to between two successive sampling intervals. It was concluded that the BL, PC and NaK groups limited the growth and to some extend the survival of the *S. aureus* strain. Limited growth and higher survival rates were observed in the NaKGlu, NaKYE and NaKKlac groups.

As described in Chapter 5 under section 5.3.4 (p. 160), YE serves as a growth promoter to bacteria and may have provided more complex nutrients that aided *S. aureus* in surviving these less than ideal conditions. *Staphylococcus aureus* is a facultative anaerobe capable of producing lactate as

part of its fermentative metabolism under low oxygen conditions (Fuchs, Pané-Farré, Kohler, Hecker, & Engelmann, 2007). By day 9 the batters packaged in tightly closed packaging were expected to have very low levels of oxygen that would be supportive of this type of metabolism. It was therefore probable that the ability of *S. aureus* to produce its own lactate, made the strain used in this study more resistant to the antimicrobial effect of the lactate added as K-lactate in the NaKKlac group. No information was found to be available on the possible antimicrobial effect of K-gluconate on *S. aureus* or bacteria in general. It was suspected that the gluconate in the NaKGlu treatment group may have served as a supplementary carbon source as *S. aureus* has been demonstrated to be capable of making use of gluconate in this regard (Strasters & Winkler, 1963).

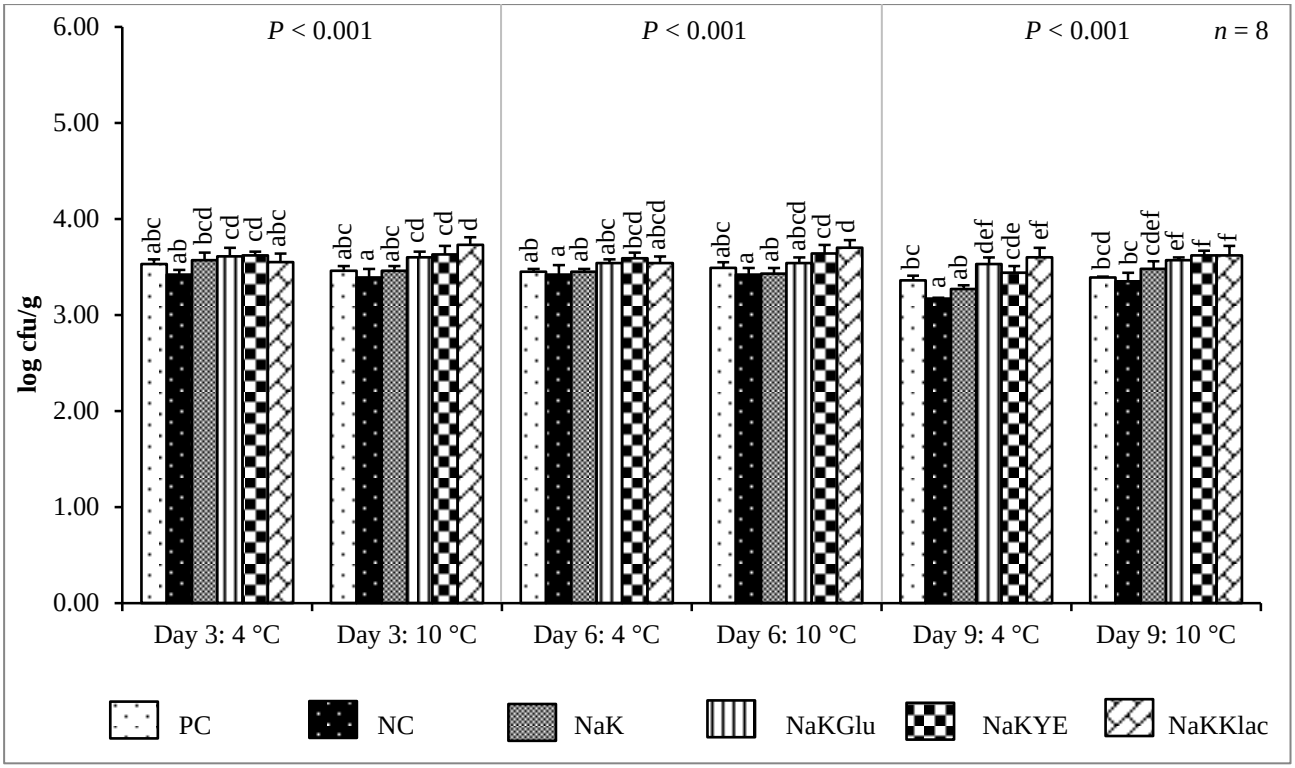

Figure 6.3. The effect of the interaction between added NaCl and/or Na replacer level and storage temperature on the growth and survival of *S. aureus* on days 3, 6 and 9. BL = 1% NaCl; PC = 2.00% NaCl; NaK = 1.00% NaCl + 1.00% KCl; NaKGlu = 1.00% NaCl + 1.00% K-gluconate; NaKYE = 0.80% NaCl + 0.80% KCl + 1% YE; NaKKlac = 1.00% NaCl + 0.80% KCl + 0.20% K-lactate. Means with different superscripts on the same day differ significantly. Error bars represent standard deviations of means.

Similar to the results of the NaCl reduction part of the study (section 6.3.1.5.), neither the control level of 2.00% added NaCl nor any of the replacer combinations could bring about meaningful reductions in *S. aureus* counts. Although the replacer combinations did not contribute to consistent growth to even higher levels, such as the 5 log cfu/g level where enterotoxin production is reported to become a major concern (Notermans & Heuvelman, 1983; USDA, 2014), the counts remained far beyond the acceptable limit of < 20 cfu/g (SANS 885, 2011). Therefore, regardless if added

NaCl levels were reduced or not, the batters could pose a serious health risk after consumption. It was again found that the inoculated *S. aureus* strain had no difficulty surviving an extended period of 9 days at 4 °C which is reportedly outside its survival range of 7 °C to 48.5 °C (Schmitt et al., 1990).

6.4. Conclusions

In this study, the survival and possible growth of *E. coli* and *S. aureus* were monitored in banger batters formulated with either reduced added NaCl levels or NaCl partially replaced with candidate NaCl replacers. In terms of the inoculated *E. coli* strain, salt reduction of 25% and 50% and partial replacement of ~ 50% NaCl on a weight-per-weight basis with potassium chloride, potassium gluconate, yeast extract or potassium lactate, did not allow for an increased contamination risk of a meat product beyond that of the inoculation level. The main risk was that *E. coli* was not susceptible to any of the less than favourable variables and survived at high numbers up to the end of shelf-life. Sub-optimal temperatures, extended storage and the anti-microbial properties of other additives in the formulations, such as sodium metabisulphite, did not have significant effects on survival, although these factors were the main bacteriostatic effects limiting any further growth across all the different formulations. A relatively high level of *E. coli* was inoculated to ascertain if outright cell death would occur while leaving room for growth if the condition were favourable. *Escherichia coli* would in all probability be present at much lower levels in real products and for future research it was suggested that much lower inoculation levels be investigated under similar conditions for effects on growth and survival.

The *S. aureus* strain was affected ($P < 0.001$) by different added NaCl levels, by partial replacement of added NaCl with various replacer combinations, sub-optimal storage temperatures and storage times. Even with reduced NaCl levels of 0.00% and 1.00% added NaCl, *S. aureus* was unable to grow consistently and to sufficiently higher numbers. At 1.50% and 2.00% added NaCl level, survival was lower ($P < 0.001$) at both temperatures from half-way through the shelf-life. The sub-optimal storage temperatures, extended storage at these temperatures and anti-microbial properties of other components in the formulations were concluded to have a synergistic interaction with the added NaCl. It was concluded that reducing the added NaCl level did not allow for an increased contamination risk beyond that of the initial inoculation level although higher added NaCl led to greater reductions over identical storage times and temperatures. Limited growth was observed for some of the replacer groups, the positive control and baseline group at both temperatures, although these growth spurts never lasted beyond two sequential sampling days. It was concluded that, while

none of the replacer combinations led to sustained increases in the risk of *S. aureus* contamination, only the partial replacement of NaCl with KCl similarly reduced the survival rate and only at an ideal storage temperature of 4 °C.

These two vastly different bacterial strains responded differently to identical challenges. Neither *E. coli* as Gram-negative bacterium, nor *S. aureus* as Gram-positive bacterium were well suited for growth under these particular conditions. Therefore, it could not be said that either one had been opportunistic with reduced and/or partially replaced NaCl levels, outside their optimum growth parameters. *Escherichia coli* did have the upper hand over *S. aureus* in being better suited for survival under these conditions. It is important to individually evaluate potential pathogens or spoilers when changes in a control system such as NaCl content are made. The main outcome from the inoculated batters was the same as for any other sufficiently compromised food product. If a contaminating microorganism is present in sufficient numbers, the overall microbial safety of the product will be compromised, regardless of what antimicrobial effect is expected by the sum of various components in the formulation. The NaCl reduction or/partial replacement strategies applied in these scenarios did not sufficiently contribute to greater overall risk.

CHAPTER 7

GENERAL DISCUSSION & CONCLUSIONS

After sugar, salt is regarded as being the second most used food additive in food processing (Seligsohn, 1981). Technologically, it is critical in manipulating and controlling the texture of processed meat products by activating the meat proteins, leading to greater water binding (Hamm, 1961). The result of which is increased tenderness of the meat and decreased fluid loss after heat cooking (Man, 2007) which is of great importance with regard to whole muscle meat products such as bacon. Another important function of salt is the direct effect it has on meat product texture. When the myofibrillar proteins are solubilised by salt, a sticky exudate on the surface of meat pieces is formed which binds together these pieces during cooking. This is the result of the formation of a matrix of heat-coagulated protein formed around the meat pieces that also entraps free water and fat globules, allowing for the existence of unique meat products such as frankfurters and bologna (Monahan & Troy, 1997). This opens up a whole new area for the utilisation of underused or less than ideal cuts of meat. Greater utilization of these meat cuts in combination with plant protein has positive implications in maximising the yield of marketable products (Nielsen, Petersen, & Møller, 1995) which contributes to the wider availability of animal protein to many more people. In South Africa, a product such as polony is the highest volume processed meat product across retail and food service sectors with per annum sales for year end August 2015 in the region of 107 000 tonnes (Andrew Cocks, SAMPA President, personal communication, October 2015).

It is well known that salt is the main source of sodium in the human diet (Ruusunen & Puolanne, 2005) and that humans illicit great hedonic pleasure from its taste (Leshem, 2009; Mattes, 1997). This directly leads to greater consumption of salty foods (Shultz, 1957; Tuorila et al., 2008) and with sustained greater consumption, greater experience of the salty taste is needed to reach the same level of hedonic response (Pangborn & Pecore, 1982). Unfortunately, this unrelenting application of, and need for, salt has led to it having serious negative effects on human health. It has long been known that constant high sodium intake leads to sustained high blood pressure (Alderman, 2000), in turn acting as a major risk factor for coronary heart disease and stroke (Aburto et al., 2013; Sacks et al., 2001; Strazzullo et al., 2009). The latest recommendation of the WHO is that no more than 2000 mg Na/day be consumed (WHO, 2006). If the meat industry wants to stimulate meat consumption, it has to face the challenges of offering more convenient and healthier options whilst identifying the new and changing roles of meat and meat products in the diet (Font-i-Furnols & Guerrero, 2014). Nowhere is this more relevant than with the efforts being made to reduce the Na content of

processed meat products. Reducing the NaCl content may require alterations in other parameters to ensure that affected foods maintain acceptable texture (Doyle & Glass, 2010) and a robust chemical and microbial shelf-life. Maintaining consumer preference and acceptance is also one of the major challenges encountered by the industry when sizeable salt reduction is involved (Bobowski et al., 2015b). Increased production costs that can affect the affordability of these altered products need to be avoided to protect the most price sensitive consumers.

In Chapter 2 of this thesis, a review about the available literature on the topic of dietary salt and Na was written. It was established that humans have a physiological need for salt and find its contributions to food so appealing that it has led to the overconsumption of it in many countries all over the world. The problem was discussed in a more localized setting with regard to the health problems and the economic burden of these problems on a developing country such as South Africa. The various functions of salt in specifically processed meat products were discussed outlining the importance of salt and identifying key areas where challenges might arise due to its partial reduction and/or replacement. A number of previously reported strategies were investigated as to how reduction and/or replacement have been experimentally attempted with a special focus on the use of ingredients that may be applied without the use of costly new processing techniques or technologies. Due to the fact that some of these two types of strategies might be interrelated, an overview of these technologies was also given. From a lack of information on the topic it was gleaned that few research groups have considered using intermediate added salt levels alone to study the effects on quality and stability. With this background information, theories on how salt content, and in effect, Na content of local products could be reduced, were formulated for practical evaluation in this thesis. An attempt was also made to identify realistic advantages that reducing and/or partially replacing salt in processed meat products could have on the affected products and how this might be advantageous to progressive processors.

The average South African was reported to have excessively high urinary Na excretion levels (Charlton et al., 2005) beyond what the WHO suggested as a limit (WHO, 2007). In addition, processed meat products were reported to be the second highest contributor to excessive Na intake (Charlton et al., 2005). In an effort to curb the effects of sustained overconsumption of Na, the South African Government drafted regulations that would limit the amount of Na in a broad range of foodstuffs. In terms of processed meat products, the initial limits fell between 800-850 mg Na/100 g product. A revised draft of the regulations published in 2013 stated these initial limits to be between 850-950 mg Na/100g product. No recent data on the general Na content of the affected products could be found on which to base a study of how many products would be affected by the regulations and to what extent Na reduction was needed. This presented an unique opportunity to

build a data basis of the Na content of processed meat products that would be affected by the regulations. As a result, Chapter 3 took the form of a survey to gather this information and to establish how many product labels displayed Na content as part of the nutritional information in relation to the upcoming labelling regulations requiring this information (Department of Health, 2014). A number of issues were identified with the regulations which included oversimplified grouping of meat product classes, incorrect grouping of meat product classes according to the products being either cured or uncured, and most importantly, Na limits deemed to be too low to guarantee food safety by the industry. These issues could have been avoided by the government by making use of input from the food industry. As a result, two different drafts of the regulations have been published to date, with the industry requiring greater input for a third draft that has already surpassed the implementation date of 30 June 2016 of the first Na regulation limits. This has made it difficult to pin-point the exact Na limits to use as a basis for this thesis, although the last official draft of regulations (Department of Health, 2013) was used throughout this thesis.

In relation to the labelling of Na content, less than 60% of product labels described NaCl, or more correctly, Na content. As the survey was conducted at the end of 2013, and the labelling regulations were only expected to come into effect closer to the end of 2016, it was reasonable to conclude that this situation would have drastically improved in the meantime. It is, however, debatable how well consumers understand this information and if it contributes to consumer satisfaction (FSA, 2008; Gilbey & Fifield, 2006; Marshall et al., 2007; Scott & Worsley, 1997). Greater consumer education through a concerted effort by the government and industry could greatly improve the effectiveness of this information. Surveying resulted in almost 500 individual meat products that were then divided into their respective categories (SANS 885, 2011) and from this information the five largest product classes were identified. A comparison of labelled Na values with actual Na values revealed that the labelled values mostly overestimated Na content. This was attributed to processors being inclined to err on the safe side. Overestimation was greatest in frozen products, posing little risk to consumers, while underestimation was greatest in dried meat products, which already pose a significant risk in terms of the overconsumption of salt in a country where dried meat products are very popular. Across the three largest product classes, only a small number of products truly exceeded the initial and final Na limits when the generous 20% tolerance levels were taken into account (Department of Health, 2014). At the time of writing it was believed that even fewer products would exceed these limits as processors started to adapt their product formulations. The Na reduction requirements of the three main classes warranted an investigation into the effects that straight forward reductions without replacement would have on meat product quality and stability.

This formed the basis of the experimental work of Chapter 4 where Na reduction to mandatory regulatory limits was implemented without any form of replacement strategy. To date, this was the first time such a strategy had been implemented in an effort to reduce Na content. Salt is critical in manipulating and controlling the texture of processed meat products (Hamm, 1961; Sofos, 1985). It decreases fluid loss in heat-processed products, increases fat binding which in turn increases viscosity, and it improves texture by promoting the binding of meat proteins to each other (Man, 2007). Through its effects on water activity, it also protects products against microbial growth (Doyle & Glass, 2010; Mossel & Thomas, 1988). Reducing the salt content requires alterations in other parameters to ensure safe and acceptable products (Doyle & Glass, 2010) while keeping these healthier products affordable to all consumers (Temple & Steyn, 2011). In light of this information and due to the fact that modern processed meat products contain a number of multi-functional ingredients, there is a need to establish the extent to which these ingredients can fulfil some of the functions of added NaCl. It also provided an opportunity to quantify product-specific shortcomings when the added NaCl level was adjusted to lower levels.

From each of the three main classes of processed meat products, a single product was selected as model for further investigation: bacon as a whole muscle, cured, non-heat treated product; polony as an emulsion, cured, cooked and chilled product; and pork bangers as comminuted, uncured non-heat treated products. The 2016 and 2019 Na limits served as intermediate added NaCl levels, except in the case of the bacon where theoretical limits were used. In addition to determining the Na content with the use of AAS, it was possible to quantify the 'hidden' Na content in each formulation from the Na content of the negative control formulations. Basic chemical parameters such as water activity, pH and moisture content yielded limited or inconsistent deviations at intermediate added NaCl levels. These deviations could not be consistently linked with deviations in other dependant parameters e.g., significantly higher water activity in the intermediate groups did not necessarily result in significantly higher microbial counts in the same groups. These discrepancies supported the need to evaluate the use of NaCl replacers in order to establish if the same variations might be prevented.

Microbial stability, lipid oxidative stability and sensory analysis results were encouraging. Processing techniques such as smoking, vacuum packaging, pasteurisation and ideal storage temperature as well as the anti-microbial properties of ingredients other than NaCl sufficiently maintained microbial stability. Lipid oxidation in the bacon and polony remained very low in part due to the anti-oxidant properties of other ingredients (Cheng et al., 2011; Honikel, 2008; Kiliç et al., 2014; Li et al., 2010; Tangkanakul et al., 2009). In addition to higher NaCl content, entrapped air and oxygen permeable packaging was believed to have contributed to the much higher levels of

lipid oxidation in the bangers. It is suggested that a more protective packaging method should be used, one which might include the use of modified atmospheres or moulded trays with vacuum packaging. Consumers detected no difference between bacon with a slightly reduced salt content and even found bacon with a low added NaCl level acceptable. Consumers found polony with intermediate added NaCl levels indistinguishable from the control. A similar result was found for the bangers with consumers only noticing the lower saltiness of the low NaCl intermediate. The results on the polony and bangers exhibited the effect that complex flavour profiles have in masking lower NaCl content due to the fact that odour intensity can increase taste (Salles, 2006).

With different added NaCl levels, a number of dynamic changes in colour were observed in both the bacon and the bangers. The bacon developed the characteristic red colour regardless of NaCl content and was more stable at lower NaCl levels (Andersen et al., 1990; Trout, 1990). Lightness and yellowness were inversely correlated with added NaCl content. The colour of the bangers was much less affected by NaCl content than by other factors such as added fat and oxygen permeable packaging (Carpenter et al., 2001) which promoted oxymyoglobin stability (Schivazappa et al., 2004). Both intermediate added NaCl levels resulted in polonies that visually only presented wetter cutting surfaces than the positive control. At the low intermediate added NaCl level, polony texture was stable for 90 days and at the high intermediate added NaCl level, polony texture was stable for 180 days. The intermediate added NaCl levels of the bacon resulted in yields and shrinkage comparable to the positive control. Only drip loss of the low intermediate and cooking and total losses of both intermediate levels were higher than for the positive control. In terms of thaw, cooking, total and refrigeration losses in the bangers, the low intermediate added NaCl level resulted in higher cooking and total losses and the higher intermediate added NaCl level resulted in cooking and total losses that were slightly lower than with 2% added NaCl. To limit similar losses reported in ham with reduced NaCl content, the use of other functional ingredients have been suggested (Ruusunen et al., 2001).

The actual Na content of the intermediate NaCl level models were substantially lower than what was formulated for in terms of product-specific limits. While every precaution was taken, it still proved difficult to estimate the precise contributions each formulation component made to total Na content. Due to the inherent requirement to limit variation in experimental work, these levels could not be adjusted in subsequent replications. In practice, initially constant reformulation will result in higher Na content that better match the regulatory limits and negates some of the afore mentioned discrepancies. If the use of replacers is to be avoided, increasing the levels of certain functional ingredients or adapting processing techniques might provide further solutions. In all likelihood,

some form of replacement strategy will be essential or more effective in formulations with highly reduced salt content.

This formed the basis of the experimental work of Chapter 5 where Na reduction was applied to pork bangers at a level of around 50% (w/w) of the original NaCl content. Consumers generally indicate concern over their health, although they do not often adopt healthier eating habits (Grunert, 2006). Consumers increasingly reject foods that compromise on the sensory experience in return for potential health benefits and it is deemed speculative and risky to depend on consumers to lower their expectations (Verbeke, 2006). Consumers can be 'retrained' to like lower saltiness (Bertino et al., 1982; Blais et al., 1986) although, in all likelihood, reduced Na products need to maintain the current level of saltiness to appease the human hedonic response to salt (Leshem, 2009; Mattes, 1997). In addition, product quality and microbial stability need to be maintained (Doyle, 2008) even though replacers often do not have the same microbiocidal effects than NaCl (Dötsch et al., 2008).

Potassium chloride, K-gluconate, yeast extract and K-lactate were selected as replacer candidates on the basis of: being the most studied and having a price-point similar to NaCl; used in some commercial products, although little information is available on its effects; used as a masking agent and flavour enhancer; and used as a Na-free organic acid preservative, respectively. The four replacer combinations had different effects on various losses. Thaw losses were consistently higher than for the current or 50% reduced NaCl content groups. Improved cooking losses were observed for all four treatment groups and improved refrigeration losses were observed for partial replacement with only KCl, KCl with YE, and KCl with K-lactate, the common denominator being KCl. This was attributed to the similar protein extraction capability of KCl to that of NaCl (Munasinghe & Sakai, 2004). Sodium and K content differed based on the substitution level and replacer combination. Sodium content was successfully reduced to levels closely matching a final Na limit of 600 mg Na/100 g for this type of product (Department of Health, 2013). A 1:1 ratio of NaCl to KCl and the use of multiple K-containing replacers in one formulation increased ($P < 0.001$) K levels which, at sufficiently high enough levels, may provide an additional blood pressure lowering effect (Abernethy, 1979; Geleijnse et al., 2003) supplementary to that of decreased Na content. The use of replacers had no effect on basic chemical parameters such as pH and moisture content. Water activity was generally, but not consistently, higher in the four replacer groups, similar to that of the 1% added NaCl group. This exemplified the fact that few substances have the ability to depress water activity to the same extent to that of NaCl (Mossel & Thomas, 1988).

Sodium chloride is known to exhibit pro-oxidative effects leading to the deterioration of lipids (Decker, 1998; Rhee, 1999; Rhee & Ziprin, 2001). 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). In this study, none of the replacer formulations improved on the lipid stability of the NaCl only formulations. Partial replacement of NaCl with only KCl or only K-gluconate did, however, result in lower ($P < 0.001$ on day 6 and $P < 0.05$ on day 9) lipid oxidation levels compared to partial replacement of NaCl with KCl and YE. Yeast extract is already widely used as flavour enhancer and masking agent, although in this application, it deteriorated shelf-life in terms of lipid oxidative stability. Similarly, YE resulted in an immediate increase in total viable counts as a result of it having a growth promoting effect on microorganisms (da Silva et al., 2012). An unexpected result was that partial replacement of NaCl with KCl and K-lactate resulted in higher ($P < 0.001$) total viable counts from day 6 onwards. This was in contrast to other reports (Pohlman et al., 2009; Tan & Shelef, 2002) and was ascribed to using a very low concentration of K-lactate in combination with reduced NaCl content.

Colour was the most consistently affected parameter with significant ($P < 0.001$) effects found at every time interval. Partial replacement with only KCl resulted in the lightest, the combination of KCl and K-lactate resulted in the best maintained redness and KCl and YE resulted in the yellowest. When only NaCl was present, redness and colour brightness deteriorated and yellowness was low, which was attributed to the negative effect of NaCl on colour stability (Andersen et al., 1990; Trout, 1990). The changes in colour were measured objectively and it is suggested that subjective evaluation by consumers will be needed in future to better gauge the extent of these effects in terms of consumer preference. Differences in consumer sensory analyses were limited to the less acceptable taste and overall liking of K-gluconate. With no differences in saltiness, these lower rankings were attributed to the larger molecular weight of K-gluconate causing a delayed and/or prolonged salty taste which consumers disliked. In addition to comparing saltiness of replacers to that of NaCl at face value, it might prove beneficial to determine the release time of replacers to that of NaCl.

The use of these replacers in an effort to reduce NaCl content resulted in a few discrepancies limited to the use of K-gluconate, KCl and YE, and KCl and K-lactate. There were also a few improvements over the negative effects normally associated with NaCl. It would be difficult to exclude any of these options from future applications without any pro-active attempts made to address specific shortcomings. For example, reduced microbial stability may be eliminated by changing storage options from refrigeration or frozen, to frozen exclusively, or manipulating or changing the current mix of other antimicrobials in a formulation. The extent of other discrepancies such as changes in colour parameters are difficult to determine from only an experimental point of view. There were, however, enough evidence in this study to suggest that the use of KCl as sole

NaCl replacer made it a suitable option and proved its worth for future consideration in other applications. In terms of specifically food safety, other areas need special attention such as the efficacy of these replacer options against specific food-borne pathogens.

This formed the basis of Chapter 6, where the effects of a reduction and/or partial replacement of NaCl in banger batters on the growth and survival of *E. coli* and *S. aureus* reference strains were investigated. Meat and meat products are highly susceptible to the exploitation of micro-organisms due to the favourite pH, moisture content and complex nutrient content (Nychas et al., 2008). Of great concern is that most meat safety issues resulting in consumer health issues and marketplace recalls stem from products contaminated with bacterial pathogens (Sofos, 2008). For fresh meat products *E. coli* 0157:H7 is a major concern (Sofos, 2008) and *S. aureus* is commonly associated with chopped meat mixes and processing plants (Gonzáles-Fandos et al., 1999). Extreme environmental conditions inflict stress on the organisms, the extent of which determines whether the organism dies, ceases to grow or experiences lag time and a reduced growth rate (Ray, 1986; Russell et al., 1995). A change in the control system, may allow for the emergence of a pathogen (Miller et al., 1998) and the reduction and/or partial replacement of NaCl may be regarded as such a change.

Neither NaCl reduction of 25% or 50%, nor partial replacement of ~ 50% NaCl with KCl, K-gluconate, KCl and YE, or KCl with K-lactate supported further growth of the inoculated *E. coli* strain. The strain was also not stressed to the point that cell death within the populations caused significant decreases in cell concentration. This was attributed to the ability of *E. coli* to enter a stationary growth phase under less than ideal conditions (Jones et al., 1987; Hengge-Aronis, 1996; Abee & Wouters, 1999) and a characteristic 'cross-stress protection' effect (Campbell et al., 2004). With no significant differences between treatment groups the main bacteriostatic effect exerted on the strain was ascribed to sub-optimal storage duration and temperature and the growth-inhibiting effects of additives such as sodium metabisulphate. It was assumed that the reduction and/or replacement of NaCl did not increase the food safety risk of a product that was already sufficiently contaminated with *E. coli.*

The *S. aureus* strain was unable to grow at sub-optimal temperatures and decreased regardless of added NaCl level. The sub-optimal storage temperature and storage duration were the main effects responsible for decreased survival rates outside the optimal range (Schmidtt et al., 1990). With partial replacement of NaCl, a greater amount of variations between treatments were found. Some limited growth was observed for the four replacer groups, although the counts decreased overall. The use of KCl had effects at both temperatures that were most similar to that of the control NaCl

level. The highest survival rate was observed for the KCl and K-lactate replacer combinations. Sub-optimal storage temperature and time also played major roles in lower survival rates. It was concluded that neither *E. coli* nor *S. aureus* was particularly opportunistic at reduced and/or partially replaced NaCl content under the less than ideal environmental conditions. Both organisms were, however, capable of maintaining sufficiently high counts and would be problematic at these levels in real products regardless of the added NaCl content. It was suggested that even lower inoculations levels may better indicate differences in survival, growth and death rates in future research.

Findings from this research offer insight into how, in response to fast-tracked legislation, the many decades old challenge of reducing salt in processed meat products can be approached with a focus on providing sensible results that can be advantageous to an industry where opportunities for, and access to, research is often improbable. A number of advantages and limitations have been identified that can act as a springboard for further research and development that can lead to real world solutions.

A number of gaps and opportunities for future research have been identified and discussed in the previous paragraphs. In addition, the use of transglutaminase enzymes to improve the texture of reduced NaCl formulations of locally adapted emulsion and restructured meat products, such as Russian sausages and polony, is suggested. In South Africa, dried meat products such as biltong and droëwors are immensely popular and by their very nature contain large amounts of salt due to the reliance on air drying at ambient temperatures without preservatives. At the time of writing, these products were excluded from the Na reduction regulations, although this will possibly change in the near future, requiring that these products be brought into line. A specific challenge could be that replacer candidates will specifically need to mimic the salting-out effect of NaCl, which is responsible for the quick moisture loss which, in turn, is responsible for the preservation of these products.

CHAPTER 8

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CHAPTER 9
SUMMARY

In light of the recent South African regulations limiting the sodium content of processed meat products, the latest draft of these regulations were used to establish to what extent commercial processed meat products deviated from these limits and required reformulation, two and a half years in advance of the first reduction limits coming into effect. Almost 60% of product labels already included information on sodium content almost three years before the applicable labelling regulations came into effect. Surveying nationally and regionally available products allowed for the identification of the five largest product classes. A comparison between labelled and determined Na content revealed that processors tended to overestimate Na content as a precautionary measure. A generous tolerance of 20% for underestimating the Na content, as stated in the labelling regulations draft, showed that only a small number of products would at the time, have exceeded the future-dated regulatory limits.

Bacon, polony and pork bangers, representing the three most populous classes were used to evaluate the efficacy of the two-part regulatory limits as intermediate added NaCl levels without replacers or alterations in processing. Water activity, pH and moisture content were inconsistently affected with no definite links to deviations in dependent parameters such as microbial stability. Microbial and oxidative stability and sensory quality results were encouraging. Current processing techniques and ingredients other than NaCl maintained quality and stability. Changes in bacon and banger colour were found, although subjective evaluation is needed to grasp the implications. Polony texture was deemed acceptable, both quantitatively and qualitatively. Total Na levels better matching the regulatory limits may further limit the minor deviations in quality and stability.

The relative success of using only 1% NaCl (w/w) in the pork bangers prompted the use of various compounds, either alone or in combination, to address the gaps in functionality of the 1% NaCl removed from the original formulation. Potassium chloride (1% w/w), K-gluconate (1% w/w), KCl (0.8% w/w) with YE (1% w/w) and lastly, KCl (0.8% w/w) with K-lactate (0.2% w/w) were compared to 1% NaCl and 2% NaCl controls. Treatments containing KCl had improved cooking losses over that of the controls. The use of K-containing compounds increased the K-content in addition to reducing Na-content. Basic chemical parameters were similar to that of the 2% NaCl control with only water activity being more similar to that of the 1% NaCl control. These replacers did not improve lipid oxidative stability and the use of YE actively deteriorated lipid oxidative stability. Colour was the most affected multi-component parameter and consumers had less

favourable hedonic responses towards the use of K-gluconate. Partial replacement with 1% KCl was the most suitable solution when additional factors such as price-point, similarity to NaCl, and ease-of-use were taken into account.

Lastly, the efficacy of the reduction and/or partial replacement of NaCl against the growth and survival of *E. coli* and *S. aureus* inoculated into banger batters were monitored. No effects on *E. coli* were observed beyond the bacteriostatic effect of sub-optimal temperatures (4 °C and 10°C) and the anti-microbial effects of the other additives in the formulations. At reduced NaCl levels, *S. aureus* was unable to grow and survival rate ultimately decreased. Partial replacement led to limited growth although survival rates eventually decreased. Survival rates were highest at 1% NaCl, 1% KCl and 0.2% K-lactate. Sub-optimal temperatures and other anti-microbial effects overrode that of partial NaCl replacement. Beyond the initial inoculation levels, reduction and/or partial replacement of NaCl did not increase the food safety risk of these bacterial species.

This research showed that conformation with the regulatory limits warrants a back-to-basics strategy using multiple approaches that deliver better results when these approaches are linked to one another.

Keywords: high blood pressure, sodium reduction, sodium replacement, processed meats, pork, microbial stability, chemical stability, sensory quality

OPSOMMING

Na aanleiding van die onlangse Suid-Afrikaanse regulasies wat die natriuminhoud van verwerkte vleisprodukte beperk, is die mees onlangse weergawe van hierdie regulasies ingespan om vas te stel tot watter mate kommersiële verwerkte vleisprodukte afwyk van hierdie perke en of herformulering benodig word, twee en ‘n half jaar voordat die eerste verlagingsperke in werking tree. Amper drie jaar voordat die toepaslike etiketregulasies in werking sou tree, is daar bevind dat byna 60% van produketikette reeds natriuminhoud beskryf het. ‘n Opname van nasionaal en plaaslik beskikbare produkte het dit moontlik gemaak om die vyf grootste produkklasse te identifiseer. ‘n Daaropvolgende vergelyking tussen etiket en ontleedte natriuminhoud het uitgewys dat vleisverwerkers geneig het om die natriuminhoud as ‘n voorsorgmatreël te oorskat. ‘n Ruim speling van 20% vir die onderskatting van die natriuminhoud, soos beskryf in die etiketregulasies, het aangedui dat slegs ‘n klein aantal produkte op daardie stadium die toekomstige regulasieperke sou oorskry.

Spek, polonie en varkwors as verteenwoordigers van die drie grootste klasse is as modelle gebruik om die doeltreffendheid van die tweeledige regulasieperke as intermediêre toegevoegde soutvlakke te evalueer sonder vervangers of veranderinge in verwerking. Wateraktiwiteit, pH en voginhoud is somtyds beïnvloed sonder enige besliste skakels tot afwykings in afhanklike maatstawwe soos mikrobiese stabiliteit. Resultate van mikrobiese en oksidatiewe stabiliteit sowel as sensoriese kwaliteit was bemoedigend. Huidige verwerkingstegnieke en bestanddele anders as NaCl het kwaliteit en stabiliteit gehandhaaf. Veranderinge in spek- en varkworskleur is gevind, alhoewel subjektiewe evaluering nodig is om die implikasies te begryp. Polonietekstuur is kwantitatief en kwalitatief as aanvaarbaar beskou. Totale Na-vlakke wat die regulasieperke beter ewenaar mag die klein afwykings in kwaliteit en stabiliteit verder beperk.

Die relatiewe sukses van die gebruik van slegs 1% NaCl (g/g) in die varkwors het aanleiding gegee tot die gebruik van verskeie ander verbindings, beide alleen of in kombinasies, om die gapings in funksie van die verlaagde 1% NaCl aan te spreek. Kaliumchloried (1% g/g), kaliumglukonaat (1% g/g), KCl (0.8% g/g) met giseskstrak (1% g/g) en laastens, KCl (0.8% g/g) met kaliumlaktaat is vergelyk met 1% NaCl (g/g) en 2% NaCl (g/g) kontroles. Behandelings wat KCl bevat het, het kookverliese verbeter teenoor die van die kontroles. Die gebruik van K-bevattende verbindings het die K-inhoud verhoog benewens die verlaging in Na-inhoud. Basiese chemiese maatstawwe was soortgelyk as die van die 2% NaCl kontrole, met slegs die wateraktiwiteit wat meer soortgelyk was as die van die 1% NaCl kontrole. Hierdie vervangers het nie die lipiedstabiliteit verbeter nie, terwyl die gebruik van gisekstrak dit aftief benadeel het. Kleur, as ‘n multi-komponent kwaliteit maatstaf,

was die meeste beïnvloed en verbruikers het minder gunstige hedonistiese reaksies gehad teenoor die gebruik van K-glukonaat. Gedeeltelike vervanging met 1% KCl was die mees geskikte oplossing wanneer verdere faktore soos pryspunt, ooreenkoms met NaCl, en gemak van gebruik in ag geneem is.

Laastens is die doeltreffendheid van die verlaging en/of gedeeltelike vervanging van NaCl teen die groei en oorleweing van *E. coli* en *S. aureus* wat in varkworsmengsels geïnokuleer is, gemonitor. Op *E. coli* is geen effekte gevind buite die bakteriostatiese effek van die sub-optimale temperature (4 °C en 10 °C) sowel as die anti-mikrobiese effekte van ander bymiddels in die formulasies nie. Teen verlaagde NaCl vlakke, was *S. aureus* nie in staat tot groei nie en die oorlewingsyfers het uiteindelik gedaal. Gedeeltelike vervanging het gelei tot beperkte groei, maar oorlewingsyfers het uiteindelik gedaal. Oorlewingsyfers was die hoogste by 1% NaCl, 1% KCl en 0.2% K-laktaat. Sub-optimale temperature en ander anti-mikrobiese effekte het die van gedeeltelike NaCl vervanging oorheers. Buiten die aanvanklike inentingsvlakke het verlaging en/of gedeeltelike vervanging van NaCl nie die voedselveiligsrisiko van hierdie bakteriese spesies verhoog nie.

Hierdie navorsing het aangetoon dat voldoening aan die regulasieperke ‘n strategie benodig wat terugneig na die basiese beginsels en dat gebruik gemaak moet word van verskeie benaderings wat beter resultate lewer wanneer hierdie benaderings met mekaar verbind word.

Sleutelwoorde: hoë bloeddruk, natriumverlaging, natriumvervanging, verwerkte vleise, varkvleis, mikrobiese stabiliteit, chemiese stabiliteit, sensoriese kwaliteit