Evaluation of Natural Preservatives for Use in a Traditional South African Sausage

By

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Declaration

I declare that the dissertation/thesis hereby handed in for the qualification M.Sc. Agric. Food Science at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at/in another University/faculty.

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S.A. Mathenjwa

November 2010

This thesis is dedicated to all my friends who love life

and

to my family, especially my parents Mr & Mrs Mathenjwa, brothers & sister
Sibusiso, Sabelo and Mazaline Mathenjwa

TABLE OF CONTENTS

Chapter	Title	Page
	Acknowledgements	i
	List of figures	ii
	List of tables	iv
	List of abbreviations	v
1	**INTRODUCTION**	**1**
2	**LITERATURE REVIEW**	**6**
	2.1. Introduction	6
	2.2. Sausage classification	7
	2.3. Boerewors manufacture	9
	2.4. Microbial composition of boerewors	10
	2.4.1. Spoilage bacteria	10
	2.4.2. Food-borne pathogens	12
	2.5. Other factors affecting the shelf-life of boerewors	14
	2.5.1. Temperature	15
	2.5.2. Water activity	16
	2.5.3. pH	17
	2.5.4. Surface area	18
	2.5.5. Gaseous environment and packaging material	18
	2.6. Traditional preservation methods of boerewors	19
	2.7. Natural preservation methods	21
	2.7.1. Lactic acid bacteria	21

2.7.2. Plant extracts 21

2.7.3. Essential oils 23

2.7.4. Garlic 23

2.7.5. Chitosan 24

2.7.6. Citrox 24

2.8. Other emerging preservation methods 25

2.8.1. Hurdle technology 25

2.8.2. Hazard Analysis Critical Control Point (HACCP) 26

2.9. Conclusions 27

3 MICROBIAL QUALITY EVALUATION OF BOEREWORS (TRADITIONAL FRESH SAUSAGE) IN BLOEMFONTEIN, SOUTH AFRICA 28

3.1. Introduction 28

3.2. Materials and Methods 29

3.2.1. Sampling procedure and preparation 29

3.2.2. Microbial analysis 30

3.2.3. Statistical analysis 32

3.3. Results and Discussion 32

3.3.1. Microbial quality of the regions 32

3.3.2. Comparison of supermarket and butcher outlets 39

3.4. Conclusions 40

4 EFFECT OF NATURAL PRESERVATIVES ON THE MICROBIAL QUALITY, LIPID STABILITY AND SENSORY EVALUATION OF BOEREWORS 41

4.1. Introduction 41

4.2. Materials and Methods 43

4.2.1. Sausage preparation 43

4.2.2. Sampling 44

4.2.3. Water activity (a_w) determination 46

4.2.4. Microbial analyses 46

4.2.5. Colour stability determination 48

4.2.6. Lipid stability determination 49

4.2.7. Sensory evaluation 49

4.2.8. Statistical analysis 50

4.3. Results and Discussion 51

4.3.1. Water activity (a_w) 51

4.3.2. Statistical effect of preservatives on different interactions 52

4.3.3. Microbial analyses 54

4.3.4. Chemical stability 61

4.3.5. Sensory evaluation 68

4.4. Conclusions 70

5 GENERAL DISCUSSION AND CONCLUSIONS 71

6 REFERENCES 76

7 SUMMARY / OPSOMMING 98

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LIST OF FIGURES

Figure	Figure title	Page
Figure 4.1.	Experimental outlay of the sampling procedure	47
Figure 4.2.	Effect of preservative types and storage time on the total bacterial counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	55
Figure 4.3.	Effect of preservative types and storage time on the coliform counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	57
Figure 4.4.	Effect of preservative types and storage time on the *Enterobacteriaceae* counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	58
Figure 4.5.	Effect of preservative types and storage time on the yeasts and moulds counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	59
Figure 4.6.	Effect of preservative types and storage time on the L* value (lightness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	62
Figure 4.7.	Effect of preservative types and storage time on the a* value (redness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	63

Figure 4.8.	Effect of preservative types and storage time on the b* value (yellowness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations	64
Figure 4.9.	Effect of preservative types and storage time on the saturation index (SI) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bar represent standard deviations	65
Figure 4.10.	TBARS-values (lipid stability) measured in mg malonaldehyde/kg meat of boerewors treated with different preservatives and stored for 9 days at 4 ^{o}C and 100 days at -18 ^{o}C. Results with different superscripts are significantly different. Error bars represent standard deviations	67

LIST OF TABLES

Table	Table title	Page
Table 2.1.	Sausage classification	8
Table 2.2.	Minimal a_w levels required for growth of food-borne microorganisms at 25°C	16
Table 2.3.	Effect of added sulphite on microbial growth in fresh pork sausage	20
Table 3.1.	The mean microbial quality of boerewors from 37 (50%) retail outlets in Bloemfontein	33
Table 3.2.	The mean microbial quality of boerewors from the different regions in 50% of Bloemfontein retail outlets	33
Table 3.3.	The presence of *E. coli* in boerewors from the different regions in 50% of Bloemfontein outlets	37
Table 3.4.	Comparison of microbial quality of boerewors purchased from butcher shops and supermarkets	39
Table 4.1.	Formulation used in the manufacture of the eight boerewors models	45
Table 4.2.	Nine-point hedonic scale used in this study for sensory analysis	50
Table 4.3.	Water activity of the boerewors treated with different preservatives at day 0	51
Table 4.4.	Analysis of variance for various treatments and their interactions	53
Table 4.5.	Mean values for the taste preference of boerewors samples manufactured with different preservatives	69

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APC	Aerobic plate counts
a_w	Water activity
°C	Degrees Celsius
cfu	Colony forming unit
Chi	10 mg/kg Chitosan treatment
Chi+S	10 mg/kg Chitosan + 100 mg/kg SO_2 treatment
Con	Control treatment
DoA	Department of Agriculture of South Africa
DOH	Department of Health of South Africa
E	*Escherichia*
Ed	Editor
e.g.	For example
et al.	(*et alii*) and others
Etc.	(*et cetera*) and so forth
FDA	United States Food and Drug Administration
g	gram
GCC	Gram-positive, catalase-positive cocci
GMP	Good manufacturing practise
GRAS	Generally recognised as safe
H	Flagella antigen
h	Hour
HACCP	Hazard Analysis Critical Control Point
HSO_3^-	Bisulphute
In vitro	In an artificial environment outside the living organism
kg	Kilogram
L.	*Listeria*
Log	Log_{10}
mg	Milligram
ml	Millilitre
mm	millimetre

MUG	4-Methyl-umbelliferyl-β-glucuronide
NaCl	Sodium chloride
NCSS	Number Statistical System, Kaysville, Utah, USA
O	Somatic antigen
p	Significance level
pH	-Log $[H^+]$
pp	Page (s)
PPC	Psychrotolerant plate count
ppm	Part per million
PVC	Polyvinyl chloride
RBCA	Rose bengal chloramphenicol agar
Ros	260 mg/kg rosemary extract treatment
Ros+Chi	260 mg/kg rosemary extract + 10 g/kg chitosan treatment
Ros+Chi+S	260 mg/kg rosemary extract + 10 g/kg chitosan + 100 mg/kg SO_2 treatment
Ros+S	260 mg/kg rosemary extract + 100 mg/kg SO_2
S	450 mg/kg SO_2 treatment
S.	*Salmonella*
SO_2	Sulphur dioxide
SO_2^-	Sulphite
SPCA	Standard plate count agar
Staph.	*Staphylococcus*
™	Trade mark
USDA	United States Department of Agriculture
VRB	Violet red bile
VRBG	Violet red bile glucose
±	Plus or minus
:	Is to
<	Less than
>	Greater than
/	per
%	Percentage

CHAPTER 1

INTRODUCTION

Sausage manufacturing by man began over two thousand years ago and the product played an important part in man's diet since then. The modern word "sausage" is derived from the Latin word "*salsus*," which means "salted" (Rust, 1987; Charimba, 2004; Wijnker, Koop & Lipman, 2006). According to Rust (1987) the preparation of sausages began with a simple process of salting and drying meats. The salt added was for preservation purposes. The meat was then flavoured with spices to improve the flavour of the product. To make the product more convenient to eat, it was placed in a container made from the gastrointestinal tract of animals. Sausages have been produced from different meats such as beef, pork, chicken, fish and buffalo meat (Raju, Shamasundar & Udupa, 2003; Sallam, Ishioroshi & Samejima, 2004; Sachindra, Sakhare, Yashoda & Rao, 2005).

Boerewors is a sausage product popular in South African and Limburgish cuisine. It came from the Afrikaans word "boer" which means farmer and "wors" which means sausage (http://en.wikipedia.org/wiki/Boerewors retrieved on 22 September 2010). The National Department of Health (DoH) of South Africa (1990) describe boerewors as any sausage sold under a name in which the word "boerewors" appears either by itself or in combination with any other word or expression. Boerewors is classified as a ground meat product. In this group the muscle structure has undergone some form of communition, such as mincing, dicing or chopping. The muscle structure is, therefore, no longer recognizable in its fibrous form, but becomes particulate in nature (Rust, 1987). The increase of consumption of traditional boerewors in South Africa and exploration of using new ingredients in the product to improve the flavour, began in the early 1960's. Different ingredients such as garlic, cheese, chilli, etc. are used to give different flavours to the sausage enjoyed by different consumers for various occasions (Krijger, 2008).

Boerewors is a fresh sausage product which makes it favourable for microbial growth to cause spoilage and growth of food pathogens (Romans, William, Carloson, Greaser & Jones, 2001). The product also has a high fat content of about 30 % and this can contribute to lipid oxidation, since it is stored in an oxygen semi-permeable package, resulting in affecting the organoleptic properties of the product. Actions, such as preservation, need to be taken to maintain the quality of the product.

The use of additives in food dates back thousands of years. Food additives are any substance(s) added to food to achieve specific properties such as improving organoleptic properties, act as emulsifying or gelatinizing agents and preserving food products. Food additives are normally not consumed as food on its own (Cengage, 2003). Sulphur dioxide (SO_2) has been used in sausages as a preservation additive against microbial growth and also to improve/maintain the colour of the sausage (Dyett & Shelley, 1966; Romans *et al.*, 2001). For a food additive to be used in a food product it needs to meet the Generally Recognized as Safe (GRAS) status. The GRAS status was designated by the United States Food and Drug Administration (FDA) in 1958, under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (FDA, 2010). The maximum allowable SO_2 level in South African fresh meat products is 450 mg/kg (DoH, 1990).

The use of chemical additives for different purposes in food products has come under the scrutiny of consumers. Gunnison & Jacobsen (1987) have stated that the use of sulphite as a preservative can trigger different allergic reactions in sulphite hypersensitive consumers. Symptoms such as asthma, urticaria, abdominal pains, nausea, diarrhea, seizures and anaphylactic shock resulting in death have been recorded. These health dangers have resulted in the need for using natural preservatives that can result in improving the quality of food.

The concept of “green” consumerism is based on that consumers are more aware of what goes into the food during processing (Rasooli, 2007). This has lead to research in the food industry for new natural preservatives that can be used to improve the food quality, by replacing conventional preservatives with natural preservatives (Rosooli, 2007).

Plant extract products have been shown to improve the quality of food as well as having health benefits. Rosemary extract has been used in meat and meat products as herbal spices. Rosemary extract compounds have liver protective and anti-tumor activity, and are also known for its antimicrobial and antioxidant activities (Offord, 2004; Belantine, Crandall, O'Bryan, Duong & Pohlman, 2006).

Chitosan, which is a byproduct of crab fish, also shows antimicrobial and antioxidant activities which is essential for maintaining the quality of food products (Ravi Kumar, 2000; Aldemir & Bostan, 2009). Chitosan claims to help with the reduction of body weight in dieting consumers - it binds the fat in the intestine before it is absorbed in the body. It also has blood anti-coagulant and anti-thrombogenic properties (Ravi Kumar, 2000). Aider (2010) has reviewed the activity of chitosan as bio-based film for packaging material, and stated that chitosan as a biofilm packaging material has the potential to preserve the quality of food products.

Bacterial growth and lipid oxidation in meat products are the main causes of food becoming unacceptable for consumption. In Chapter 2 of this study, the different factors that affect the shelf-life of meat and meat products, such as boerewors, and the different methods that have been studied to improve the shelf-life of fresh sausages, without altering the properties of the products, will be reviewed. Boerewors is currently conventionally preserved with 450 mg/kg SO_2, which is added to the product to increase the shelf-life and maintain organoleptic properties such as colour (Dyett & Shelley, 1966). Natural antimicrobial systems are set to become an important component in food preservation methodology due to different reasons, e.g. consumers becoming aware and rejecting the use of chemical preservatives and other chemical additives in food, and healthy eating habits of consumers (natural, organic or green consumerism) (Dillon & Board, 1994; Smid & Gorris, 1999; Rasooli, 2007).

The hygienic standard of boerewors produced in Bloemfontein, will be evaluated in the third chapter. Hygienic quality is an essential aspect in the food industry determining shelf-life as well as food safety. There are certain quality standards that are stipulated either by the industry and/or governmental and foodservice organizations. The importance of these standards is that they provide guidance for production of food based on different physical, chemical and microbial characteristics.

The effectiveness of the natural and conventional preservatives will be evaluated in Chapter 4. The effect of rosemary extract and chitosan as natural preservatives in boerewors will be studied to determine whether SO_2 can be replaced by one, or both of these preservatives or in combination with lower concentrations of SO_2, and which will not have a detrimental effect on sulphite sensitive consumers.

Purpose and objectives of the study

The purpose of this study was to evaluate alternative natural preservatives, rosemary extract and chitosan, as single preservatives or in combination with lowered levels of SO_2, in the production of boerewors. These natural preservatives should have the same or comparable antimicrobial action, chemical stability (lipid and colour) and sensory properties than SO_2 in conventionally produced boerewors.

Objectives

a.) To evaluate and compare the microbial quality of boerewors in 50% of Bloemfontein butcheries and supermarkets by means of total bacteria count, coliform count, presence of *E. coli* and *Staphylococcus aureus*, *Enterobacteriaceae* count, psychrotolerant bacteria count and yeasts and moulds count.

b.) To manufacture boerewors models containing no preservatives (control), SO_2 (conventional boerewors), rosemary extract, chitosan, and combinations of all the mentioned preservatives. The effect of these preservatives will be evaluated by:

- Microbial analysis (the effect on total bacteria count, coliform count, *E. coli* (Gram-negative pathogen) and *Staph. aureus* (Gram-positive pathogen)
- Colour stability
- Lipid stability
- Sensory quality

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Consumption of meat in South Africa has been estimated for red meat to be between 25.73 kg/person/year and white meat to be 29.69 kg/person/year in 2007-2008 (DoA, 2009). Chicken meat, beef meat, beef sausage (Boerewors), minced meat and other pork products (ham and bacon) are the popular meat products consumed in South Africa (Nel & Steyn, 2002). Boerewors is a fresh sausage meat product typically produced in South Africa. It is grilled or fried over medium heat prior to consumption.

Quality of the meat and meat products is an important aspect that has been under supervision by the National Department of Health (DoH) of South Africa (2001). There are different factors that affect the quality of meat and meat products, which include temperature, water activity (a_w), pH and microbial composition (Eisel, Linton & Muriana, 1997; Garbutt, 1997; Romans *et al.*, 2001). All these factors, therefore, have an influence on the spoilage potential of food. Spoilage of food involves a complex process and excessive amounts of food are lost due to microbial spoilage. This results in high economic losses and may even pose health hazards (Al-Sheddy, Al-Degal & Bazaraa, 1999; Liu, Yang & Li, 2006).

The use of antimicrobial processes such as preserving fresh sausages with SO_2 (450 mg/kg SO_2), is essential for improving the shelf-life of boerewors (DoH, 2001). However, consumers are more alert about food and food products which are chemically preserved due to their negative health effects (allergic reactions) and are seeking naturally preserved food products (McDonald, 1992; Bañón, Díaz, Rodríguez, Garrido & Price, 2007). These

consumer led trends has fuelled renewed searches for preservatives derived from plant, animal and microbial sources (Dillon & Board, 1994; Lanciotti, Patrignani, Bagnolini, Guerzoni & Gardini, 2003). Plant derived preservatives (grape, rosemary extract, etc.) and animal derived preservatives (e.g. chitosan from fish) have been shown to have antioxidant and antimicrobial properties (Bañón, *et al*., 2007).

In this literature review, factors that affect the shelf-life of boerewors and other related products are reviewed. Attention will be given to the microbial profile of meat as a critical point for food safety and quality (Sofos, 2008). The review will also evaluate methods to eliminate/substitute chemical additives in fresh sausages and related products with natural preservatives and other emerging preservation methods.

2.2. SAUSAGE CLASSIFICATION

There are different types of sausages produced by the meat industry. These can be classified as fresh sausage, dry and semidry sausage; cooked sausage; cooked, smoked sausages; uncooked, smoked sausage and cooked meat specialities (Rust, 1987; Romans *et al.*, 2001). The different sausages based on specific characteristics are described in Table 2.1. Boerewors forms part of the fresh sausages which are made from selected cuts of fresh meat, it is not cured and has to be stored in a refrigerated or frozen state prior to being consumed (Romans *et al.*, 2001).

During the making of boerewors, synthetic or natural casings are used to case the grounded meat or minced meat (Kim, 2006). Casing may be used in the manufacture of either fermented sausages, cooked/sterilized sausage and also in fresh sausage e.g. Boerewors (Houben, 2005; Wijnker *et al.*, 2006). Casings are used to protect sausage contents, to reduce the loss of moisture during storage, and to hinder the penetration of microbes into the product stuffing. Casing can be done manual or with an automatic sausage filler of various constructions (Kim, 2006).

Table 2.1. Sausage classification (Rust, 1987; Kim, 2006).

Classification	Characteristics	Examples
Fresh sausage	Fresh meat (mainly pork); uncured, comminuted, seasoned and usually stuffed into casings; must be cooked fully before serving	Fresh pork sausage Bratwurst Boerewors (South Africa)
Dry and semidry sausages	Cured meat; fermented air dried, may be smoked before drying; served cold	Gonoa salami Pepperoni Leñ bologna Summer sausage Droë wors or Dry wors (South Africa)
Cooked sausage	Cured or uncured meats; comminuted, seasoned, stuffed into casings, cooked and sometimes smoked; served cold	Liver sausage Braunschweiger Liver cheese
Cooked, smoked sausages	Cured meats; comminuted, seasoned, stuffed into casings smoked and fully cooked; do not require further cooking, but some are heated for serving	Frankfurters Bologna Cotto salami
Uncooked, smoked sausage	Fresh meats; cured or uncured, stuffed, smoked, but not cooked; must be fully cooked before serving	Smoked, country-style pork sausage Mettwurst Kielbasa
Cooked meat specialties	Specially prepared meat products; cured or uncured meats, cooked but rarely smoked, often made in leaves, but generally sold in sliced, package form; usually served cold	Loaves Head cheese Scrapple

2.3. BOEREWORS MANUFACTURE

Different ingredients are used in the preparation of boerewors, which includes animal tissue (lean meat of beef or/and pork), moisture for mixing purposes in the sausage mixture and for extraction of protein, and fat which contribute to palatability of the product. The non-meat ingredients that can be added to the product include salt (1-5%) which acts as a preservative against bacterial growth; and spice and flavourings are added for seasoning. To prevent oxidation of fat, antioxidants may be added (Rust, 1987). Natural animal casing or synthetic casing is used to stuff the sausage mixture (Rust, 1987; Houben, 2005). During the manufacture of boerewors the meat is cut into small pieces or minced in a grinder or bowl cutter. The temperature used during the trimming or cutting is important and should be -2°C or lower to ensure a clean cut (Rust, 1987).

The regulations (DoH of South Africa, 1990) governing the composition and labelling of raw boerewors, raw species sausage and raw mixed-species sausage, state that raw boerewors shall be manufactured from the meat of an animal of the bovine, ovine, porcine or caprine species or from a mixture of two or more thereof, shall be contained in an edible casing, and:

a) shall contain a minimum of 90% total meat content and not more than 30% fat content;
b) shall contain no offal except where such offal is to be used solely as the casing of the raw boerewors;
c) shall contain no mechanically recovered meat; may contain a maximum of 0.02 grams of calcium per 100 gram of the product mass.

Secondly, in or in connection with the manufacture of raw boerewors, no ingredients shall be added except:

a) cereal products or starch;
b) vinegar, spices, herbs, salt or other harmless flavourants;
c) permitted food additives;
d) water

2.4. MICROBIAL COMPOSITION OF BOEREWORS

Meat in general is an ideal growth medium for a wide range of microorganisms, (Garbutt, 1997; Russo, Ercolin, Mauriello & Villani, 2006; Zhang, Kong, Xiong & Sun, 2009). The major types of microorganisms in meat are bacteria, yeasts and moulds. The meat microbiota composition includes *Enterobacteriaceae*, lactobacilli, pseudomonads, *Brochothrix thermosphacta* and *Shewanella putrefaciens* which are associated with the spoilage of fresh meat (Garbutt, 1997; Huffman, 2002). According to Nortje, Voster, Greebe & Steyn (1999) and Huffman (2002), the organisms implicated in meat- and poultry-borne diseases are *Salmonella*, *Escherichia coli* O157:H7, *Clostridium botulinum*, *Clostridium perfringens*, *Aeromonas hydrophila*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes* and *Bacillus cereus*.

2.4.1. Spoilage bacteria

There are many factors that can influence the presence of certain microbial groups on meat and meat products. After slaughtering, the carcass may be contaminated with bacteria arising from the skin, faeces and the intestine of animals as well as air, water, soil, personnel and other factors such as cross-contamination during the slaughtering process. The total bacterial counts may reach levels between 10^2 and 10^4 cfu/cm^2 on the carcass (Huffman, 2002; Russo *et al.*, 2006; Wijnker *et al.*, 2006). According to Sachindra *et al*. (2005) the microbial ecology of meat and meat products will depend mainly on the type of environment, the kind of meat and raw materials, equipment, packaging and storage temperature.

Fresh sausage microbial profiles have been characterized by the presence of aerobes, facultative anaerobes and mesophiles, which are responsible for spoilage and potentially pathogenic bacteria (Cocolin, Rantsion, Iacumin, Urso, Cantoni & Comi, 2004). Aerobic colony counts range from $1.5 \times 10^3 – 2.1 \times 10^8$ cfu/g for fresh sausage and for frozen sausage from $1.4 \times 10^3 – 3.1 \times 10^7$ cfu/g (Farber, Malcolm, Weiss & Johnstone, 1988). In deboned

meat the aerobic counts have been shown to range from 1.4 x 10^5 – 1.5 x 10^7 cfu/g (Nel, Lues, Buys & Venter, 2004).

The presence of *Enterobacteriaceae* is often used as hygiene indicators of foods of animal origin. Their presence on processed food may give a better indication than coliforms of inadequate treatment or post-process contamination from the environment, and may help to indicate the extent of faecal contamination (Nel *et al.*, 2004; Crowley, Cagney, Sheridan, Anderson, McDowell, Blair, Bishop & Duffy, 2005). *Escherichia coli*, *Shigella* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Morganella* spp. and *Yesinia* spp. forms part of the *Enterobacteriaceae* family and have been demonstrated to show growth in fresh and frozen meat (Nel *et al*., 2004; Coma, 2008). The presence of *E. coli* in high numbers also indicates the presence of organisms originating from faecal population. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers (Nel *et al*., 2004). Nel *et al*. (2004) has stated that the maximum limit of *E. coli* in meat and meat products should not be more than 10 cfu/g as proposed by the National Department of Health (DoH) of South Africa (2001).

Members of the *Pseudomonas* group are the Gram-negative bacteria associated with the spoilage of food such as meat, milk and fish. *Pseudomonas* spp. are psychrotrophic bacteria that cause spoilage of meat and meat products stored at low temperatures (Ercolini, Russo, Blaiotta, Pepe, Mauriello & Villani, 2007; Olofsson, Ahrné & Molin, 2007). The most dominating species of *Pseudomonas* which causes spoilage of meat stored in a refrigerator (8 ºC or lower), is the *Pseudomonas fragi* followed by *Pseudomonas lundensis* and *Pseudomonas fluorescens* (Olofsson *et al.*, 2007).

There are different yeasts and moulds species that have been isolated from meat and meat products. According to Dillon & Board (1994) yeast species originate from the field in which the animals grazed and are transferred via the fleece or hide to the carcass surface. Examples of the yeast species include *Candida famata, Candida sake, Cryptococcus albidus* var. *albidus, Crypotococcus infirmominatus* and *Rhodotorula rubra* which were isolated from minced lamb and lamb meat products (Dalton, Board & Davenport, 1984; Dillon & Board,

1994). The yeasts that have been isolated from ingredients required for the development of a fresh sausage includes species such as *Candida*, *Crypotococcus*, *Debaryomyces*, *Pichia*, *Rhodotorula* and *Torulopsis* (Dalton *et al.*, 1984). Dalton *et al.* (1984) showed that yeast counts of sulphited fresh British sausage sampled from retail outlets ranged from 5.0×10^3 – 4.7×10^8 cfu/g and for those sampled from factories, from 2.5×10^4 – 4.0×10^4 cfu/g. The yeast counts of the unsulphited fresh sausages sampled from the factories ranged from 2.0×10^4 – 3.0×10^4 cfu/g. These studies showed that yeast cells are more resistant to sulphite than bacterial cells. There are six genera of moulds that can be isolated from slaughter animal carcasses or cuts, which include *Thamnidium*, *Mucor*, *Rhizopus*, *Cladosporium*, *Penicillium* and *Sporotrichum*.

2.4.2. Food-borne pathogens

Microorganisms isolated during the process of ground beef processing, includes many types of pathogenic microorganisms, most notably *Salmonella* spp., *Staph. aureus, Listeria monocytogenes, E. coli* and *Campylobacter jejuni* (Farber *et al*., 1988; Eisel *et al.*, 1997). According to the United States Department of Agriculture (USDA, 1999), sausage makers should ensure that their products are not contaminated by pathogens such as *Listeria*, *E. coli* O157, *Salmonella*, *Trichinae* and *Staphylococcus* enterotoxin.

Escherichia coli is a highly recognized food pathogen that causes gastro-intestinal diseases in humans, especially *E. coli* O157:H7, which is frequently detected in the intestinal tracts and hide of cattle and pigs. This pathogen is also associated with ground beef products and other bovine products. The consumption of food and water contaminated with faecal matter of animals sometimes result in infections caused by *E. coli* strains (Li & Logue, 2005; Arthur, Kalchayanand, Bosilevac, Brichta-Harhay, Shackelford, Bono, Wheeler & Koohmaraie, 2008; Ateba & Bezuidenhout, 2008; Wong, MacDiarmid & Cook, 2009). According to Ateba & Bezuidenhout (2008), there is little information available on the prevalence of *E. coli* O157:H7 in the faeces of animals in South Africa and only a few outbreak cases caused by the pathogen are documented in South Africa since patients seldomly report their cases in hospital.

Staphylococci-contaminated food products that include red meat have been implicated in food-poisoning outbreaks (Shale, Lues, Venter & Buys, 2005). The presence of *Staph. aureus* can be used as indicator of personal hygiene and also is known to produce harmful enterotoxins. According to Shale *et al.* (2005) the South African legislation stipulates that a maximum count of 10^2 cfu/g in meat is acceptable. The amount of *Staph. aureus* required for production of toxin is $10^5 – 10^8$ cfu/g (Farber *et al.,* 1988; Nel *et al*., 2004; Shale *et al*., 2005). Studies conducted on the incidence of *Staph. aureus* in ground beef, broiler and processed meat in Pretoria, South Africa, showed that *Staph. aureus* was present in 23.4% samples of ground beef, 39.5% samples of broiler meat and 7.1% samples of processed meat products (Voster, Greebe & Nortje, 1994). In deboned meat cuts the counts of *Staph. aureus* has been shown to range from $3.8 \times 10^3 – 2.42 \times 10^5$ cfu/g (Nel *et al*., 2004). The prevalence of *Staph. aureus* in the meat and meat products is due to the fact that it is part of the microbiota of animals and humans (Voster *et al*., 1994; Nel *et al*., 2004). High counts of *E. coli* and *Staph. aureus* have been found in the intestine of cattle and broiler chickens. This may result in contamination of the meat during the slaughtering process due to the negligence of good manufacturing practice (GMP) and/or Hazard Analysis Critical Control Point (HACCP) systems (Voster *et al*., 1994; USDA, 1999).

Studies conducted by Mreme, Mpuchana & Gashe (2006) about the prevalence of *Salmonella* in raw minced meat, raw fresh sausage and raw burger patties from retail outlets in Gaborone, Botswana, showed that the prevalence of *Salmonella* was the highest in fresh sausages (26%) followed by minced meat (20%). Burger patty samples had very low prevalence with only 7% of the samples being positive for *Salmonella*. The most prevalent serogroups of *Salmonella* isolated in minced meat and sausages were B and C followed by E/G. The *Salmonella enterica* serovars isolated were *S.* Typhi, *S.* Enteritidis, *S.* Anatum, *S.* Reading, *S.* Melagridis, *S.* Typhimurium, *S.* Paratyphi B, *S.* Newport, *S.* Bovis-morbificans, *S.* Braenderup, *S.* Infantis, *S.* Tennessee and *S.* Montevideo. The presence of *S.* Typhi and Paratyphi in meat products indicates human origin and therefore poor personal hygiene during handling of the meat products. Farber *et al*. (1988) showed that there is no correlation between the presence of *Salmonella* spp. and other organisms such as *E. coli* and *Staph. aureus* on fresh sausage and frozen sausage.

Listeria monocytogenes is recognized as a human pathogen, and the occurrence of *L. monocytogenes* results in listeriosis, which is a gastrointestinal food infection that leads to bacteremia and meningitis in humans (Gombas, Chen, Clavero & Scott, 2003; Madigan, Martinko & Parker, 2003). This organism has been detected in a variety of ready-to-eat food products such as deli-style salad, processed meat, smoked fish, ice cream and cheese (Gombas *et al.*, 2003; Hoffman, Gall, Norton & Wiedmann, 2003; Madigan *et al.*, 2003). The levels of this organism that has been detected in food is not clear, but it has been suggested that levels of > 10^3 cfu/g *L. monocytogenes* may result in listeriosis (Gombas *et al.*, 2003).

Campylobacteriosis is transmitted through consumption of food contaminated with *Campylobacter* species (Hussain, Mahmood, Akhtar & Khan, 2007; Little, Richardson, Owen, de Pinna & Threlfall, 2008). *Campylobacter jejuni* is known to cause diarrhea/dysentery in children, and undercooked food such as poultry or other meats, raw milk and surface water have been implicated. Studies conducted in the United Kingdom (Little *et al.*, 2008) and Pakistan (Hussain *et al.*, 2007) on the prevalence of *Campylobacter* in raw red meat, showed that the meat was frequently contaminated with *Campylobacter jejuni*, followed by *Campylobacter coli*. The incidence of *Campylobacter* has been suggested to be due to cross-contamination during slaughtering processing in abattoirs, manual skinning and evisceration (Hussain *et al.*, 2007).

2.5. OTHER FACTORS AFFECTING THE SHELF-LIFE OF BOEREWORS

Bacterial growth in meat and meat products is affected by various intrinsic factors and extrinsic factors (Cannon, Morgan, Heavner, McKeith, Smith & Meeker, 1995). Intrinsic factors include pH, nutrient availability, water activity and oxidation/reduction potential while extrinsic factors include temperature, particle surface area, gaseous environment and package material (Cannon *et al.*, 1995; Eisel *et al.*, 1997; Romans *et al.*, 2001). The most important factors which have an influence on the shelf-life of sausage products, will be discussed in the following sections.

2.5.1. Temperature

Temperature is one of the most important factors that has an effect on the rate and extent of microbial growth. According to Madigan *et al.* (2003), microorganisms can be divided into four groups in relation to their temperature optima: psychrophiles, with low temperature optima; mesophiles, with mid-range temperature optima; thermophiles, with high temperature optima, and hyper-thermophiles, with very high temperature optima. Psychrophilic pathogens such as *L. monocytogenes*, mesophilic pathogens such as *Staph. aureus* and thermophilic pathogens such as *Clostridium botulinum* have been isolated from most food products (Romans *et al.*, 2001; Hoffman *et al.*, 2003).

Temperature has been shown to affect the lag phase and the generation time of microbial growth. When temperature is reduced below the optimum growth of a particular microorganism, the lag time/phase and generation time increases and growth rate decreases until, as the temperature approaches the minimum for growth, cell division ceases (Herbert & Sutherland, 2000).

According to Savic (1985) one easy way to increase sausage shelf-life is to lower the temperature of all rooms needed in the processing and storage of meats and sausages. With lower temperatures there are several important genera that can be isolated from meat and meat products, which include *Pseudomonas, Archromobacter, Micrococcus, Lactobacillus, Streptococcus, Leuconostoc, Pediococcus, Flavobacterium* and *Proteus.* The presence of these pychrotrophic bacteria in meat has raised concern about the quality and the safety of the meat and meat products and also resulted in economic losses (Steinbruegge & Maxcy, 1988). It has also been shown that some psychrophiles such as *L. monocytogenes* and *Yersinia enterocolitica* are capable of growing at temperatures as low as 2 °C (Romans *et al.*, 2001). According to Romans *et al.* (2001) meat shelf-life can be increased when it is stored or kept at -2 °C, which is the freezing point of meat. *Escherichia coli* has been used as a reference or indicator organism for the maximum temperature required as safe for holding or storing raw

meat and other unpreserved foods (Gill, Badoni & Jones, 2007). The presence of *E. coli* in meat products indicates temperature abuse during storage of meat and meat products, since *E. coli* has been shown to have a minimum growth temperature of 7 °C (Gill *et al.*, 2007). Boerewors is normally stored at 4 °C (Rust, 1987; Cocolin *et al*., 2004; Kim, 2006).

2.5.2. Water activity

Water activity (a_w) is a parameter which is commonly used to measure the amount of water available in food for microbial growth (Mossel, Corry, Struijk & Baird, 1995). According to Mossel *et al.* (1995) the Gram-negative bacteria are generally more sensitive to reduced a_w than Gram-positive bacteria. Most microorganisms are grouped according to their minimal requirement of a_w (Table 2.2.).

Table 2.2. Minimal a_w levels required for growth of food-borne microorganisms at 25°C (Mossel *et al*., 1995; Romans *et al.*, 2001; Kim, 2006).

Groups of Microorganisms	Minimal a_w
Most bacteria	0.91-0.88
Most yeasts	0.88
Regular moulds	0.80
Halophilic bacteria	0.75
Xerotolerant moulds	0.71
Xerophilic moulds and osmophilic yeasts	0.62-0.60

Fresh sausage such as boerewors has a a_w equal to or higher than 0.97, and most spoilage and pathogenic microorganisms such as *Pseudomonas* spp. and *E. coli* require a a_w of 0.96 – 0.97 for growth. *Staphylococcus aureus* is capable to survive at a a_w of 0.86. These facts make boerewors highly perishable products (Romans *et al.*, 2001; Cocolin *et al*., 2004; Thomas, Anjaneyulu & Kondaiah, 2008).

2.5.3. pH

Most of the microorganisms grow at a neutral pH of 7.0 (Cannon *et al*., 1995; Romans *et al.*, 2001; Kim, 2006). A reason for this is that the proteins are more heat stable at their iso-electric point, which is normally near neutral. The majority of bacteria function most efficiently in neutral environments and they can repair and recover easily when grown in neutral pH (Mossel *et al.*, 1995).

The normal pH of meat range from 5.2 – 5.7; the pH of meat products range from 4.8 – 6.8 while that of fresh sausage has been suggested to be at a pH of not less than 5.5 (Romans *et al.*, 2001; Cocolin *et al*., 2004). Each organism has a specific pH range in which growth is optimum. Bacteria can be grouped according to their required pH optimum. Bacteria that show growth in lower pH values are called acidophiles while those that grow in high pH values are called alkaliphiles (Madigan *et al.*, 2003). Few bacteria can grow in a lower pH range of about pH 4.0 while yeasts and moulds can thrive at this pH (Kim, 2006). Most bacteria grow well in food products at pH 5.0 or higher (Romans *et al.*, 2001; Madigan *et al.*, 2003).

Fresh sausages are more perishable when compared to fermented sausages. This is due to the higher pH (5.5) and a_w (0.97) of fresh sausage, and since the fermentation process by lactic acid bacteria is not involved in the making of the products (Cocolin *et al.*, 2004; Thomas *et al*., 2008).

2.5.4. Surface area

During the making of a boerewors sausage one of the processes that occurs is the mincing/grinding of the meat. The ground meat is then cased or stuffed in a natural casing or synthetic casing (Dyett & Shelley, 1966; Kuri, Madden & Collins, 1995; Romans *et al.*, 2001; Kim, 2006). It has been shown that ground beef poses more risks for contamination compared to intact muscle tissue because it can be contaminated throughout, due to the increased surface area and mixing during the grinding operation, and the transfer of product to product during processing may result in cross-contamination (Voster *et al*., 1994; Eisel *et al*., 1997). Microorganisms isolated during the process of ground beef processing, include many types of pathogenic microorganisms which have been isolated from raw beef, most notably *Salmonella* spp., *L. monocytogenes, E. coli* and *Campylobacter jejuni* (Eisel *et al*., 1997).

2.5.5. Gaseous environment and packaging material

The growth of bacteria is also determined by the presence of molecular oxygen. Most bacteria, yeasts and moulds grow well in the presence of oxygen and they are referred to as aerobic bacteria. Those organisms that do not need oxygen for growth are referred to as anaerobic bacteria, which include bacteria such as *Clostridium* that produce toxins in meat products stored under vacuum packaging (Romans *et al.*, 2001; Madigan *et al.*, 2003). The growth of spoilage and pathogenic microorganisms will depend on the type of storage packaging material (Rantsiou, Iacumin, Cantoni, Comi & Cocolin, 2005). Fresh sausages are normally packed in oxygen-permeable polyvinyl chloride (PVC) film or atmosphere packaging. With PVC film the product is exposed to oxygen, which will reduce the shelf-life of the sausage. With modified atmosphere packaging, oxygen concentrations are lower and have been replaced by carbon dioxide which will increase the shelf-life of meat products (Cannon *et al.*, 1995).

2.6. TRADITIONAL PRESERVATION METHODS OF BOEREWORS

Currently in South Africa boerewors is preserved with SO_2, which is added to the product in a sulphite salt form, namely sodium metabisulphite. The sulphite salts all share the ability to generate molecular SO_2, which correlate well with many of their preservative activities. For this reason it is possible to calculate treatment levels in foods and are, therefore, quoted as part per million (ppm) or mg/kg SO_2 (Gould & Russell, 2003). The DoH of South Africa (2001) stipulates that the product may contain SO_2 in a concentration that does not exceed 450 mg/kg or 450 ppm.

Dyett & Shelley (1966) showed that the use of SO_2 as preservative in fresh sausage showed lower total bacterial counts compared to the non-sulphur preserved sausages. The presence of 450 ppm has been suggested to inhibit the growth of pathogenic organisms such as *Staph. aureus* and *Salmonella* Typhimurium. Another study done by Bank & Board (1982), clearly demonstrated that sulphite caused a shift away from the Gram-negative dominated biota of unpreserved meat toward a Gram-positive biota largely consisting of *Brochothrix thermosphacta,* lactic acid bacteria, micrococci and yeasts. The Gram-positive biota grew more slowly than the Gram-negative ones, so that the shelf-life of the fresh sausage was extended when sulphite was added.

The antimicrobial effect of sulphite on fresh pork sausage stored at temperatures of 22 °C has been demonstrated by Dyett & Shelley (1966). Even at a high temperature such as 22 °C, the strong antimicrobial effect of sulphite was noticeable (Table 2.3). This result suggested that sausages containing a sulphite concentration greater or equal to 450 mg/kg had a lower aerobic count. The study also showed that the presence of 400 – 500 mg/kg SO_2 in minced meat had a negative effect on the growth of Gram-negative bacteria (Dyett & Shelley, 1966).

Table 2.3. Effect of added sulphite on microbial growth in fresh pork sausage (Dyett & Shelly, 1966; Gould, 2000).

Incubation time at 22 °C (days)	**Total aerobic count (millions/g) in sausage containing metabisulphite (SO_2; mg/kg), added at:**				
	0 mg/kg	**150 mg/kg**	**300 mg/kg**	**450 mg/kg**	**600 mg/kg**
1	136	78	2	3	0.2
2	256	243	73	5	2
3	608	691	445	62	39

Sulphur dioxide is a very reactive molecule and there are several factors that affect its effectiveness as an antimicrobial agent against Gram-positive microbes such as lactic acid bacteria. The pH is one of these factors since SO_2 exists in various forms and has two dissociation constants. Between pH 5.0 and 9.0 this substance exists as a mixture of bisulphite (HSO_3^-) and sulphite (SO_2^-). Below pH 5.0 the mixture changes to one of bisulphite ions and molecular SO_2 in solution. As the pH decreases, the proportion of molecular SO_2 increases and it is in this form that it has the most potent antimicrobial effect (Carr, Davies & Sparks, 1976). Other factors that affect the effectiveness of SO_2 are carbonyl compounds (keto- or aldehyde-groups) that bind with it. Thus for SO_2 to be effective, not only must the substrate be acidic, but fairly free of oxygen and sulphite binding compounds (Carr *et al.*, 1976).

The use of conventional preservatives such as SO_2 in meat products has raised consumer concern (Bañón, *et al*., 2007; Sebranek & Bacus, 2007). Sulphur dioxide in meat products is not completely destroyed during cooking. Some SO_2 is also liberated as gas during cooking, and this could give rise to respiratory problems (asthmatic people), thiamine absorption deficiency and disruption of carbohydrate metabolism, particularly with individuals who have an allergic reaction to SO_2 (McDonald, 1992; Bañón, *et al.*, 2007).

Other symptoms such as urticaria, abdominal pains, nausea, diarrhea, seizures and anaphylactic shock resulting in death have been recorded (Gunnison & Jacobsen, 1987).

2.7. NATURAL PRESERVATION METHODS

2.7.1. Lactic acid bacteria

The use of starter cultures such as lactic acid bacteria i.e. homofermentative lactobacilli and/or pediococci, and Gram-positive, catalase-positive cocci (GCC), i.e. non-pathogenic, coagulase-negative staphylococci and/or *Kocuria*, improves the quality and safety of the final product and standardize the production process (Leroy, Verluylen & De Vuyst, 2006). The starter culture is used mainly for fermented sausages, due to its abilities to lower the pH of the product and produce bacteriocin (Kim, 2006). Bacteriocins are antimicrobial peptides produced by lactic acid bacteria. Nisin and pediocin are well known bacteriocins. Nisin is produced by *Lactococcus lactis* and pediocin is produced by *Pediococcus acidilactici*, which have been shown to be effective against *L. monocytogenes* and other Gram-positive pathogens on meat surfaces (Siragusa, Cutter & Willett, 1999; Coma, 2008).

2.7.2. Plant extracts

Plant extracts such as rosemary, grape seed, tea and ginkgo biloba extract are used in a variety of food applications to preserve food quality. Some plant extracts such as tea and grape seed extracts have been suggested to add neutraceutical and health benefits (Jayaprakasha, Selvi & Akariah, 2003).

Grape seed extracts are rich in monomeric phenolic compounds such as catechin, epicatech and epicatechin-3-*O*-gallate, and in dimeric, trimeric and tetrameric procyanidins.

These compounds have been suggested to give grape seed extract antioxidant and antimicrobial activities (Bañón *et al*., 2007). Studies conducted on grape seed extract shows that it produces a high antioxidant activity in fish oil and frozen fish, cooked pork patties and cooked turkey (Bañón *et al.*, 2007).

The application of green tea at a concentration of at least 200 g/kg has been shown to increase the shelf-life of raw, frozen and cooked meat patties (Bañón *et al.*, 2007). Green tea also is rich in phenolic compounds such as epicatechin, epicatechin gallate, epidogalloctechin, teaflavin gallate, teaflavin monogallate A and B, and teaflavin digallate. Green tea and grape seed extracts have, however, been shown to be partially effective in increasing the shelf-life of meat. They are more effective when used in combination with lower concentrations of SO_2 (Bañón *et al.*, 2007).

Natural antimicrobials and antioxidants such as rosemary (*Rosmarinus officinals* L.) extract has been shown to be one of the strongest antioxidants in preventing microbial contamination in red meat packaged in modified atmosphere (Belantine *et al.*, 2006; Martínez, Cilla, Beltrán & Roncalés, 2007). The rosemary extract has been shown to contain several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmanariquinone and rosmaridiphenolic, which break free radical chain reactions by hydrogen donation (Georgantelis, Ambrosiadis, Katikou, Blekas & Georgakis, 2007). Rosemary extract has been used as a natural preservative in products such as fresh pork sausage in Greece (Georgantelis *et al.*, 2007).

Clove, rosemary, cassia bark and liquorice spice extracts have been shown to possess antimicrobial activities against *L. monocytogenes*. The combination of rosemary and liquorice spice extracts inhibits not only the growth of *L. monocytogenes*, but are also effective against species such as *E. coli*, *Pseudomonas fluorescens* and *Lactobacillus sake* in vacuum packaged hams and modified atmosphere packaged fresh pork chops (Zhang, Kong, Xiong, & Sun, 2009).

2.7.3. Essential oils

The use of essential oils is not only important for aromatic and medical purposes. Studies conducted on essential oils have shown that some essential oils have antimicrobial properties (Busatta, Vital, Popiolski, *et al.*, 2008). The problem associated with the use of essential oils is that they need to be applied in high concentrations for it to be effective. This may result in poor organoleptic properties of the food (Busatta *et al*., 2008; Coma, 2008). Some Gram-negative bacteria have been shown to be more resistant to essential oils than Gram-positive bacteria. This has been attributed to the double cell wall in Gram-negative bacteria which contains lipopolysaccharides (Oussalah, Callet, Saucier & Lacroix, 2007; Busatta *et al*., 2008).

Marjoram (*Origanum majorana*) has been shown to have antimicrobial activities *in vitro* due to the presence of terpinen-4-ol. The effectiveness of marjoram as an antimicrobial has been studied by Busatta *et al*. (2008). They showed that the amount of organisms reduced during a storage period of 35 days, and marjoram reduced the count of *E. coli* in fresh sausages *in vitro*. It was concluded that marjoram can be used to prolong the shelf-life of fresh sausages due to its bacteriostatic action at lower concentrations and its bacteriocidal action at higher concentrations.

2.7.4. Garlic

Garlic has also been studied because of its wide spectrum of action as an antibacterial, antiviral, antifungal and antiprotozoal agent and also for its medical benefits on the immune and cardiovascular systems (Sallam *et al.*, 2004). Fresh garlic and its powder has been shown to maintain the aerobic plate count for chicken sausages below the maximum permissible limit (MPL) or the upper limit during cold storage at 3 °C. The MPL is suggested to be 7 $\log_{10}$ cfu/g (Sallam *et al*., 2004; Martínez *et al.*, 2007). According to Coma (2008), garlic oil

and oregano are effective against species such as *Staph. aureus*, *Staph. enteritidis*, *L. monocytogenes*, *E. coli* and *Lactobacillus plantarum* in whey protein-based film.

2.7.5. Chitosan

Chitosan is a deacetylated form of chitin, and is composed of polymeric 1,4-linked 2-amino-2-deoxy-β-D-glucose. It is derived from the shell of crabs and shrimps and the cell wall of fungi (Roller, Sagoo, Board, O'Mohony, Caplice, Fitzgerald, Fogden, Owen & Fletcher, 2002). Chitosan has been shown to exhibit antimicrobial activities against a range of foodborne microorganisms (Georgantelis *et al.*, 2007). Chitosan has the ability of disrupting the permeable barrier of the outer membrane of Gram-negative bacteria, and also bind the trace elements. This has expanded the use/application of chitosan as an antimicrobial substance in foods (Georgantelis *et al.*, 2007; Coma, 2008; Kanatt, Chander & Sharma, 2008). A study conducted by Kanatt *et al.* (2008) showed that when chitosan is used in combination with mint extract, it was more effective against organisms such as *Bacillus cereus*, *Staph. aureus*, *E. coli*, *Ps. fluorescens* and *S.* Typhimurium. Chitosan also has antioxidant properties. It was observed that 1% chitosan used in minced meat resulted in a 70% decrease in the 2-thiobarbituric acid value of meat after 3 days of storage at 4 °C (Georgantelis *et al.*, 2007). Other functional properties of chitosan include its ability to lower serum cholesterol and the intestinal lipid binding effect (Soultos, Tzikas, Abrahim, Georgantelis & Ambrosiadis, 2008)

2.7.6. Citrox

Citrox is a phyto-pharmaceutical of organic origin, and is highly concentrated with safe antimicrobial and fungicidal ingredients, effective in killing common pathogens such as *E. coli* and *Samonella*. Citrox contains vegetable glycerin, citrus seed extract and pulp and Wysong oxherphol™ antioxidant (vitamin E, tocopherol epimers, fat-soluble vitamin C, organic chelators, and natural botanical oleoresins) and contains no additives as ingredients (Wysong Corporation, 2003). The literature is depleted on the effectiveness of citrox.

Wysong Corporation (2003), however, claims that citrox is effective against more than 800 bacterial and viral strains, 100 strains of fungi and yeasts, as well as a number of single-celled and multi-celled organisms. Included in the list are *Candida albicans* (common yeast infections), parasites, *Staph. aureus*, *S.* Typhi, and *E. coli*. The mode of action of citrox has been suggested to occur in the cytoplasmic membrane of the microbe, whereby it prevents the uptake of crucial amino acids in the membrane causing disorganization, and this allows the cell's contents to leak or lyse, thus inactivating the microbe. Citrox has also been suggested to prevent uptake of amino acids, but the exact mechanism are unknown. It is speculated that there is an inhibition of the enzymatic activities of the affected cell membrane (Wysong Corporation, 2003).

2.8. OTHER EMERGING PRESERVATION METHODS

There are a few methods that have been implemented in the food industry to increase the quality and shelf-life of food products. Hurdle technology and Hazard Analysis Critical Control Point (HACCP) are two of the major methods.

2.8.1. Hurdle technology

Hurdle technology according to Karthikeyan, Kumar, Anjaneyulu & Rao (2000), is a combination of factors which are used to increase the shelf-life of food products. The most important hurdle technology factors used for food preservation includes temperature, a_w, pH, redox potential, preservative, and competitive microorganisms such as lactic acid bacteria (Karthikeyan *et al*., 2000; Kim, 2006). The microbial stability and safety of most food depends on a combination of these factors such as reducing a_w, lowering pH and reheating which have been shown to be sufficient for inhibiting the growth of yeasts and moulds in fresh pork sausages for 3 days. When potassium sorbate solution was added as a preservative, the growth of yeasts and moulds were inhibited for about 9 days at ambient and refrigeration temperatures (Thomas *et al.*, 2008).

Hurdle technology has been applied in meat and meat products such as Chinese sausage, Chinese raw ham and traditional Chinese meat products (Karthikeyan *et al.*, 2000). Huffman (2002) has reviewed the use of this technology to decontaminate carcasses and fresh meat before processing into meat products. The technology has been divided into pre-harvest reduction of bacteria on livestock and post-harvest decontamination techniques. The pre-harvest reduction of bacteria on livestock involves the diet of the cows (feed of the animals should have lower counts of *E. coli* O157:H7), competitive exclusion (addition of probiotics in feed), drinking water treatment, chlorine administration to animals in the form of sodium chloride (NaCl) and other pre-harvest technologies (vaccines, bacteriophage). The post-harvest decontamination techniques involved the use of chemical dehairing, hot water rinsing, steam pasteurization, steam vacuum, chemical rinses and spraying treatment with lactoferrin prior to chilling of the carcass. According to Huffman (2002) this technique or treatments resulted in the reduction of spoilage and pathogenic microorganisms in fresh meat.

2.8.2. Hazard Analysis Critical Control Point (HACCP)

According to the Food and Drug Administration (FDA, 2001), HACCP involves seven principles that can be implemented to increase the safety of food products:

- Hazard analysis
- Identifying the hazard
- Establish preventive measures with critical limits for each control point
- Establish procedures to monitor the critical control points
- Establish corrective actions to be taken when monitoring shows that a critical limit has not been met
- Establish procedures to verify that the system is working properly
- Establish effective recordkeeping to document the HACCP system

Studies conducted by Smith, Hussain & Millward (2002) to determine the impact of using a HACCP plan in retail butchers' premises, showed that if the HACCP plan is followed, it results in hygienic premises. Inclusion of HACCP training associated with

support to the personnel results in an even better improvement in the hygiene of the premises. The study of Cates, Anderson, Karns & Brown (2001) showed that the use of a traditional inspection system of the slaughtering process of poultry is not as efficient in the control of the presence of pathogenic microbes, compared to the HACCP-based slaughtering project.

2.9. CONCLUSIONS

Boerewors forms a very important part of the diet of the South African consumer. The shelf-life of the product is, therefore, an essential aspect. Boerewors is a perishable product due to the high surface area created in the muscle tissue of the animal during communution which involves grinding or cutting of meat. Pathogenic organisms such as *E. coli*, *Staphylococcus*, *Salmonella* and *L. monocytogenes* and spoilage organisms such as *Pseudomonas* sp., *Brochothrix thermosphacta*, yeasts and moulds have an opportunity to prevail in the product. This is due to contamination of the product which could occur as pre- or post-contamination of the meat product. Pre-contamination which may involve the animals in the field, the environment, feed of the animal, etc. and post-contamination may involve the process involved in the manufacturing of boerewors, the ingredients (meat, casing and additives) used and the personnel involved in the production of the product.

The use of traditional SO_2 as a preservative of boerewors products to increase the shelf-life can have a detrimental effect on the asthmatic consumer, even though the legislation of South Africa allows the use of SO_2 in meat and meat products in concentrations of not more than 450 mg/kg. There is a lack of knowledge about the effect of SO_2 on food products consumed by South African consumers. Sulphur dioxide is a well known antimicrobial agent, effective against pathogen and spoilage organisms, but it is a chemical preservative. Consumers have become more informed about the use of chemical additives in food products and they prefer organic foods or naturally preserved food products. The use of natural preservatives, such as grape seed extract, rosemary extract, and chitosan, have shown antimicrobial and antioxidant properties in some foods. The evaluation of some of these natural preservatives in the production of boerewors will, therefore, proof worthwhile.

CHAPTER 3

MICROBIAL QUALITY EVALUATION OF BOEREWORS (TRADITIONAL FRESH SAUSAGE) IN BLOEMFONTEIN, SOUTH AFRICA

3.1. INTRODUCTION

Boerewors is a popular traditional fresh sausage consumed in South Africa. According to the South African Foodstuff, Cosmetic and Disinfectant Act, Regulation No R2718), boerewors is composed of 90% total meat (beef or/and pork) and a fat content of not more than 30%. Other ingredients in the sausage are cereal products (starch), vinegar, spices and water, which are contained in an edible casing. The product is preserved with 450 ppm SO_2 (Department of Health [DoH] of South Africa, 1990; DoH, 2001).

The microbial quality of the product is dependant on the chemical and physical characteristics. Food can be categorised as perishable (mostly fresh foods), semi-perishable (e.g. potatoes) and stable or non-perishable (e.g. flour) products (Madigan, Martinko & Parker, 2003). Fresh sausages have been categorised as perishable food products due to their high water activity and pH (Romans, William, Carloson, Greaser & Jones, 2001; Cocolin, Rantsion, Iacumin, Urso, Cantoni & Comi, 2004). Spoilage and pathogenic organisms have frequently been isolated from fresh sausage products (Farber, Malcolm, Weiss & Johnstone, 1988; Cohen, Filliol, Karraouan, Badri, Carle, Ennaji, Bouchrif, Hassar & Karib, 2008). Pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* and spoilage organisms such as *Pseudomonas*, *Proteus*, *Sporotrichium* and *Candida*, cause spoilage in food (Voster, Greebe & Nortjé, 1994; Madigan *et al.*, 2003). Presence of these organisms in the sausage products has been suggested to be through contamination of meat, spices, other ingredients, equipment and handlers during processing (Sachindra, Sakhare, Yashoda & Rao, 2005; Cohen *et al.*, 2008).

According to Warburton, Weiss, Purvis & Hill (1987), at least 238 incidents of foodborne disease were associated with sausages between 1973 and 1978 in Canada. The annual foodborne disease estimation for the United States, has been estimated to be 63,000 cases per year due to consumption of meat (especially ground meat) contaminated with *E. coli* O157:H7 and about 110,000 cases per year caused by other enteropathogenic *E. coli* (Madigan *et al.*, 2003). In South Africa there is a lack of information about the prevalence of foodborne diseases from meat products such as traditional boerewors. There is a need for quantifying the situation regarding the microbiological quality, foodborne diseases and verifying the necessity for the implementation of methods to prevent and control outbreaks in South Africa (Voster *et al.*, 1994).

The purpose of surveying the quality of food is essential for providing data that can be used for setting a regulation standard and improving food safety in South Africa and/or for the implementation of hazard analysis critical control point (HACCP) or good manufacturing practices (Brown, Gill, Hollingsworth, Nickelson, Seward, Sheridan, Stevenson, Sumner, Theno, Usborne & Zink, 2000; Phillips, Jordan, Morris, Jenson & Sumner, 2006). The objective of this study was to assess the microbial quality of boerewors (Farmers-sausage) produced from the different regions of Bloemfontein (north, south, east, west and central), comparing them to international guidelines on fresh sausages, and also comparing the boerewors microbial quality produced from supermarkets and small butchery shops in Bloemfontein, South Africa.

3.2. MATERIALS AND METHODS

3.2.1. Sampling Procedure and Preparation

All 74 of the butcheries (supermarkets and butcher shops) in Bloemfontein were classified in demographic areas north, south, east, west and central. Boerewors were purchased from 37 (50%) of these butcheries. The butcheries were randomly selected using

the random number generation analysis tool of Microsoft Excel™. Each butchery was sampled once, the samples were transported in a cooler box with ice blocks and analysed within 4 hours of sampling. Ten gram from each sample was aseptically removed using a sterile scissor and forceps and was placed in 90 ml phosphate water buffer in sterile WhirlPak bags and homogenized for 2 min in a stomacher.

3.2.2. Microbial analyses

The homogenised samples were serially diluted (1:10) in phosphate buffer and 1 ml of the appropriate dilution was plated as follows for the different microbial evaluations:

3.2.2.1. Aerobic plate counts (APC)

The dilutions were pour-plated on standard plate count agar (SPCA; Oxoid CM0463). The plates were incubated at 32 °C for 48 h. After incubation the colonies were enumerated by means of a colony counter (Harrigan, 1998).

3.2.2.2. Psychrotolerant plate counts (PPC)

The dilutions were pour-plated on standard plate count agar (SPCA; Oxoid CM0463). The plates were incubated at 4 ± 1 °C for seven days (Nortjé, Nel, Jordaan, Naudé, Holzapfel & Grimbeek, 1989).

3.2.2.3. Enterobacteriaceae counts

The dilutions were pour-plated and overlayed with violet red bile glucose agar (VRBG; Oxoid CM0485). The plates were incubated at 37 °C for 24h. Colonies of *Enterobacteriaceae* produced purple red colonies with a diameter of 0.5 mm or greater and sometimes surrounded by a red zone of precipitated bile (Harrigan, 1998).

3.2.2.4. Coliform counts and Escherichia coli

The dilutions were pour-plated on violet red bile agar containing MUG (VRB+MUG; Oxoid CM0978). Plates were incubated at 37 °C for 24h. After incubation the coliforms were enumerated by means of a colony counter, while the presence of *E. coli* was confirmed by fluorescence of the colonies under an ultraviolet light (Harrigan, 1998).

3.2.2.5. Staphylococcus aureus

Enumeration of *Staph. aureus* was conducted by using the spread plate (0.1 ml aliquots) method on a pre-dried surface of Baird-Parker agar (Oxoid CM0275) and the plates were incubated at 37 °C for 24h. *Staphylococcus aureus* typically forms colonies that are 1.0 - 1.5 mm in diameter, black, shiny, convex with a narrow white entire margin and surrounded by clear zones extending 2-5 mm into the opaque medium (Harrigan, 1998).

3.2.2.6. Yeasts and Moulds

The dilutions were pour-plated on rose bengal chloramphenicol agar (RBC; Oxoid CM0549). The plates were incubated at 25 °C for 4 - 5 days. After incubation the colonies were enumerated by means of a colony counter (Harrigan, 1998).

3.2.3. Statistical analysis

The microbial counts obtained for the boerewors samples were transformed to log cfu/g and subjected to analysis of variance (ANOVA; NCSS, 2007). The microbial quality of the boerewors samples obtained from the different regions in Bloemfontein were compared by means of the Tukey-Kramer multiple comparison test (NCSS, 2007).

The microbial quality of the boerewors samples obtained from the supermarkets and small butcher shops were statistically compared using a two-sample t-test on counts transformed to log cfu/g. The minimum level of statistical significance was set at 5% (NCSS, 2007).

3.3. RESULTS AND DISCUSSION

3.3.1. Microbial Quality of the Regions

The mean microbial counts obtained for all the retail outlets sampled in this study, are given in Table 3.1., while the mean microbial counts for the retail outlets in different regions are given in Table 3.2.

3.3.1.1. Aerobic plate count (APC)

The mean aerobic plate count (APC) of the 37 retail outlets was 6.59 ± 1.09 log cfu/g (Table 3.1). The northern region showed the highest mean APC of 6.97 ±1.44 log cfu/g (Table 3.2). The lowest mean APC was seen in the western region with a count of 6.38 ±0.96 log cfu/g (Table 3.2). However, there was no significant difference ($p>0.05$) in mean APC between the various regions (Table 3.2).

Table 3.1. The mean microbial quality of boerewors from 37 (50%) retail outlets in Bloemfontein.

Boerewors organism recoveries (Mean ± SD log cfu/g)	
APC*	6.59 ± 1.09
PPC*	7.13 ± 1.09
Enterobacteriaceae count	3.88 ± 1.18
Coliform count	4.60 ± 1.11
Yeasts & Moulds count	5.02 ± 0.89

*APC: Aerobic plate count; PPC: Psychotolerant plate count

Table 3.2. The mean microbial quality of boerewors from the different regions in 50% of Bloemfontein retail outlets.

	Regions (Mean ± SD log cfu/g)					Significance level (p-value)
	Northern	**Southern**	**Central**	**Western**	**Eastern**	
n*	6	13	6	6	6	
APC*	6.97 ± 1.44	6.45 ± 1.14	6.92 ± 1.15	6.38 ± 0.88	6.41 ± 0.84	0.7778
PPC*	7.00 ± 1.03	7.11 ± 1.22	7.26 ± 0.98	6.73 ± 1.16	7.56 ± 0.90	0.7697
Enterobacteriaceae	3.05 ± 0.75	4.27 ± 0.77	3.65 ± 1.58	4.11 ± 0.98	3.84 ± 1.75	0.3107
Coliforms	3.96 ± 0.30	4.67 ± 0.93	4.43 ± 2.25	4.87 ± 0.51	4.99 ± 0.73	0.5342
Yeasts & Moulds	5.04 ± 0.99	5.02 ± 0.91	5.09 ± 0.86	4.79 ± 1.13	5.16 ± 0.63	0.9704

*n: Number of samples; APC: Aerobic plate count; PPC: Psychrotolerant plate count

The South African Foodstuffs, Cosmetics and Disinfectant Act (Act Number 54 of 1972) and regulations made under the act "Regulations Governing Microbial Standards for Foodstuffs and Related Matters", R692, 16 May 1997 (DoH, 2001), does not specify any microbial quality limits for minced beef, boerewors, raw meat or processed beef/pork products. The National Directory of Veterinary Service of South Africa only stipulate the microbiological standards regarding the maximum allowed levels for chilled export and frozen export meat which are 4.00 log cfu/g and 5.00 log cfu/g (Department of Agriculture [DoA], 2007a).

The accepted upper limit regarding aerobic plate counts for fresh sausages is 7.00 log cfu/g (Nortjé, Voster, Greebe & Steyn, 1999). About 29.73% (11 out of 37) retail outlets did

not meet these limits. According to Shapton & Shapton (1991), the maximum APC recommended for fresh sausages is 6.00 log cfu/g according to the Brazilian standard. When the APC of this study is compared with the Brazilian standard, about 67.57% retail outlets (25 out of 37) did not meet this specification.

However, in a study conducted in the same regions of Bloemfontein in 2004, the APC for boerewors were shown to have a mean of 5.81 log cfu/g ranging from 4.55 – 6.89 log cfu/g (Charimba, 2004). These APC mean counts were lower than that observed in the current study. Possible reasons for the difference could be ascribed to samples being taken in different seasons. The current study was performed in summer while the other study was undertaken in the winter months. In a study on Egyptian fresh sausages, the APC ranged from 4.04 – 8.00 log cfu/g. The high counts have been suspected to be due to mishandling of the product during processing and improper storage conditions (El-Khateib, 1997).

3.3.1.2. Psychrotolerant plate counts (PPC)

The mean PPC for all retail outlets were 7.13 ± 1.09 log cfu/g (Table 3.1). The western region showed the lowest mean PPC of 6.73 ±1.27 log cfu/g (Table 3.2) The eastern region showed the highest mean PPC of 7.56 ±0.90 log cfu/g but the counts were not significantly different between these regions ($p>0.05$).

The PPC are essentially for assessing the microbial activity on meat and meat products stored at lower temperatures. There are, however no specific guidelines or standards that regulates the presence of PPC in meat and meat products (DoA, 2007b). This may be because there is no specific or uniform methodology set out for enumerating psychrotelerant bacteria (Harrigan, 1998; Jay, 2002).

According to Sunki, Annapured & Rao (1978) and Nortjé *et al.* (1989), bacterial spoilage of meat and meat products is caused mostly by psychotrophic Gram-negative

bacteria. Psychrotolerant bacteria such as *Pseudomonas* spp., *Brochothrix thermosphacta*, *Shewanella* spp. or *Aeromonas* spp. and other *Enterobacteriaceae* have been shown to have the ability to grow in chill temperatures (Nortjé, Nel, Jordaan, Badenhorst & Holzapfel, 1990; Holley, Peirson, Lam & Tan, 2004).

A microbial survey conducted on raw ground meat in Ohio, United States, had a mean PPC of 7.79 log cfu/g and a mean APC of 7.92 log cfu/g (Charmber, Brechbill & Davi according to Elmali & Yaman, 2005). These counts were similar to those observed in this study (Table 3.1).

The mean PPC counts of the regions were higher than the mean APC for all the regions. Álvarez-Astorga, Capita, Alonso-Calleja, Morena & García-Fernandez (2002) made a similar observation with chicken and chicken products. This was ascribed to the psychrotolerant bacteria dominating other bacteria during the storage of meat and meat products at lower temperatures.

3.3.1.3. Enterobacteriaceae counts

The mean *Enterobacteriaceae* count for all the retail outlets were 3.88 ± 1.18 log cfu/g (Table 3.1). The *Enterobacteriaceae* count showed no significant difference ($p > 0.05$) between regions, although the northern region had the lowest mean of 3.05 ±0.75 log cfu/g and the southern region had the highest mean of 4.27 ±1.04 log cfu/g (Table 3.2).

The *Enterobacteriaceae* count are used as a hygiene indicator of foods from animal origin (Arthur, Bosilevac, Nou, Shackelford, Wheeler, Kent, Jorni, Pauleng, Allen & Koohmaraie, 2004; Crowley, Cogney, Sheridan, Anderson, McDowell, Blair, Bishop & Duffy, 2005). No standards for *Enterobacteriaceae* in fresh sausages are available in South Africa. The Swedish standard for the presence of *Enterobacteriaceae* in fresh sausages is 5.00 log cfu/g (Shapton & Shapton, 1991). About 18.91% (7 out of 37 butcheries) did not

meet these specifications (Table 3.1). The retail outlets of the northern region were the only outlets that did meet these specifications (Table 3.2).

3.3.1.4. Coliform count and Escherichia coli

The mean coliform count for all the retail outlets was 4.60 ± 1.11 cfu/g (Table 3.1). The northern region showed the lowest mean count of 3.96 ±0.30 cfu/g, and the eastern region showed the highest mean count of 4.99 ±0.73 cfu/g (Table 3.2). There were no significant ($p>0.05$) differences between the mean coliform counts of the different regions.

Since no standards exist for South African meat products, the Swedish guidelines were also followed for the coliform count which is 5.00 log cfu/g (Shapton & Shapton, 1991). Of the 37 outlets, 13 (35.13%) did not meet this specification (Table 3.1). Again, the northern region was the only region that conformed to these guidelines (Table 3.2).

Other guidelines for coliforms have been proposed by researchers which state that about 2.70 log cfu/g of coliforms in fresh and frozen sausage is satisfactory and counts which are over 2.70 and less than 3.70 log cfu/g are acceptable, but further investigation is required (Golden & Elliott according to Farber, Malcolm, Weiss & Johnstone, 1988).

The incidence of *E. coli* in 50% of the outlets in the five Bloemfontein regions, is given in Table 3.3. *Escherichia coli* was detected in 18.92% (7 out of 37) butcheries. The central region showed the highest incidence of *E. coli* with about 50% (3 out of 6) of the outlets positive. The northern and eastern regions had 16.67% (1 out of 6) outlets which were positive for *E. coli*, while the southern region had 15.38% (2 out of 13) outlets positive. The western region was the only region with no *E. coli* incidence. The reason for the high incidence of *E. coli* in the central region may possibly be that this is the region where the lower/middle class purchases their food and the hygienic standard of premises and/or equipment may be compromised.

Table 3.3. The presence of *E. coli* in boerewors from the different regions in 50% of Bloemfontein outlets.

***E. coli* incidence**			
Regions	**n**	**Number of butcheries detected**	**Percentage of butcheries (%)**
Northern	6	1	16.67
Southern	13	2	15.38
Central	6	3	50
Western	6	0	0
Eastern	6	1	16.67
Total	**37**	**7**	**18.92**

n: Number of samples

According to Nel, Lues, Buys & Venter (2004), the maximum limit of *E. coli* in meat and meat products should not be more than 10 cfu/g (1 log cfu/g). According to the DoH of South Africa (2001), *E. coli* type I and other pathogens should not be present in any of the perishable meat products. In boerewors samples where *E. coli* was detected, high counts of APC and/or *Enterobacteriaceae* were also observed. Although Arthur *et al*. (2004) stated that there is no correlation between the level of pathogens and the counts of aerobic bacteria and *Enterobacteriaceae*, and that these indicator organisms cannot be used for direct indications for the presence or absence of *E. coli* O157:H7, the indicator organisms can be useful as a guideline for reduction of *E. coli* contamination.

3.3.1.5. *Staphylococcus aureus*

The counts for *Staph. aureus* were less than 1 log cfu/g (10 cfu/g) for all the regions. According to Shale, Lues, Venter & Buys (2005) the South African legislation stipulates that a maximum count of 2.00 log cfu/g in meat is acceptable for *Staph. aureus*. Farber *et al.* (1988) have also suggested that counts of 2.00 log cfu/g are satisfactory, but counts which are more than 2.00 log cfu/g and less than 3.00 log cfu/g are acceptable but require investigation. In the case where counts are more than 3.00 log cfu/g, production should be stopped. All the outlets in the Bloemfontein regions conformed to the South African legislation.

In other studies, counts of 0.21 and 3.80 log cfu/g for *Staph. aureus* have been observed in ground meat and raw meat balls. The high number of *Staph. aureus* in raw meat balls was suspected to be due to the ingredients used to make the product and personal hygiene (Elimali & Yaman, 2005).

3.3.1.6. Yeasts and Moulds

The mean yeasts and moulds count for all retail butcheries was 5.02 ± 0.89 cfu/g (Table 3.1). The eastern region had the highest mean count of 5.16 ±0.63 cfu/g, but which was insignificant ($p>0.05$) while the western region showed the lowest mean count of 4.79 ±1.23 cfu/g, which was also insignificant ($p>0.05$) (Table 3.2). According to the Brazilian guideline for yeasts and moulds count, fresh sausages should have counts less than 2.00 log cfu/g but this guideline are under review (Shapton & Shapton, 1991). Not one of the samples analysed in this study conformed to this guideline (Tables 3.1 and 3.2).

The high counts of yeasts and moulds in the boerewors may have resulted from the initial high counts of yeasts and moulds in the minced meat that was used to prepare the sausages. Counts of yeasts and moulds with a mean of 3.57 log cfu/g have been reported in ground beef (Elimali & Yaman, 2005). The high counts of yeasts and moulds in meat and meat products have been associated with the general hygienic standard of the environment,

since these organisms are capable of multiplying in warm moist areas and on equipment which is not easily cleanable (O'Toole, 1995).

3.3.2. Comparison of Supermarket and Butcher Outlets

The microbial quality of boerewors purchased from butcher and supermarket outlets, is given in Table 3.4. The mean APC, coliform and *Enterobacteriaceae* count of the butcher outlets was higher when compared to that of the supermarkets although there were no significant differences ($p>0.05$) between the mean counts of the different retail types. The coliform mean count for butcheries was significantly ($p<0.05$) higher when compared to that of supermarkets. The supermarket mean PPC and yeasts and moulds counts showed insignificantly ($p>0.05$) higher counts when compared to the butcher shops.

Table 3.4. Comparison of the microbial quality of boerewors purchased from butcher shops and supermarkets.

	Retail Type		
	Butcher shops	**Supermarkets**	**Significance level (p-value)**
n*	20	17	
APC*	6.60 ± 1.10	6.58 ± 1.12	0.9564
PPC*	7.12 ± 1.16	7.14 ± 1.02	0.9516
Enterobacteriaceae	4.08 ± 1.26	3.61 ± 1.05	0.2315
Coliforms	**4.99 ± 0.91**	**4.09 ± 1.17**	**0.0124**
Yeasts & Moulds	4.85 ± 0.85	5.25 ± 0.93	0.1800

*n: number of samples; APC: Aerobic plate count; PPC: Psychrotolerant plate count

The results obtained in this study were contradictory to those of Kammenou, Metaxopoulos & Drosinos (2003) which studied the microbial quality of minced beef from butcher shops and supermarkets. They found high mean APC and *Enterobacteriaceae* counts for minced meat purchased in supermarkets. The possible explanation was that minced meat is prepared in butcher shops upon purchase and supermarkets are prepared in advance. However, with boerewors, the product is prepared in advance in both the butcher shops and

supermarkets. The results in this study are, therefore, an indication that the hygienic conditions in supermarkets are better than those in butcheries.

3.4. CONCLUSIONS

The results of this study demonstrated that the microbial quality of boerewors sold in the different regions of Bloemfontein retail outlets did not differ significantly between the regions. The incidence of *E. coli* in the central region may indicate that the socio-economic status has an effect on the quality, though the socio-economic status was not investigated in this study. There is, therefore, a need for further investigating the correlation between the quality of meat products and socio-economic status.

The significantly high coliform counts in boerewors purchased from the butcher shops indicate that there is poor hygienic conditions compared to the supermarkets. This may be related to staff being trained in hygienic practices in the corporate supermarket environment, while no training or very little training takes place in butcher shops.

CHAPTER 4

EFFECT OF NATURAL PRESERVATIVES ON THE MICROBIAL QUALITY, LIPID STABILITY AND SENSORY EVALUATION OF BOEREWORS

4.1. INTRODUCTION

In recent years there has been an increase in consumer awareness regarding the use of chemical additives in food and food products (Tiwari, Valdramidis, O' Donnell, Muthukumarappan, Bourke & Cullen, 2009). This has resulted in an increase in research on natural additives, such as using plant and animal derivatives (Ennajar, Bouajila, Labrini, Mathieu, Abderraba, Raies & Romdhane, 2009). The description of food quality differs between consumers and the food industry. Consumers describes food quality based on visible (intrinsic and extrinsic attributes) characteristics such as appearance, colour, odour, flavour and size of the food and this description is affected by the end use of the product by consumers, whereas the food industry describes food quality based on the microbiological, physiochemical and also sensory attributes of the food (Northern, 2000; Rodríguez, Lupín & Lacaza, 2006; Waskar, Devangare, Gosavi, Ravikanthi, Maini & Rekhe, 2009).

Boerewors is a traditional fresh sausage produced in South Africa and made from pork and beef meat. It is currently preserved with 450 mg/kg SO_2 (450 ppm SO_2; DoH, 1990). Sulphur dioxide has been shown to lower the total bacteria counts and also inhibit the growth of Gram-negative bacteria (Dyett & Shelley, 1966; Roller, Sagoo, Board, O'Mahony, Caplice, Fitzgerald, Fogden, Owen & Fletcher, 2002). Sulphur dioxide is known for giving the sausages the bright red colour, which is an essential parameter that consumers use when purchasing meat and meat products (Peña-Edgido, García-Alonso & Garćia-Moreno, 2005; Ismail, Lee, Ko & Ahn, 2009). In countries such as the United States the use of sulphite agents in meat and other foods (fresh fruits and vegetables) are not permitted (Roller *et al.*, 2002; Peña-Edgido *et al*., 2005). This is due to its relation to health problems such as allergies (asthmatic attacks) and thiamine (Vitamin B_1) absorption deficiency. Thiamine is an

essential co-factor needed in carbohydrate metabolism (McDonald, 1992; Roller *et al*., 2002; Bañón, Díaz, Rodríguez, Garrido & Price, 2007).

Natural preservatives such as rosemary (*Rosmarinus officinalis* L.) extracts have been shown to have anti-flammatory, anitcarcinogenic, antidiuretic and hepatolonic protective properties (Ho, Ferraro, Chen, Roser & Huang, 1994; Offord, 2004). Rosemary is composed of carnosel, carnosol, carnisic acid, rosmaric acid, urosolic acid, betulinic acid, α-pinene, camphor, and bornyl acetate. Rosemary extract also possesses antioxidant and anti-microbial properties. The diterpenes, carnosol and carnosic acid, have been shown to account for the antioxidant properties (Offord, 2004, Rižnar, Čelan, Knez, Škerget, Bauman & Glaser, 2006). The phenolic compounds in rosemary extract are believed to enhance anti-microbial properties by affecting the functioning of the bacterial cellular membrane, the synthesis of DNA, RNA, protein and lipids, and the functioning of the mitochondrion (Belantine, Crandall, O'Bryan, Duong & Pohlman, 2006).

Chitosan is one of the novel preservatives that has been studied, and has Generally Recognised as Safe (GRAS) status. It is a deacetylated form of chitin derived from the shell of crabs and shrimps and the cell wall of fungi (Roller *et al*., 2002; Soultos, Tzikas, Abrahim, Georgantelis & Ambrosiadis, 2008; Aldemir & Bostan, 2009). It consists of polymeric 1,4-linked 2-amino-2-deoxy-β-D-glucose (Roller *et al*., 2002). Chitosan has been shown to have a minimum inhibitory concentration (MIC) ranging from 0.01 to 1.0%, which is effective against the growth of bacteria, yeasts and fungi (Roller *et al*., 2002; Soultos *et al.*, 2008). Chitosan also possess antioxidant activities, water binding, chelating and emulsifying capacities. It also has health benefits and acts as a dietary fibre, binding intestinal lipid and lowering the effect of serum cholesterol (Roller *et al.*, 2002; Georgantelis, Ambrosiadis, Katikou, Blekas & Georgakis, 2007; Soultos *et al.*, 2008). The mechanisms of chitosan as an anti-microbial agent has been suggested to be due to the oligomeric chitosan that can bind DNA and resulting in inhibition of mRNA synthesis, and also due to cell membrane disruption whereby the cationic groups of chitosan interact with the anionic groups of the microbial cell membrane (Aldemir & Bostan, 2009).

Lipid oxidation is one of the main parameters that affect the quality of meat and meat products. Lipid oxidation results in the development of unacceptable organoleptic characteristics such as rancid flavour, colour, texture and odour deterioration. Products produced from the oxidation reactions may also pose health risks (carcinogenic, low absorption of fat soluble vitamin), whereas microbial growth causes spoilage and foodborne diseases (Rižnar *et al.*, 2006; Georgantelis *et al*., 2007). Controlling both lipid oxidation and preventing microbial growth will have an increase in shelf-life. The use of natural preservatives or additives in food products can provide beneficial effects to consumers and also to the food industry.

There are different methods that can be used to reduce oxidation and microbial growth in meat such as lowering the temperature, pH, a_w, inactivation of enzymes, using suitable packaging material, and controlling packaging atmosphere (Cannon, Morgan, Heavner, McKeith, Smith & Meeker, 1995). Not all of these methods are suitable for preserving the quality of fresh sausages such as boerewors. The objectives of this study was to study the effectiveness of rosemary extract (derived from a plant) preservative, chitosan (derived from crabs, shrimps and fungi) preservative and SO_2 as a conventional preservative, applied individually and in combination with each other, on the microbial quality, colour stability, lipid stability and sensory attributes of boerewors.

4.2. MATERIALS AND METHODS

4.2.1. Sausage preparation

Boerewors models were manufactured following typical industrial procedures (Hugo, Roberts & Smith, 1993) and in compliance with the South African regulations for boerewors (Government Notice No. R.2718 of 23 November, 1990; Foodstuffs, Cosmetics and Disinfectants Act No. 54 of 1972; DoH, 2001). Table 4.1 shows the formulation of the eight boerewors models. Fresh meat (beef [70/30] and pork [50/50]), was purchased from a butchery in the Bloemfontein District. The models consisted of 90 % total meat content

formulated to contain 30 % fat and 60 % lean meat. The beef and pork meat were comminuted through a 13 mm steel plate.

The rusk, spices, Worcester sauce and vinegar were mixed in 85 g ice water and left to stand for 5 minutes to allow for hydration. The spice mixture consisted of coriander (13.846 g), monosodium glutamate (4.388 g), black pepper (4.050 g), nutmeg (3.375 g), cloves (1.000 g), thyme (0.675 g), sodium chloride (37.500 g), ascorbic acid (0.155 g), dextrose (2.511 g) and the preservative for each treatment as given in Table 4.1. The spice mixture, rusk, vinegar and Worcester sauce were thoroughly mixed with the cubed meat and minced once more through a 4.5 mm steel plate. The different models were then stuffed in 28/32 hog casings which was previously washed in lukewarm tap water. In the conventional boerewors, 0.0682% w/w of sodium metabisulphite was added as preservative which is equivalent to 450 mg/kg SO_2 (S), which is the standard concentration used in preserving boerewors. Rosemary extract (Ros) (Flavor'Plus^{TM} Ref. # 050501, SharonBolel Chemical Marketing, South Africa) was added at a concentration of 0.026% w/w which is equivalent to 260 mg/kg for all rosemary containing models (Table 4.1). This concentration of rosemary extract has been suggested by Georgantelis *et al.* (2007) to be effective as an antimicrobial and antioxidant agent. Chitosan containing models (Table 4.1) were formulated by using 1.0 % w/w chitosan (Sigma-Aldrich, USA). This is the concentration level that was found most effective (Soultos *et al.*, 2008). The level of SO_2 was reduced to 100 mg/kg SO_2 in models where SO_2 was used in combination with other preservatives types (Bañón *et al.*, 2007).

4.2.2. Sampling

The boerewors models were cut into 60 – 80 g pieces, placed in polyester trays and wrapped with air-permeable polyethylene film. The models were stored at 4 °C under fluorescent light for 9 days. For microbial analysis and colour determination, analyses were performed on days 0, 3, 6 and 9. Three samples per model were used for microbial and chemical analysis. The whole experiment was repeated three times with three different batches of meat purchased in different weeks. The experimental outlay is given in Fig. 4.1. Water activity was performed

Table 4.1. Formulations used in the manufacture of the eight boerewors models (Hugo *et al.*, 1993).

Ingredient	Control (%)	SO_2* (%)	Rosemary (%)	Chitosan (%)	Rosemary + SO_2 (%)	Chitosan + SO_2 (%)	Rosemary + Chitosan (%)	Rosemary + Chitosan + SO_2 (%)
Beef 70/30	60.0	59.9318	59.974	59.0	59.9588	59.9848	58.974	58.9588
Pork 50/50	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Vinegar	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Rusk	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Spice mixture	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Worcester sauce	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Water	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Preservative	0.0	0.0682	0.026	1.0	0.026 + 0.0152	1.0 + 0.0152	0.026 + 1.0	0.026 + 1.0 + 0.0152
Total	100	100	100	100	100	100	100	100
Preservative content	0.00 mg/kg	450 mg/kg	260 mg/kg	10 g/kg	260 mg/kg + 100 mg/kg	10 g/kg + 100 mg/kg	260 mg/kg + 10 g/kg	260 mg/kg + 10 g/kg + 100 mg/kg
Salt content	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%	1.5%
Ascorbic acid content	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg	62.0 mg/kg

* Sulphur dioxide was added as sodium metabisulphite

on day 0 only for all the treatments. TBA analyses were performed on days 0, 9 and 100 and were performed in duplicate.

4.2.3. Water activity (a_w) determination

The water activity of the sausage samples was measured with a Novasina Thermoconstanter TH 200 water activity meter at room temperature and the means of results was recorded.

4.2.4. Microbial analyses

The effect of the preservatives against a wide spectrum of micro-organisms, namely total bacteria, *Enterobacteriaceae*, coliforms and *E. coli* (Gram-negative bacteria), *Staphylococcus aureus* (Gram-positive bacteria) and yeasts and moulds, were evaluated.

Microbial analysis was performed on days 0, 3, 6 and 9 on all eight treatments. Ten grams of a boerewors model were weighed into a WhirlPak™ bag, 90 ml of 0.1 M phosphate buffer was added and homogenized (Steward Stomacher 400) for 1 min. Further serial dilutions were prepared to 10^{-10} for total bacterial counts, and 1 ml of the appropriate dilutions was plated by the pour-plate method and using standard plate count agar (SPCA; Oxoid CM0463). Plates were incubated at 32 °C for 48 h. After incubation the colonies were enumerated by means of a colony counter (Harrigan, 1998).

For determination of coliforms and *E. coli*, *Enterobacteriaceae*, yeasts and moulds and *Staphylococcus aureus*, serial dilutions were prepared to 10^{-5}. Violet red bile agar with MUG (VRB+MUG; Oxoid CM0978) was used for enumeration of coliforms and *E. coli* and incubated at 37 °C for 24 h. The presence of *E. coli* was confirmed by fluorescence of the colonies under an ultraviolet light (Harrigan, 1998). For *Enterobacteriaceae*, dilutions were

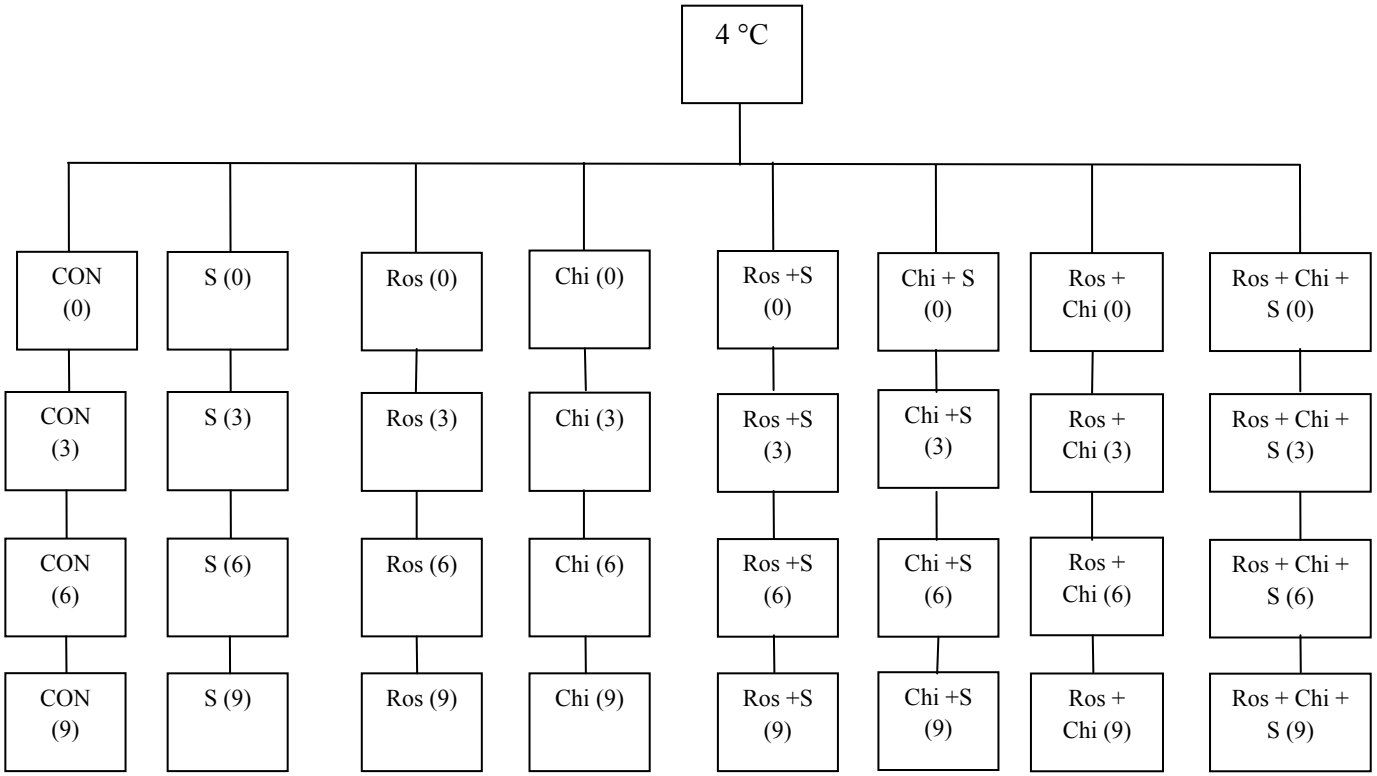

Figure 4.1. Experimental outlay of the sampling procedure. Factorial design (treatment X storage time X batch X repetitions); Microbial analysis = (8X4X3X3); Colour stability = (8X4X3X3) and lipid stability = (8X3X2X6).

pour-plated and overlayed with violet red bile glucose agar (VRBG; Oxoid CM0485). The plates were incubated at 37°C for 24 h. Colonies of *Enterobacteriaceae* produced purple red colonies with a diameter of 0.5 mm or greater and sometimes surrounded by a red zone of precipitated bile (Harrigan, 1998).

Staphylococcus aureus were enumerated by using the spread plate method on a pre-dried surface of Baird-Parker agar (Oxoid CM0275) and the plates were incubated at 37 °C for 24 h. *Staphylococcus aureus* typically forms colonies that are 1.0 – 1.5 mm in diameter, black, shiny, convex with a narrow white entire margin and surrounded by clear zones extending 2 – 5 mm into the opaque medium (Harrigan, 1998).

The pour-plate method was also used for determination of yeasts and moulds counts using rose bengal chloramphenicol agar (RBC; Oxoid CM0549) and plates were incubated at 25 °C for 4-5 days (Harrigan, 1998).

4.2.5. Colour stability determination

On days 0, 3, 6 and 9 each sausage stored at 4 ^{o}C was opened and colour (L*, a* and b*) values measured on 6 different positions on each sausage after 30 minutes bloom using a Minolta CR-400 chromometer to determine the effect of preservative type on colour stability. The saturation index (SI) which is related to the colour intensity of the meat, was calculated according to the formula: $SI = (a^{*2} + b^{*2})^{0.5}$(Luño, Beltrán & Roncalés, 1998).

4.2.6. Lipid stability determination

A 5 g sample was removed from each portion of sausage and used for thiobarbituric acid reactive substance (TBARS) analysis using the aqueous acid extraction method of Raharjo *et al.* (1992) to determine the effect of preservative type on lipid oxidation. TBARS were measured on the day of production, after 6 days of storage at 4 ^{o}C overwrapped with oxygen permeable polyethylene film, and after 100 days of frozen storage at -18 ^{o}C.

4.2.7. Sensory Evaluation

For sensory analysis, packets of boerewors from the seven treatments were removed from the freezer and defrosted in a refrigerator at 4 °C, one day before it was to be evaluated. Boerewors from a specific treatment was pan-fried to an internal temperature of above 70 ^{o}C, removed from the pan and kept warm in stainless steel containers on hot trays.

A 75-member consumer panel, students and staff from the Agricultural Faculty of the University of the Free State, ages ranging from 19 years to 60 years, both men and women, was used to taste/evaluate and give their acceptability opinion on the cooked boerewors samples from the seven treatments. The questionnaire consisted of a nine-point hedonic scale (Table 4.2), ranging from zero (1), denoting not acceptable, to nine (9), denoting extremely acceptable. Respondents were asked to respond to the question "how much do you like or dislike the sample?"

The boerewors samples were coded with randomized, three-digit codes and rotated to prevent bias. To avoid the halo effect, the tasting of the eight samples was done in two sessions over a period of two weeks. At each session, each respondent received a 20 mm piece of boerewors per treatment, from four treatments. The following session included the last three treatments, as well as a sample from the control treatment. Testing was done in individual

booths, under red lights, to mask any colour differences, and at an ambient temperature of 20 – 22 °C. Chilled sparkling water was provided as a palette cleanser.

Table 4.2. Nine-point hedonic scale used in this study for sensory analysis (Lawless & Heymann, 1998; Stone & Sidel, 2004).

OVERALL LIKING								
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Dislike extremely	**Dislike very much**	**Dislike moderately**	**Dislike slightly**	**Neither like nor dislike**	**Like slightly**	**Like moderately**	**Like very much**	**Like extremely**

4.2.8. Statistical analysis

The microbial counts obtained of total bacteria, yeasts and moulds and coliforms for each treatment were transformed to log cfu/g. These transformed values as well as results for TBARS and colour measurements were subjected to analysis of variance (NCSS, 2007). Differences were considered significant at the $p<0.05$ level. The very important interaction between preservatives type and storage time were further investigated by means of the Tukey-Kramer multiple comparison test (NCSS, 2007).

The sensory analysis data was collected in spread sheets using Microsoft Excel 2007 and the statistical analyses were done using NCSS (2007). The significance of the overall acceptance measured for each boerewors sample was tested by means of analysis of variance (ANOVA). The different preservatives were used as the main effects at a significance level of 95% ($p\leq0.05$). If the main effect was significant, the Tukey-Kramer multiple comparison test was applied to determine the direction of the differences between mean values.

4.3. RESULTS AND DISCUSSION

4.3.1. Water activity (a_w)

The water activity of the different boerewors models on day 0 is shown in Table 4.3. The water activity was evaluated to determine whether the different preservatives used in the models affected the a_w of the sausage products. The water activity of the different sausages showed no significant difference.

These results suggested that the different concentrations of the preservatives used for the different boerewors treatments, did not affect the water activity at day 0. This in turn means that the results of the microbial, lipid stability and sensory characteristics will only be due to the effectiveness of the different preservatives.

Table 4.3. Water activity of the boerewors treated with different preservatives at day 0.

Preservatives	Water activity (Aw)
Con	0.947 ± 0.004
S	0.951 ± 0.005
Ros	0.955 ± 0.003
Chi	0.958 ± 0.008
Ros+S	0.955 ± 0.003
Chi+S	0.953 ± 0.002
Ros+Chi	0.956 ± 0.005
Ros+Chi+S	0.951 ± 0.002
Significance level	NS

4.3.2. Statistical effect of preservatives on the different interactions

Table 4.4 shows the analysis of variance for the treatments of batches, storage time, and preservative type as well as the interactions among these treatments in relation to microbial quality, colour stability, lipid stability and their interactions.

There were significant differences in the microbial quality between the batches, storage time and preservatives ($p<0.001$), but no significant difference for the storage time for the coliform counts was observed (Table 4.4). The interactions between batch X storage time, batch X preservative type, storage time X preservative type, batch X storage time X preservative type had a significant ($p<0.001$) effect on the microbial quality of the different treatments. This means that the storage time of 0 – 9 days had an effect on the microbial growth, whereby the growth of the total bacteria, the *Enterobacteriaceae* and the yeasts and moulds increased significantly ($p<0.001$) whereas the increase of coliform counts was not significant. The preservative types also had an effect on the growth of bacteria in the different treatments.

There were significant differences ($p<0.001$) in all measured colour parameters (L*, a*, b* and SI values) for batch, storage time and preservative type. The interactions between the batch X storage time, batch X preservative type, storage time X preservative type and batch X storage time X preservative type were statistically significant ($p<0.001$) for a*, b* and SI values (Table 4.4). These results indicate that the different preservative types and the storage time used contributed to the colour stability of the different sausages.

There were significant differences ($p<0.001$) in the lipid stability between the batches, storage time and preservative type (Table 4.4). The interactions between batch X storage ($p<0.001$), batch X preservative type ($p<0.01$), storage time X preservative type, batch X storage time X preservative type had a significant ($p<0.001$) effect on the lipid stability of the different treatments.

Table 4.4. Analysis of variance for various treatments and their interactions.

Microbial quality				
Treatments	**Total count**	**Coliform counts**	***Enterobac-teriaceae***	**Yeasts & Moulds**
Batch	***	***	***	***
Storage Time	***	**NS**	***	***
Preservative Type	***	***	***	***
Batch X Storage Time	***	***	***	***
Interactions:				
Batch X Preservative Type	***	***	***	***
Storage Time X Preservative Type	***	***	***	***
Batch X Storage Time X Preservative Type	***	***	***	***
Colour:				
	L	**A**	**b**	**SI**
Batch	***	***	*	***
Storage Time	***	***	***	***
Preservative Type	***	***	***	***
Interactions:				
Batch X Storage Time	***	***	***	**
Batch X Preservative Type	***	***	***	***
Storage Time X Preservative Type	NS	***	***	***
Batch X Storage Time X Preservative Type	NS	***	***	***
Lipid Stability:				
	TBARS			
Batch	***			
Storage Time	***			
Preservative Type	***			
Interactions:				
Batch X Storage Time	***			
Batch X Preservative Type	**			
Storage Time X Preservative Type	***			
Batch X Storage Time X Preservative Type	**			

NS = Not Significant
* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

4.3.3. Microbial analyses

The results of the microbial analyses of the boerewors treatments preserved with different preservatives are presented in Figures 4.2, 4.3, 4.4 and 4.5.

4.3.3.1. Total bacterial count

A significant ($p<0.001$) increase in total bacterial count for the control (Con), rosemary (Ros) and rosemary in combination with 100 mg/kg SO_2 (Ros+S) treatments was observed from day 1 – 9. The total bacteria count for the Con treatment showed significantly ($p<0.001$) higher counts when compared to the Chitosan (Chi), Chi+S and Ros+S+Chi treatments at days 3 – 6 (Figure 4.2). The treatment preserved with 450 mg/kg SO_2 (S) showed lower counts when compared to the 260 mg/kg Rosemary (Ros) which were significantly different ($p<0.001$) at days 3 – 9, but the counts of the S treatment were not significantly different in maintaining the total bacteria count during a storage time of 1 – 6 days than those of the Chi treatment and the other Chi containing treatments (Figure 4.2).

The S, Chi, Chi+S, Ros+Chi and Ros+S+Chi treatments maintained the total bacterial counts (Figure 4.2) from day 1 – 9 under the acceptable microbial quality standard of 6.00 log cfu/g (Shapton & Shapton, 1991).

The combination of natural preservatives with conventional preservatives, were effective in reducing and maintaining the total bacterial counts. Interestingly, the Ros+S showed significantly ($p<0.001$) higher counts than the S, Chi+S, Ros+Chi and Ros+Chi+S, but the counts were not significantly different to that of the Chi treatment ($p>0.001$) on the 9^{th} day. The counts for the Con, Ros and Ros+S treatments continued to increase to levels of more than 7 log

cfu/g. These counts were significantly higher ($p<0.001$) than the S, Chi+S, Ros+Chi and Ros+Chi+S treatments on day 9 (Figure 4.2).

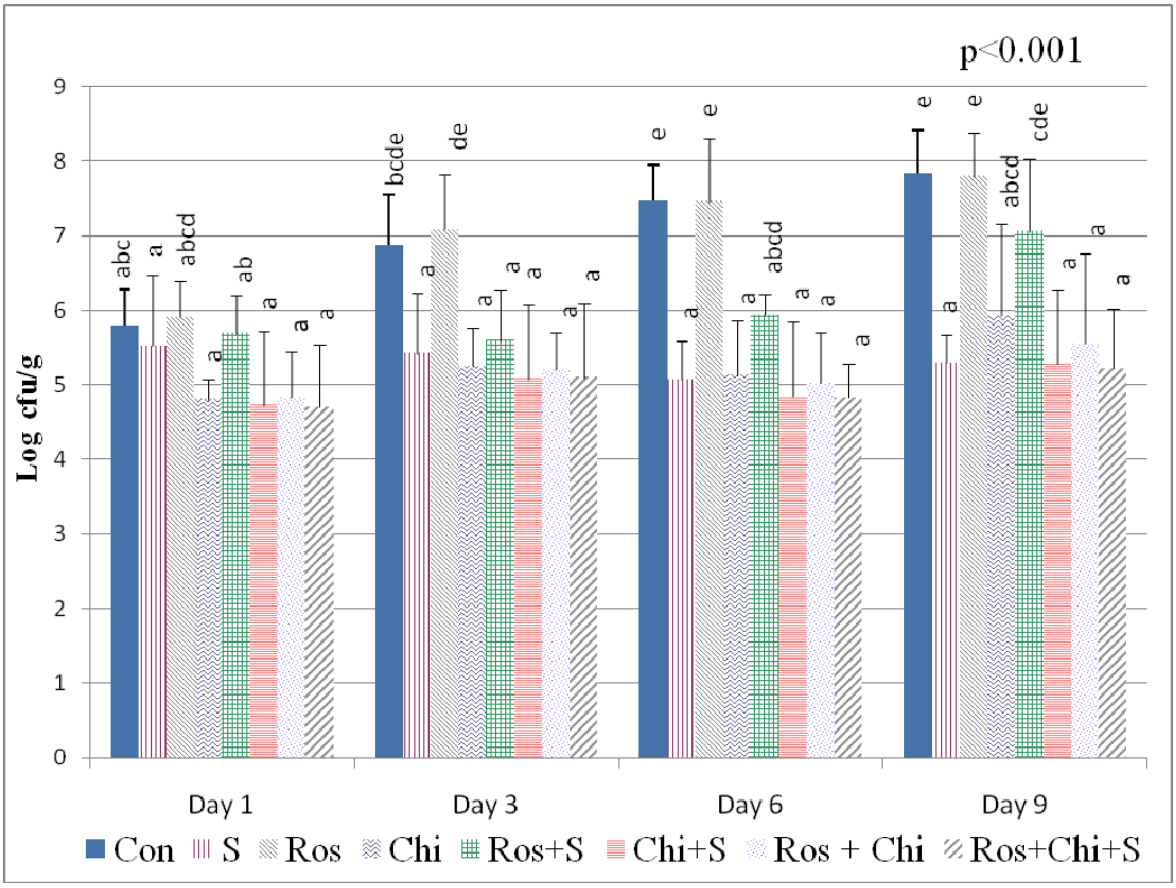

Figure 4.2. Effect of preservative types and storage time on the total bacterial counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

Georgantelis *et al.* (2007) observed similar effects of the different preservatives. They studied the effect of rosemary extract, chitosan and α-tocopherol on the microbial quality of pork sausages stored at 4 °C. Chitosan and chitosan in combination with rosemary extract had significantly lower counts when compared to the treatment with rosemary extract only and the control at days 5, 10 and 15. In this study, rosemary extract showed a synergistic effect with S

and Chi in reducing the total counts but when it was used on its own, rosemary extract was not effective. Seydim, Guzel-Seydim, Action & Dawson (2006) found a similar effect when they used rosemary extract for reduction of total counts in vacuum packaged ground ostrich meat. Rosemary extract was more effective when it was used in a mixture or in combination with sodium lactate (Seydim *et al.*, 2006). In another study, chitosan coated grilled pork with 2 – 2.5% chitosan packaged in vacuum was shown to lower total viable counts to less than 1 log cfu/g (Yingyuad, Ruamsin, Reekprkhon, Douglas, Pongamphai & Siripatrawan, 2006).

4.3.3.2. Coliform count

A significant difference ($p<0.001$) was observed for coliform counts between the treatments during the storage time of 9 days (Figure 4.3). On day 1 the Chi+S treatment showed a significantly ($p<0.001$) lower coliform count than Con and the Ros+Chi+S treatment had a significantly ($p<0.001$) lower coliform count than Con on day 6 of storage at 4 ^{o}C. During the 9 day storage time there was an insignificant decrease in coliform counts in S, Chi and chitosan in combination with other preservatives types. The storage time had no significant effect on the coliform counts of the different treatments as shown in Table 4.4. The Ros treatment had, however, insignificantly higher counts when compared to the S and Chi treatments.

Zhang, Kong, Xiong & Sun (2009) observed that rosemary spice in combination with liquorice were more effective in maintaining the coliform count in modified atmosphere packaged fresh pork, and with higher concentrations of 40 – 80 mg/ml, the inhibition was better. Aldemir & Bostan (2009) observed a decrease in coliform counts in meatballs preserved with chitosan and the decrease was significant with higher concentrations of 1 – 2% chitosan when compared to the control.

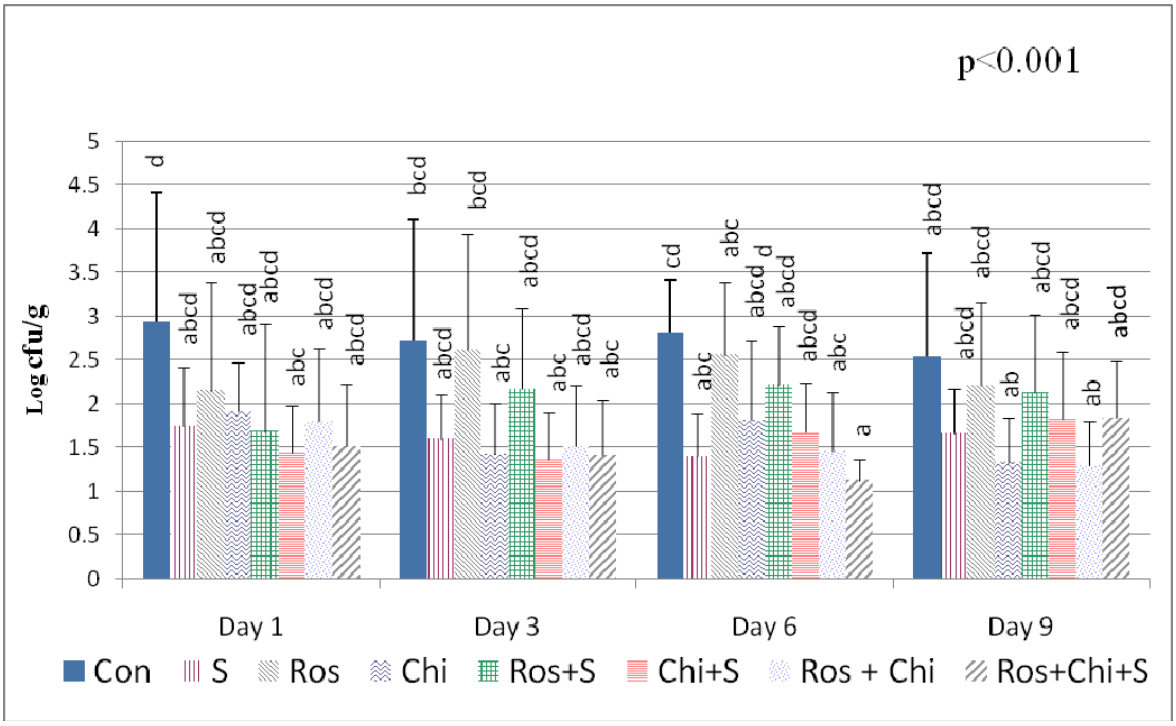

Figure 4.3. Effect of preservative types and storage time on the coliform counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

4.3.3.3. Enterobacteriaceae count

The S and Chi+S treatments had significantly lower *Enterobacteriaceae* counts than the Ros treatment on day 1 (Figure 4.4). At day 6, significantly lower counts were observed for the S and Chi treatments when compared to the Ros treatment. The S, Chi and chitosan in combination with other preservative type (Chi+S, Ros+Chi and Ros+Chi+S) treatments, maintained the counts of *Enterobacteriaceae* at 1 – 1.5 log cfu/g during the storage time of 1 – 6 days.

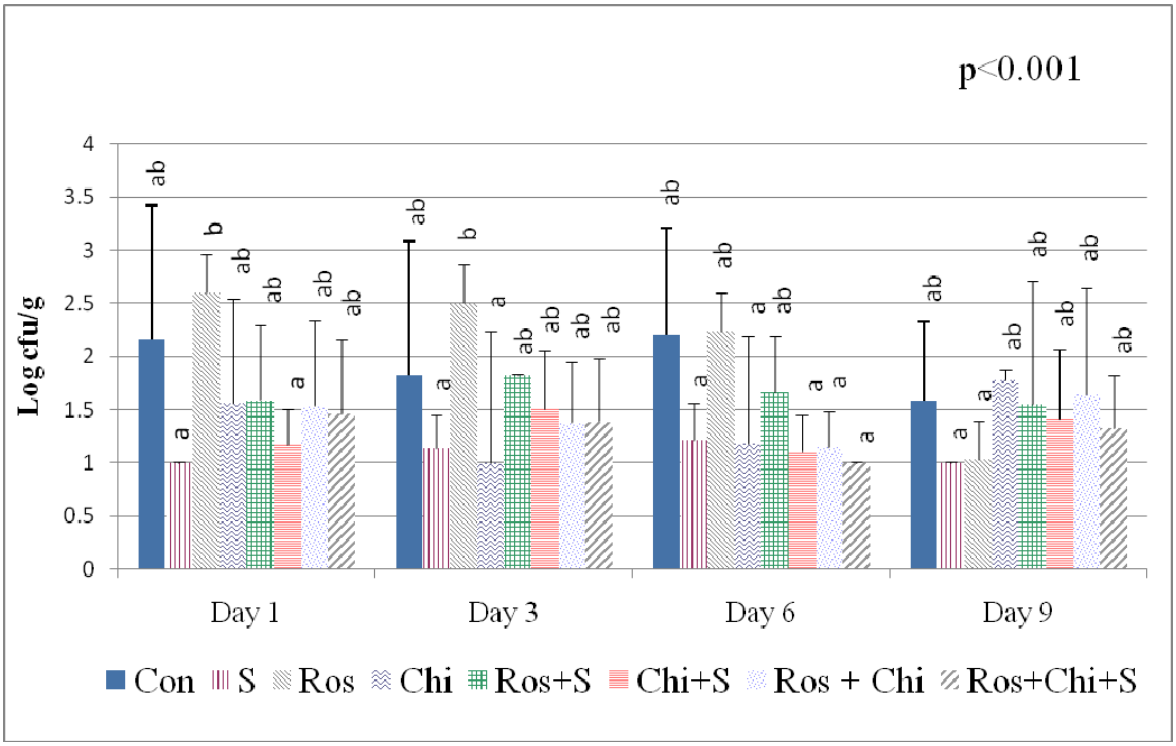

Figure 4.4. Effect of preservative types and storage time on the *Enterobacteriaceae* counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

Enterobacteriaceae is known as an indicator for hygienic quality of food products. These counts for the eight treatments were low, and met the standard of < 5.00 log cfu/g for *Enterobacteriaceae* counts for all the storage days (Shapton & Shapton, 1991). In a study of Soultos *et al.* (2008) a fresh pork sausage stored at 4 °C for 7 days and preserved with 1% chitosan gave counts of 2.64 – 3.28 log cfu/g *Enterobacteriaceae.* These counts were in agreement with those observed in this study for chitosan. A synergistic effect of chitosan in combination with rosemary was observed. Georgantentelis *et al.* (2007) also observed a similar synergistic effect of a combination of chitosan and rosemary in fresh pork sausages.

4.3.3.4. Yeasts and moulds count

The Chi+S and Ros+Chi+S treatments showed significantly ($p<0.001$) higher reductions of yeasts and moulds counts when compared to the Con and Ros treatments after 9 days storage time (Figure 4.5). The S, Ros and Con treatments showed a significant increase in the yeasts and moulds count during the 9 days storage time. The Chi treatment had a significantly ($p<0.001$) lower yeasts and moulds count when compared to that of Con, Ros and S treatment after 6 – 9 days storage time.

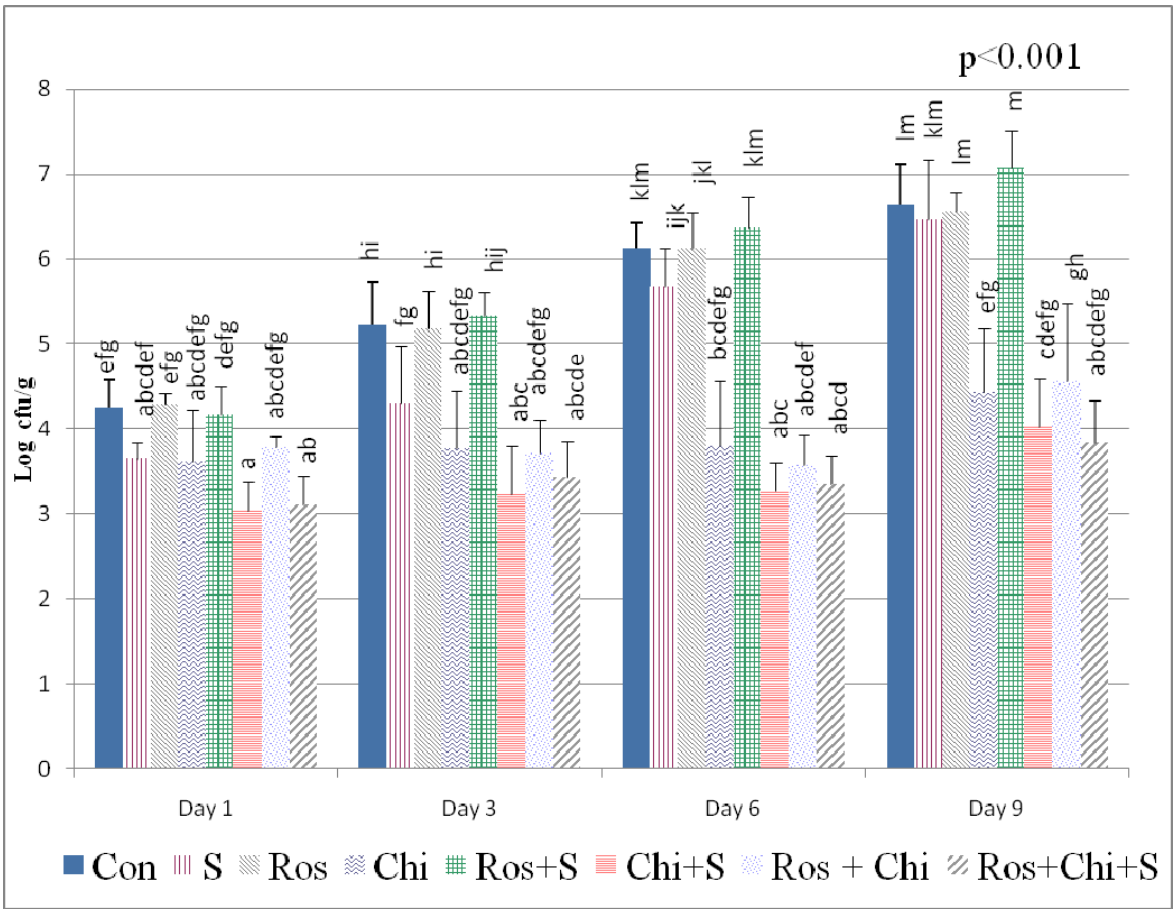

Figure 4.5. Effect of preservative types and storage time on the yeasts and moulds counts of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

The chitosan and chitosan containing treatments reduced the yeasts and moulds counts in this study. This was in contradiction with the observation of Aldemir & Bostan (2009) who found that chitosan was ineffective against yeasts and moulds in meatballs. This may be due to the lower concentration of 50 – 500 mg/kg chitosan that was used in their study. Soultos *et al.* (2008) observed yeasts and moulds counts ranging from 2.41 – 5.02 log cfu/g in fresh pork sausage preserved with 1% chitosan for 28 days at 4 °C. The rosemary extract and sulphite were not very effective in reducing the yeasts and moulds counts in this study. Yeasts and moulds are known to be resistant to SO_2 (Roller *et al.*, 2002).

4.3.3.5. E. coli and Staphylococcus aureus

In this study, the boerewors contained no *E. coli* and *Staphylococcus aureus.* The counts were less than 1 log cfu/g (<10 cfu/g) for all the boerewors treatments. These results suggested that the raw material used to formulate the different sausage treatments, were of good microbial quality. Wang (1992) observed that different concentrations of chitosan of about 1 – 2.5% were effective against *E. coli* and *Staph. aureus* organisms. The phenolic compounds of the rosemary have been shown to be effective against Gram-positive and Gram-negative bacteria, and the effectiveness has been linked to carnosic acid and carnosal (Rožman & Jeršek, 2009). The competition that exists among the microorganisms may also be a contributing factor to the absence of these species in the control treatments.

4.3.4. Chemical stability

4.3.4.1. Colour stability

The results of the colour stability of the boerewors treated with different preservatives are presented in Figures 4.6, 4.7, 4.8 and 4.9. The lightness (L*) value colour of the different boerewors treatments is shown in Figure 4.6. There were no significant differences in L* value for all the treatments though S and Chi+S treatments showed higher L* values when compared to the other treatments from days 1 – 6. There was also a insignificant decrease in the L* value during the 9 days of storage of all the sausage treatments. The Chi+S on days 3, 6, and 9 demonstrated a significantly ($p<0.001$) lower L* value compared to S on day 1. Chi+S, Ros+Chi and Ros+Chi+S showed a significantly ($p<0.001$) lowerer L* value on day 9 than S on day 1.

There was a significant decrease in the redness (a*) values of all the treatments during the storage time of 1 – 9 days (Figure 4.7). The S treatment showed a significantly higher ($p<0.001$) a* value to that of the Ros, Con and Ros+Chi+S treatments from days 3 – 9. The a* value for the S and Chi+S treatments were comparable to each other, while the Ros treatment showed a significantly ($p<0.001$) higher reduction of a* value when compared to the S treatment at 3 – 9 days storage time.

The redness of meat is an important aspect which consumers use to purchase meat and meat products (Boles, Mikkelsen & Swan, 1998). The redness colour originates when meat myoglobin is exposed to oxygen resulting in the formation of red myoglobin. Rosemary extract has been shown to reduce redness in broiler meat, fresh pork and frozen pork patties (Mc Carthy, Kerry, Kerry, Lynch & Buckley, 2001; Mirshekar, Dastar & Shabanpour, 2009). A synergistic effect of rosemary with ascorbic acid has been observed in modified packaged fresh pork sausage, whereby the redness colour of the product was maintained for 12 days (Martínez, Cilla,

Beltrán & Roncalés, 2007). In this study the effect of chitosan in maintaining the redness colour was comparable to the 450 mg/kg SO_2 treatment at day 9. The application of different molecular weight chitosan in a reduced fat Chinese-style sausage were shown to maintain the redness colour of the products from day 0 to 9 (Lin & Chao, 2001). The redness of 1% chitosan preserved beef patties packaged in an oxygen permeable film (PVC) and stored at refrigerated temperatures has been shown to have greater redness than that of control packages in the same material at days 3 – 5 (Suman, Mancin, Joseph, Ramanathan, Konda, Dady & Yin, 2010).

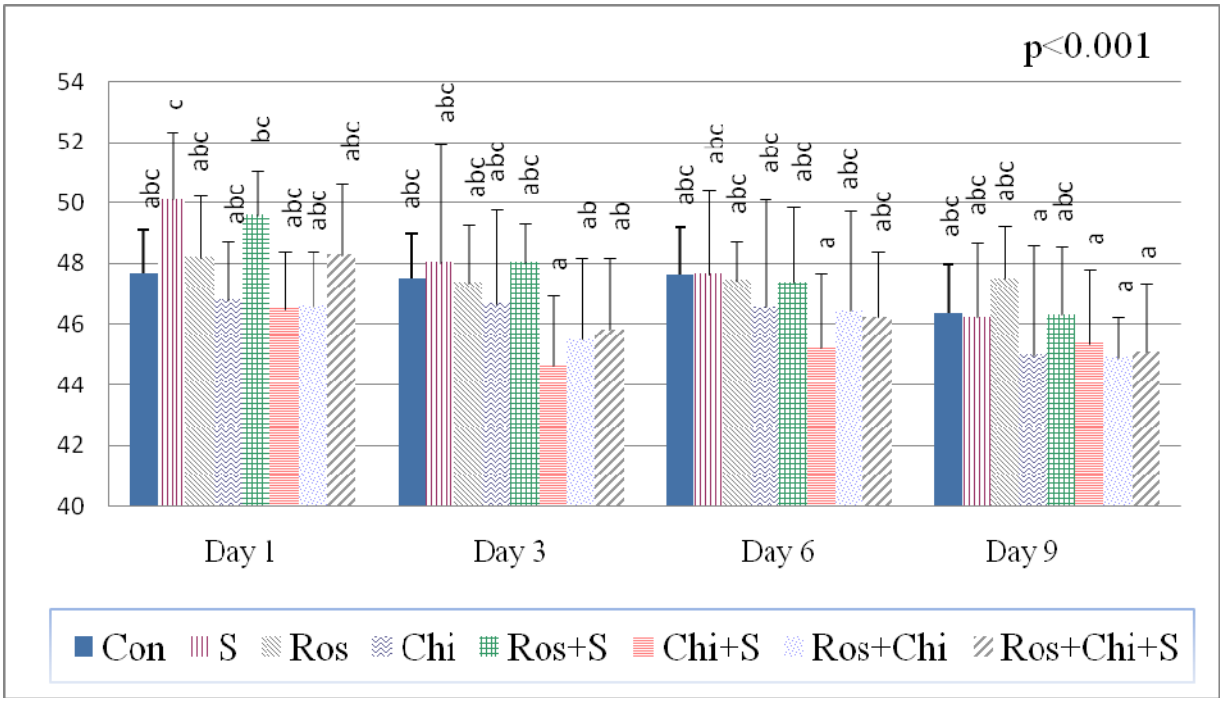

Figure 4.6. Effect of preservative types and storage time on the L* value (lightness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

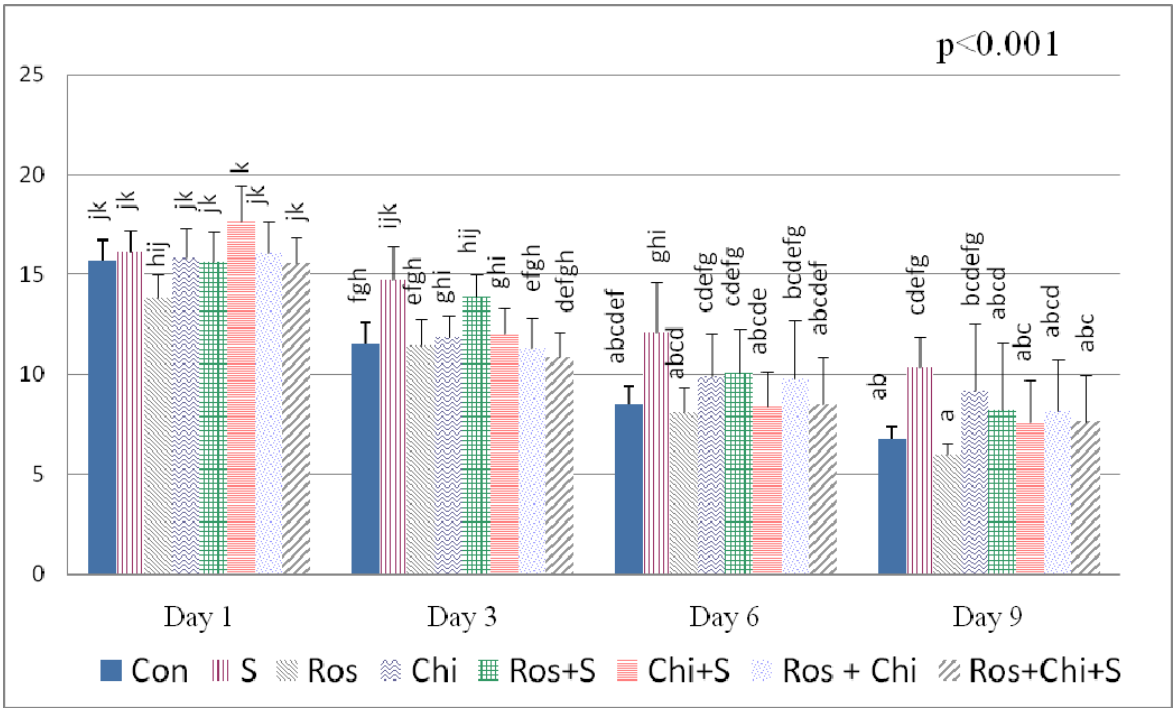

Figure 4.7. Effect of preservative types and storage time on the a* value (redness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

There was decrease in the yellowness (b*) value of the treatments for a period of 3 days which was not significant. But after 3 days a significant decrease in the b* value of the treatments, especially the Con and Ros treatments, was observed (Figure 4.8). The S and Ros+S treatments were comparable to each other at days 1 – 9. Studies have shown that rosemary and chitosan do not have much effect on the yellowness value in most meat products (Lin & Chao, 2001; Seydim *et al.*, 2006; Mirshekar *et al.*, 2009).

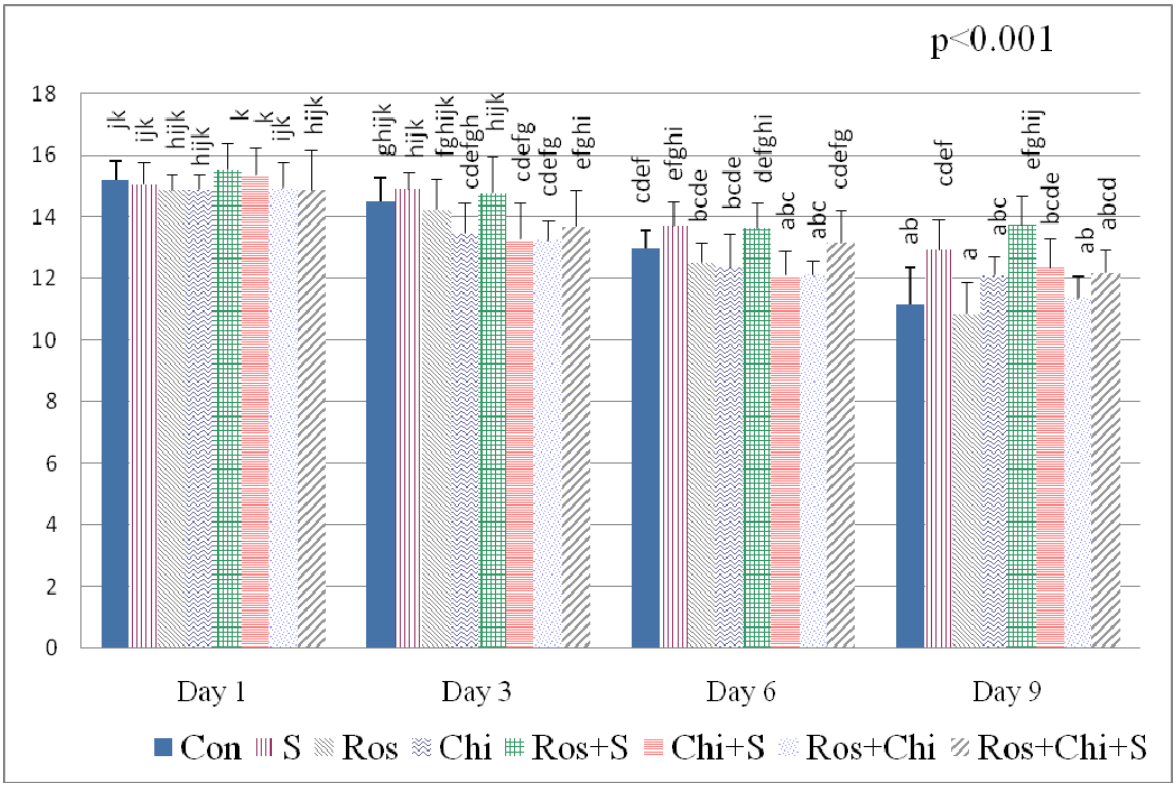

Figure 4.8. Effect of preservative types and storage time on the b* value (yellowness colour) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

The saturation index (chroma) corresponds to the colour intensity, an index representing the relative concentration of haemoglobin in the red blood cell. There was a significant decrease in the saturation index of all the treatments during the storage time of 9 days (Figure 4.9). The Chi+S treatment showed a significantly ($p<0.001$) higher saturation index at day 1 when compared to the Ros treatment. At days 3 – 9 the S treatment showed a significantly ($p<0.001$) higher saturation index value when compared to the Con and Ros treatments. The S treatment showed a higher colour intensity when compared to the other treatments while the Ros treatments showed lower intensity, since its a* and b* values were lower.

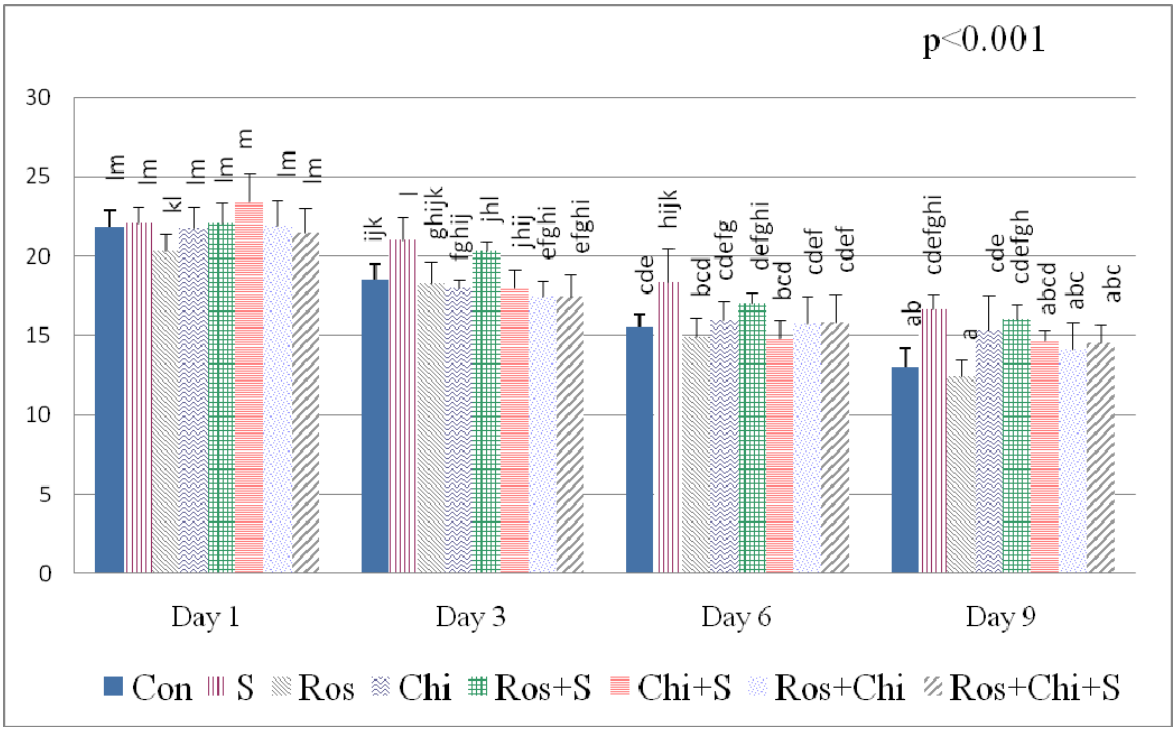

Figure 4.9. Effect of preservative types and storage time on the saturation index (SI) of boerewors stored at 4 °C. Results with different superscripts are significantly different. Error bars represent standard deviations.

Interestingly, the Ros+S treatment saturation index was comparable to that of the S treatment. The bright red colour of beef meat is linked to the high a*- and the saturation index value whereas the pork colour is linked to the L* and Hue value ($H = \tan^{-1}$ [b/a]) which is affected by the pH and oxygen concentration (Zanardi, Novelli, Ghiretti, Dorigoni & Chizzolini, 1999). The ability of chitosan to give a better colour appearance to meat has been linked to its capacities to bind water and lipid in meat and this contribute to the high a* value, b* value and colour intensity (Knorr, 1983; Soultos *et al.*, 2008).

4.3.4.2. Lipid stability

The results of the lipid stability of the boerewors treated with different preservatives are presented in Figure 4.10. The higher the TBARS value, the higher the rancidity of the product. At a TBARS value of ≥ 1, rancid off-flavours become detectable by taste panels (Buckley & Connolly, 1980; Boles & Parrish, 1990). Recent studies in meat such as beef, however, indicate that TBARS values of 2 or greater are considered to be rancid (Campo, Nute, Hughes, Enger, Wood & Rickerolsory, 2006; Suman *et al.*, 2010).

When comparing the treatments stored at 4 °C for the period of 9 days the TBARS values of Chi were significantly ($p<0.001$) higher than the Con, S and Ros treatments at day 1. The Ros+S and S treatments showed insignificant lower TBARS at day 9 than Con, Ros, Chi and chitosan in combination with other preservative types.

When comparing the treatments stored at -18 °C for the period of 100 days, the S treatment maintained the TBARS values from day 1 – 100. A synergistic effect between rosemary and SO_2 was observed in the Ros+S treatment, whereby TBARS values were maintained at low values which were comparable to that of the S treatment during a storage time of 100 days. The Chi and Ros+Chi treatments showed significant ($p<0.001$) increases in TBARS values during the 100 days storage time when compared to the S and Ros treatments.

The rosemary extract had good antioxidant properties when compared to the chitosan, and this has been speculated to be due to the composition of rosemary with phenolic compounds such as carnosol and carnosic acid that have been shown to have high antioxidant properties (Rižnar *et al.*, 2006). Georgentelis *et al.* (2007) also observed similar trends whereby the fresh pork sausage preserved with rosemary had lower oxidation products of 0.16 mg malonaldehyde (MDA)/kg meat to that of chitosan of 0.37 MDA/kg meat treatment after 20 days storage at 4 °C.

In beef patties preserved with rosemary stored for a period of 20 days at 2 °C, the rosemary showed lower TBARS values when compared to those preserved with ascorbic acid. Rosemary extract has also been observed to reduce or maintain the TBARS in broiler meat at 0.5 – 0.6 mg MDA/kg meat for a period of 120 days (Mirshekar *et al.*, 2009).

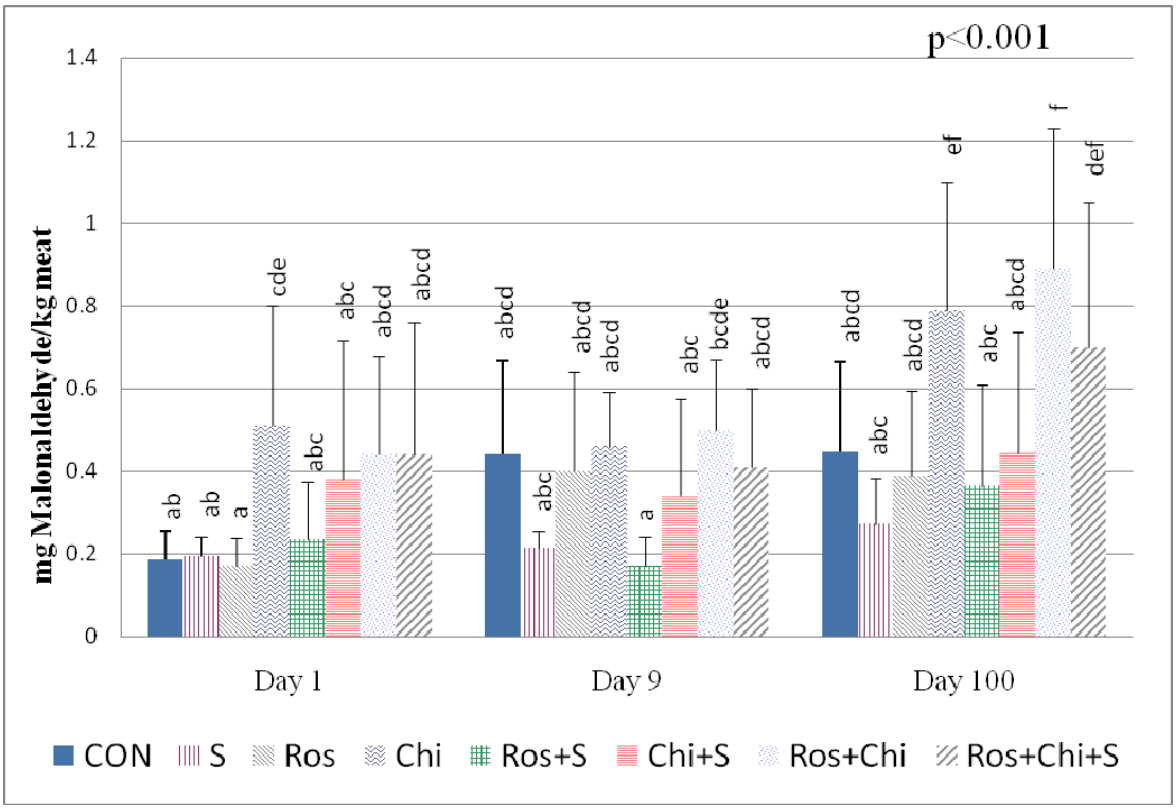

Figure 4.10. TBARS-values (lipid stability) measured in mg malonaldehyde/kg meat of boerewors treated with different preservatives and stored for 9 days at 4 ^{o}C and 100 days at -18 ^{o}C. Results with different superscripts are significantly different. Error bars represent standard deviations.

Kannatt, Chander & Sharma (2008) stated that chitosan does not have a significant antioxidant activity but when chitosan is used in combination with mint in salami, it maintained the TBARS of the salami for two weeks at 0.28 – 0.29 mg MDA/kg meat. Chitosan has been shown to be effective in maintaining the TBARS values at less than 2 MDA/kg in fresh pork sausage and beef patties (Soultos *et al.*, 2008; Suman *et al.*, 2010).

All the treatments showed lower TBARS values of less than 1 in the current study. The low TBARS values of the treatments at day 100 may be due to further oxidation of MDA to other organic products of lipid oxidation (alcohol & acids) which are not determined by the reaction with TBA (Soultos *et al.,* 2008). Another possible reason may be due to the decomposition of MDA by bacteria such as pseudomonad's and *Enterobacteriaceae*, which posses the ability to selectively attack and utilize carbonyl compounds, including MDA (Soultos *et al.,* 2008). Other factors such as temperature have an effect on the oxidation rate of meat and meat products. For example, during the cooking process there is a significant increase in the TBA values because the cooking method disrupts the muscle membrane system, thereby exposing the lipid component to oxygen and/or other reaction catalysts such as iron (Kamil, Jeon & Shahidi, 2002).

4.3.5. Sensory evaluation

The results of the taste preference of the boerewors treatments are shown in Table 4.5. The S treatment was preferred by most consumers. The preference was significantly ($p<0.001$) different to that of the Chi treatment and chitosan containing treatments. The Con, Ros and Ros+S treatments were more preferred by consumers compared to the Chi treatments.

The high score for the S treatment was probably due to the fact that consumers are used to a sausage with this kind of preservative (450 mg/kg SO_2). Rosemary also showed a positive sensory score due to improving the flavour of the sausages which was in accordance to findings in other studies (Sánchez-Escalante, Djenane, Torrescano, Giménez, Beltrán & Roncalés, 2001; Rižnar *et al.*, 2006).

Table 4.5. Mean values for the taste preference of boerewors samples manufactured with different preservatives.

Treatment	Sensory Score
No preservative (control)	6.7^{bc}
S (450 mg/kg SO_2)	6.8^{c}
Ros	6.3^{bc}
Chi	5.2^{a}
Ros + S	6.2^{bc}
Chi + S	5.2^{a}
Ros + Chi	5.8^{ab}
Ros + Chi + S	6.0^{ab}
p	<0.001

Samples that share the same superscript letter (a, b, c) are not significantly preferred to one another (equally liked), although there is a small difference in means.

Kanatt, Chander & Sharma (2008) performed a sensory analysis on pork cocktail salami preserved with a mixture of chitosan and mint. There were no significant differences between treated and untreated treatments based on taste, colour, flavour and texture. This may be due to the mint properties. In the current study, however, the presence of chitosan resulted in lower sensory scores. Interestingly, rosemary extract in combination with the chitosan improved the sensory attribute of the sausages. The chemical composition of chitosan should be further investigated to determine which compounds affects the sensory taste of the sausages, since in this study a synergistic effect of rosemary and chitosan was observed in improving the taste of the sausages.

4.4. CONCLUSIONS

In the current study, chitosan and rosemary extract were evaluated in terms of antimicrobial, antioxidant, colour and sensory properties as individual natural preservatives and in combination with each other and the conventional SO_2 preservative. Chitosan was the most effective in reducing and maintaining the microbial growth, also in combination with the other preservative types. The rosemary extract showed good antioxidant properties and sensory attributes while the SO_2 was effective in maintaining the colour of the sausages and had good sensory attributes.

Chitosan and combinations of Chi+S and Ros+Chi gave comparable antimicrobial and antioxidant properties to the conventionally preserved sausages (S). Although rosemary extract was not identified as a good antimicrobial, its antimicrobial and antioxidant properties were improved when used in combination with the SO_2. More research is, however, needed to find the perfect combinations.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Boerewors is a traditional fresh sausage that is enjoyed by many South African consumers (Nel & Steyn, 2002). The product undergo communition, such as mincing, dicing or chopping, which makes the product more susceptible to microbial and oxidative spoilage. Other factors that contribute to the quality of the sausage are temperature, pH, water activity, surface area, gaseous environment and packaging materials (Cannon, Morgan, Heavner, McKeith, Smith & Meeker, 1995). The sausage is preserved with 450 mg/kg SO_2 which is added as sodium metabisulphite (DoH, 2001).

The use of conventional (chemical) preservatives in food products has raised consumer concerns. The need for innovative research on natural compounds from plants, animals and even microorganisms as bio-preservative methods has increased in recent years (Roller, Sagoo, Board, O'Mohony, Caplice, Fitzgerald, Fogden, Owen & Fletcher, 2002; Tiwari, Valdramidis, O' Donnell, Muthukumarappan, Bourke & Cullen, 2009). Plant extracts such as rosemary, green tea and grape seed extracts have been studied due to containing phenolic compounds that have been shown to have antimicrobial and antioxidant properties in meat and meat products (Georgantelis, Ambrosiadis, Katikou, Blekas & Georgakis, 2007). Other natural preservatives such as chitosan, have gained a lot of interest due to its high antibacterial activity and also its ability to reduce lipid oxidation in meat (Roller *et al.*, 2002; Georgantelis *et al*., 2007). The use of natural preservatives also contributes positively to consumer's health because of its neutraceutical properties when compared to conventional preservatives (Jayaprakasha, Selvi & Akariah, 2003).

In Chapter 3 the aim was to evaluate the microbial quality of boerewors sausages produced in the Bloemfontein area. Fifty-seven of the 75 butchery outlets (supermarkets and

butcher shops) from the different regions (north, south, east, west and central) were surveyed. When the mean aerobic plate counts in the different regions were compared to the guideline of not more than 7.00 log cfu/g for fresh sausages (Nortjé, Vorster, Greebe & Steyn, 1999), all the regions complied to this guideline. The psychrotrophic count of boerewors was also evaluated in this study since boerewors is stored at low temperatures until consumed. The mean counts for four of the five regions were even higher than the APC which implies that this product can spoil fast when stored at refrigeration temperatures. Counts of *Enterobacteriaceae*, coliforms and presence of *E. coli* are indicators of hygienic practices during the production line. Since no South African standards exist for these counts, the Swedish standard of 5.00 log cfu/g for fresh sausages (Shapton & Shapton, 1991) was used for comparison purposes. The mean counts for all the regions complied to this guideline although individual butcheries (18.91% outlets for *Enterobacteriaceae* counts, 35.13% outlets for coliform counts and 18.92% outlets for *E. coli* incidence) did not comply to this guideline. The yeasts and moulds counts of all the butcheries in this study did not comply to the Brazilian guidelines of less than 2.00 log cfu/g. The high counts of yeasts and moulds in meat and meat products are associated with the general hygienic conditions of the environment, since these organisms are capable of multiplying in warm moist areas and on equipment which is not easily cleanable. The supermarkets showed a better microbial quality when compared to the small butchery shops and the small butcheries had the highest incidence of *E. coli*.

The results of this study demonstrated that the microbial quality of boerewors sold in the different regions of Bloemfontein retail outlets did not differ significantly between the regions. The incidence of *E. coli* in the central region may indicate that the socio-economic status has an effect on the quality, though the socio-economic status was not investigated in this study. The significantly high coliform counts in boerewors purchased from the butcher shops indicate that poor hygienic conditions exist in butcher shops compared to the supermarkets. That may be related to staff being trained in hygienic practices in the corporate supermarket environment while no training or very little training takes place in butcher shops. Emphasis should be placed on the creation of internal standards by the different food produce outlets to manage the quality of food (Codron, Giraud-Héraud & Soler, 2005).

The preservation of food products is driven by the need to extend the shelf life of the food. The different methods used for achieving this objective should be appropriate for the different food products due to differences in physiochemical and microbial properties of the food (Rasooli, 2007). There are different factors (intrinsic and extrinsic) that affect the quality of fresh sausages, such as boerewors. The use of SO_2 as a preservative in boerewors can help to obtain properties such as delaying the onset of oxidative rancidity reactions during storage, reducing colour loss in meat, and antimicrobial activity (Gould & Russell, 2003). The use of chemical preservatives is, however, under scrutiny by consumers. Sulphur dioxide is no exception, since it has been shown to affect the absorption of vitamin B_1 and cause respiratory problems in sulphite sensitive consumers (McDonald, 1992; Bañón, Díaz, Rodríguez, Garrido & Price, 2007).

The objective of Chapter 4 was, therefore, to evaluate alternative natural preservatives that can achieve the same positive effects (being an antioxidant and antimicrobial) than SO_2. Rosemary extract is a plant derived preservative that has been used as a herb. In some studies, however, it has been shown to have antimicrobial and antioxidant activities in food (Balentine, Crandall, O'Bryan, Duony & Pohlman, 2006; Rižnar, Čelan, Knez, Škerget, Bauman & Glaser, 2006; Martínez, Cilla, Beltrán & Roncalés, 2007). Chitosan is an animal derived preservative from the shell of crab and shrimps and cellwall of fungi. It has been shown to posses antimicrobial and antioxidant properties (Yingyuad, Ruamsin, Reekprkhon, Douglas, Pongamphai & Siripatrawan, 2006; Aldemir & Bostan, 2009). These two natural preservatives were selected to evaluate whether they can be used as substitutes for the conventional SO_2 in boerewors or be used in combination with lowered concentrations of SO_2.

In this study, rosemary extract as individual preservative at a 260 mg/kg concentration has shown to have good antioxidant properties and it also had a comparable sensory taste score to the boerewors that was preserved with SO_2. These results agreed with other studies (Rižnar et al., 2006; Georgantelis *et al*., 2007; Mirshekar, Destar & Shabanpour, 2009). Chitosan as sole preservative at a concentration of 10 g/kg showed good antimicrobial properties and good colour

attributes by preventing red colour loss. It, however, had the lowest sensory taste score. The 260 mg/kg rosemary extract was not as effective as the SO_2 as an antimicrobial agent and in maintaining the colour of the sausages.

The combination of 260 mg/kg rosemary extract and 10 g/kg chitosan used in this study did improve the microbial quality, but were not as effective as SO_2 as antioxidant agents, colour stabilizers and sensory improvers. There was, however, a good synergistic effect between the lowered concentrations of 100 mg/kg SO_2 and the natural preservatives used in this study. Rosemary extract and SO_2 retarded oxidation and gave high sensory scores, while the SO_2 and chitosan showed good antimicrobial activities. The combination of chitosan + rosemary extract with 100 mg/kg SO_2 showed good antimicrobial activity, colour stability and sensory properties but as antioxidant agents it was not comparable to that of the 450 mg/kg SO_2 preserved boerewors.

The quality of the boerewors was affected by the natural preservatives used in this study. The effectiveness of these natural preservatives alone and in combination with the conventional preservative was positive in improving the microbial, chemical and sensory quality of the boerewors. The challenge to introduce a new naturally preserved sausage product in the market can be affected by quality attributes such as colour and sensory properties, but correct information supplied to the consumer can improve the shift from consumers searching the product, to experience the product and then eventually to credence the product (Northern, 2000).

Another method that may also be investigated to preserve boerewors using natural preservatives, include using different kinds of packaging materials and methods. There is an increase in research based on chitosan-containing packaging films and coating which has been shown to improve the quality of food (Kittur, Kumar & Tharanathan, 1998; Yingyuad *et al.*, 2006). These films have an ability to prevent the growth of bacteria in the food products without affecting the physiochemical properties of the meat. The other advantage is that these films are

easily degradable in the environment (Cooksey, 2001; Quintavella & Vicini, 2002; Yingyuad *et al.*, 2006).

Future research may include:

- Increase the sample size of the survey and determine the effect of the socio-economic status on the quality of sausages.
- Explore the different interactions between the temperature X storage time X preservative type, of the different treatments.
- Explore the effect of the natural preservatives (chitosan and rosemary extract) in different sausage types produced in South Africa, e.g. polony and viennas.
- Explore other natural preservatives such as mints to improve the antimicrobial, antioxidant and sensory quality of the boerewors (Kanatt, Chander & Sharma, 2008) in combination with chitosan and rosemary extract.
- Explore the use of natural films, such as chitosan-containing film, as packaging materials to improve the quality of fresh meat (Yingyuad *et al.*, 2006).

CHAPTER 6

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CHAPTER 7

SUMMARY

Boerewors is a popular dish in South Africa, and it is classified as a ground meat product. It is a fresh sausage product and the quality of the sausage is affected by the growth of microorganisms and oxidation spoilage. Boerewors is currently preserved with 450 mg/kg sulphur dioxide (SO_2). The SO_2 has antimicrobial, antioxidant and sensory properties in the boerewors but SO_2 may also have negative effects on consumer health. Natural preservatives have become important in the food industry because consumers are moving away from consuming chemically preserved food. Rosemary extract is a natural preservative that has been shown to have antimicrobial and antioxidant properties. Another natural preservative that has been studied is chitosan which is a deacetylated form of chitin derived from the shell of crabs and shrimps and the cell wall of fungi and also has been shown to have antimicrobial and antioxidant properties.

One of the aims of the study was to evaluate the microbiological quality of boerewors produced in different regions of Bloemfontein and comparing the quality of boerewors from small butcheries with supermarkets. The boerewors of 50% of the butcheries in Bloemfontein were subjected to microbial evaluation by using aerobic plate counts (APC), psychrotolerant plate counts (PPC), *Enterobacteriaceae* counts, coliform counts, yeasts and moulds counts and determination of the presence of *Staphylococcus aureus* and *Escherichia coli*. No *Staph. aureus* was found in any of the boerewors samples. The results of this study demonstrated that the microbial quality of boerewors sold in the different regions of Bloemfontein retail outlets did not differ significantly between the regions. The incidence of *E. coli* in the central region may indicate that the socio-economic status has an effect on the quality, though the socio-economic status was not investigated in this study. The significantly high coliform counts in boerewors purchased from the butcher shops indicate that poor hygienic conditions exist in butcher shops

compared to the supermarkets. That may be related to staff being trained in hygienic practices in the corporate supermarket environment, while no training or very little training takes place in butcher shops.

The second aim was to determine the effectiveness of rosemary extract, chitosan and fungi) and sulphur dioxide (SO_2) as a conventional preservative, applied individually and in combination with each other, on improving the quality of boerewors. Eight boerewors treatments were formulated as follows: control (Con), 450 mg/kg SO_2 (S), 260 mg/kg rosemary extract (Ros), 10 g/kg chitosan (Chi), 260 mg/kg rosemary extract + 100 mg/kg SO_2 (Ros+S), 10 g/kg chitosan +100 mg/kg SO_2 (Chi+S), 260 mg/kg rosemary extract + 10 g/kg chitosan (Ros+Chi) and 260 mg/kg Rosemary extract + 10 g/kg Chitosan + 100 mg/kg SO_2 (Ros+Chi+S). Water activity (a_w), microbial properties, colour stability, lipid stability and sensory analysis were conducted on the different sausage treatments. There were no significant differences between the a_w of the different treatments. Chitosan and chitosan in combination with other preservative types had a significant effect ($p<0.001$) on reducing the total bacterial counts, coliforms and *Enterobacteriaceae* counts which were comparable to the results of the SO_2 treatment. Chitosan, however, had a better effect on decreasing yeasts and moulds counts than SO_2. Chitosan also showed good colour properties which were comparable to that of the 450 mg/kg SO_2 preserved sausage. Rosemary extract showed a comparable lipid stability to those of the S treatment and it showed significantly ($p<0.001$) lower TBARS values when compared to the Chi treatment. The Ros treatment had a positive effect on the sensory taste of the sausages when compared to the Chi treatment, but the S treatment was still preferred by the sensory panel. The reduced level of 100 mg/kg SO_2 showed good synergistic effects in combination with chitosan as antimicrobial, colour stabilizer and in combination with rosemary as antioxidant and improving the sensory score of the sausages.

Keywords: boerewors, natural preservatives, rosemary extract, chitosan, sulphur dioxide (SO_2), microbial quality, lipid stability, colour stability, sensory properties

OPSOMMING

Boerewors is ‘n gewilde dis in Suid-Afrika wat as ‘n gemaalde vleisproduk geklassifiseer word. Dit is ‘n vars worsproduk en die kwaliteit van die wors word beïnvloed deur die groei van mikroörganismes en oksidasie-bederf. Boerewors word huidig met 450 mg/kg swaeldioksied (SO_2) gepreserveer. Die SO_2 het antimikrobiese, anti-oksidant en sensoriese eienskappe in die boerewors maar SO_2 kan ook ‘n negatiewe effek op die verbruiker se gesondheid hê. Natuurlike preserveermiddels het belangrik geword in die voedselindustrie omdat verbruikers begin wegbeweeg van die inname van chemies-gepreserveerde voedsel. Roosmarynekstrak is ‘n natuurlike preserveermiddel wat antimikrobiese en anti-oksidant eienskappe vertoon. ‘n Ander natuurlike preserveermiddel wat bestudeer is, is chitosan wat ‘n gedeasetileerde form van chitien is wat vanaf die skulp van krappe en die selwand van fungi afkomstig is. Dit vertoon ook anti-mikrobiese en anti-oksidant eienskappe.

Een van die doelwitte van die studie was om die mikrobiese kwaliteit van boerewors wat in die verskeie streke van Bloemfontein geproduseer word, te evalueer en om die kwaliteit van die boerewors vanaf slaghuise met supermarkte te vergelyk. Die boerewors van 50% van die slaghuise in Bloemfontein is aan mikrobiese evaluasie onderwerp deur middel van aerobiese plaattellings, psigrotolerantetellings, *Enterobacteriaceae* tellings, kolivormtellings, giste en skimmel tellings en die teenwoordigheid van *Staphylococcus aureus* en *Escherichia coli*. Geen *Staph. aureus* is in enige van die boerewors monsters opgespoor nie. Die resultate van die studie het gedemonstreer dat die mikrobiese kwaliteit van boerewors wat in die verskeie areas van Bloemfontein verkoop word, nie betekenisvol van mekaar verskil het nie. Die voorkoms van *E. coli* in die sentrale area mag ‘n aanduiding wees dat die sosio-ekonomiese status van die verbruiker ‘n invloed op die kwaliteit mag hê, alhoewel dit nie verder in hierdie studie ondersoek is nie. Die betekenisvolle hoë kolivormtelling in die boerewors vanaf slaghuise, is ‘n aanduiding van swak higiëniese werksomstandighede teenoor die beter higiëne in supermarkte. Dit mag ook verwant wees aan supermarkpersoneel wat opleiding in higiëniese praktyke in die korporatiewe supermark omgewing ontvang, terwyl geen of min opleiding in slaghuise plaasvind.

Die tweede doelwit was om die effektiwiteit van roosmarynekstrak, chitosan en swaeldioksied as konvensionele preserveermiddel, individueel toegedien en in kombinasie met mekaar, te ontleed in terme van die invloed op die kwaliteit van die boerewors. Agt boerewors behandelings is as volg geformuleer: kontrole (Con), 450 mg/kg SO_2 (S), 260 mg/kg roosmarynekstrak (Ros), 10 g/kg chitosan (Chi), 260 mg/kg roosmarynekstrak + 100 mg/kg SO_2 (Ros+S), 10 g/kg chitosan +100 mg/kg SO_2 (Chi+S), 260 mg/kg roosmarynekstrak + 10 g/kg chitosan (Ros+Chi) and 260 mg/kg roosmarynekstrak + 10 g/kg Chitosan + 100 mg/kg SO_2 (Ros+Chi+S). Water aktiwiteit (a_w), mikrobiese eienskappe, kleurstabiliteit, lipiedstabiliteit en sensoriese analiese is op die verskillende worsbehandelings uitgevoer. Daar was geen betekenisvolle verskille tussen die a_w van die verskillende behandelings nie. Chitosan en chitosan in kombinasie met ander preserveermiddel tipes het ‘n betekenisvolle ($p<0.001$) effek op die vermindering van die totale bakterietellings, kolivorm- en *Enterobacteriaceae* tellings gehad en was vergelykbaar met die resultate van die SO_2 behandeling. Chitosan het egter ‘n beter effek as SO_2 op die vermindering van giste en skimmels tellings gehad. Chitosan het ook goeie kleureienskappe, vergelykbaar met die 450 mg/kg SO_2 gepreserveerde wors, vertoon. Roosmarynekstrak het ‘n vergelykbare lipiedstabiliteit as die S behandeling getoon en het ‘n betekenisvolle ($p<0.001$) laer TBARS waardes vertoon wanneer dit met die Chi behandeling vergelyk is. Die Ros behandeling het ‘n meer positiewe effek op die smaak van die wors gehad wanneer dit met die Chi behandeling vergelyk is, maar die S behandeling is nog steeds deur die sensoriese paneel verkies. Die verminderde vlak van 100 mg/kg SO_2 het goeie sinergistiese effekte in kombinase met chitosan as antimikrobiese middel en kleurstabiliseerder getoon, terwyl dit goeie anti-oksidant en sensoriese eienskappe in kombinasie met roosmarynekstrak getoon het.

Sleutelwoorde: boerewors, natuurlike preserveermiddels, roosmarynekstrak, chitosan, swaeldioksied (SO_2), mikrobiese kwaliteit, lipiedstabiliteit, kleurstabiliteit, sensoriese eienskappe