EVALUATION OF A NATURAL PRESERVATIVE IN A BOEREWORS MODEL SYSTEM

by

Charles Petrus Benjamin van Schalkwyk

Submitted in fulfilment of the requirements for the degree of

MAGISTER SCIENTIAE AGRICULTURAE (FOOD SCIENCE)

in the

Department of Microbial, Biochemical and Food Biotechnology
Faculty of Natural and Agricultural Sciences
University of the Free State

Supervisor: Prof. A. Hugo
Co-supervisor: Prof. C.J. Hugo

May 2010

DECLARATION

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

____

C.P.B. van Schalkwyk
May 2010

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to the following persons and institutions:

Our Heavenly Father for the talents he granted me.

Prof. A. Hugo, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for his supervision, assistance, time (even on week ends and late nights), encouragement and endless patience.

Prof. C.J. Hugo, Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, for her keen interest, soft – spoken encouragement and ever readiness and willingness to help. My endless gratitude to Proff. Hugo, for making my dream a reality!

Mrs. Carina Bothma, for her kind assistance with the sensory analysis.

Ms Arina Jansen for her kind assistance with the TBARS analysis.

All the other staff at the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, that assisted me and gave me their support. It is greatly appreciated.

Management of Crown National in granting me study leave to work on this project; and for their financial assistance.

My ouers vir hulle aanmoediging en belangstelling – dit het my gehelp om aan te gaan wanneer ek soms die gevoel gekry het hierdie taak is net “te veel” vir my; dit het my gehelp om weer krag en moed te skep en weer aan te gaan. Hierdie verhandeling word aan julle opgedra.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIT	Allyl isothiocyanate
ANOVA	Analysis of variance
atm	Atmospheres
ATP	Adenosine Tri – Polyphosphate
a_w	Water activity
B.	*Brochothrix*
B.C.	Before Christ
C.	*Clostridium*
CC	Cranberry concentrate
CoA	Co enzyme A
CM	Chitosan and mint mixture
cfu	Colony forming units
cm	Centimeter
ºC	Degrees Celsius
E.	*Escherichia*
e.g.	For example
EOs	Essential oils
et al.	*(et alii)* and others
g	Gram
GC-MS	Gas chromatography-mass spectrometry
GMP	Good Manufacturing Practices
GRAS	Generally Regarded As Safe
GSE	Grape Seed Extract
GTE	Green Tea Extract.
h	Hours
kg	Kilogram
L.	*Listeria*
LAB	Lactic acid bacteria
log	Log_{10}

MAB	Mesophilic aerobic bacteria
M	Moles
SH-	Sulph-hydryl
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
MRSA	Methicillin-resistant *Staphylococcus aureus*
ppm	Part per million
RBCA	Rose bengal chloramphenicol agar
S.	*Salvia*
SPCA	Standard plate count agar
Staph.	*Staphylococcus*
TAPC	Total Aerobic Plate Count
TBARS	Thiobarbituric Acid Reactive Substance
µl	Micro liter
USA	United States of America
VRB+MUG	Violet red bile agar + MUG
w/v	Weight per volume
w/w	Weight per weight
Y.	*Yersinia*
Y & M	Yeasts and Moulds

CHAPTER 1

INTRODUCTION

1.1. Background to the study

Boerewors is a fresh sausage that contains a mixture of beef and pork. The mixture is flavoured with salt, pepper and various spices, especially coriander. The mixture is stuffed into cleaned pork, beef or sheep intestines (casings). According to law, boerewors is defined as being the clean, healthy and wholesome muscle and fat of beef, mutton or pork or a mixture of 2 or more. It must contain at least 90% meat and a maximum of 30% fat. It may contain rusk, spices, harmless flavourants, vinegar and allowed preservatives (sulphur dioxide [SO_2], 450 mg/kg) (DoH, 1990).

A preservative is regarded as a category of food additives. The other food additive categories include nutritional additives, flavouring agents, colouring agents, texturing agents and miscellaneous additives (Branen, 1989). A definition for additives, according to the Food Protection Committee of the Food and Nutrition Board (U.S.) is: “a substance or mixture of substances, other than basic foodstuff, which is present in a food as a result of any aspect of production, processing, storage, or packaging. The term does not include chance contaminants” (Branen, 1989).

Preservatives are used to prevent or retard both chemical and biological deterioration of foods. Those preservatives used to prevent chemical deterioration include antioxidants (prevent autoxidation of pigments, flavours, lipids, and vitamins); anti-browning compounds (prevent enzymatic and non-enzymatic browning); and antistaling compounds (prevent texture changes). The primary additives used to prevent biological deterioration, are the antimicrobials (prevent food poisoning from various bacteria and moulds) (Branen, 1989; 1993).

Sulphur dioxide and its various salts claim a long history of use, dating back to the times of the ancient Greeks. They have been used extensively as antimicrobials and to prevent enzymatic and non-enzymatic discolouration in a variety of foods (Davidson & Juneja, 1989). Until recently, the use of SO_2 has had GRAS (Generally Regarded as Safe) status. Investigations have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites (Ough, 1993; Roller *et al.*, 2002). This has caused a great deal of research in all areas concerning sulphites and SO_2. Other materials can act as antimicrobial agents, but none has been found to replace the antioxidant capabilities of SO_2 (Ough, 1993).

Antimicrobials continue to be one of the most important food preservatives. Current research is on synthetic, natural occurring, and biologically derived antimicrobials in food systems (Dillon & Board, 1994; Sökmen *et al.*, 2004). Research is especially needed on application of naturally occurring and biologically derived antimicrobials in food systems. One reason for this is that consumers are rejecting the use of chemical preservatives, but still demand foods with an acceptable shelf-life (Dillon & Board, 1994).

For a natural antimicrobial compound to be used as a biopreservative in food systems, it needs to be produced economically on a large scale, it must not cause unacceptable organoleptic changes and it must be toxicologically safe. The future of natural antimicrobial agents is most likely to be in combination with other preservative systems or with physical treatments, such as heating or freezing processes (Dillon & Board, 1994).

Selection of the proper antimicrobial is dependent upon several factors, including the chemical and antimicrobial properties; the properties and composition of the food product in question; the type of preservation system, other than chemicals, used in the food product; the type, characteristics, and the number of micro-organisms; the safety of the antimicrobial; and the cost effectiveness of the antimicrobial (Branen, 1993).

Due to the economical impacts of spoiled foods and the consumer's concerns about the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (Sökmen *et al.*, 2004). Examples of natural preservatives that have been studied in meat products similar to Boerewors, on its own at various additions and in combination with reduced levels of sulphite additions, include: chitosan (Roller *et al.*, 2002), allyl isothiocyanate (Nadarajah *et al.*, 2005), ascorbate, green tea and grape seed extracts (Banon *et al.*, 2007), spice and herb extracts (Shan *et al.*, 2009), and cranberry concentrate (Wu *et al.*, 2009).

Chapter 2 of this research dissertation will review the literature on the background and history of boerewors, the types of micro-organisms and spoilage of the product and current methods of preservation. It will also give a review of current conventional methods of preserving meat, their usage, efficacy, advantages and disadvantages.

Since consumers are rejecting the use of chemical preservatives, but still demand foods with acceptable shelf life (Dillon & Board, 1994), the literature study will give a review of natural preservation methods for meat; usage, efficacy, advantages and disadvantages. A study of antimicrobial agents in plants and animals reveals that few, if any, act alone; they almost invariably act in concert with each other. This trend in evolution must not be overlooked by those who seek natural systems for food preservation. Successful exploitation will most probably stem from a combination of natural and established systems with hopefully a reduction in the levels of the latter (Dillon & Board, 1994).

Chapter 3 will describe the materials and methods used in this thesis. Chapter 4 will describe the findings of the testing of Citrox as a potential antimicrobial in Boerewors. Citrox comprises a range of phyto-alexins derived from the pith and rind of green Bergamont oranges and fruit acids (Neall, 2006). Its anti-viral, -bacterial, -mould and -yeast effect is due to the synergistic activity of these bioflavonoid and organic acid compounds. It offers the following benefits (Neall, 2006):

- It is a totally natural organic compound,
- It has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative), viruses, moulds, yeasts and fungi,
- It is non-mutagenic, non-carcinogenic, non-toxic, non-corrosive, non-tainting and non-volatile,
- It has extended action (residual effect), but does not possess a knock down (shock) action,
- It has the ability to break down biofilm,
- It is effective even in the presence of organic and debris matter,
- Its mechanism of action is by the destruction of the cell wall.

According to the supplier in South-Africa, Citrox is being used to decontaminate and sanitize meat, chicken and fish after slaughter / catch and processing (Neall, 2006).

In Chapter 5 the general discussion and conclusions of this study will be given, while all the references used in this study will be given in Chapter 6. Chapter 7 will give a short summary of the study both in English and Afrikaans.

1.2. Purpose and objectives of the study

The purpose of this study will be to test Citrox, on its own, at two dosage levels, and in combination with reduced SO_2 levels, as a natural preservative in boerewors in terms of microbial, chemical and sensory effectiveness.

Objectives:

To test the effect of “Citrox” on:

- The total aerobic bacteria present in boerewors
- The coliforms present in the boerewors
- The presence of a representative of the Gram-negative bacteria (*Escherichia coli*), if present in the boerewors
- The presence of a representative of the Gram-positive bacteria (*Staphylococcus aureus*), if present in the boerewors
- The yeasts and moulds present in the boerewors
- The colour and colour stability of boerewors as expressed by the colour-a* (redness) value, the colour-L* (lightness) value and the colour-b* (yellowness) value
- The lipid stability of the boerewors as expressed by TBARS (Thiobarbituric Acid Reactive Substance) values
- The sensory analyses as determined by a taste panel

The results will be compared to similar studies done on other natural preservatives in similar products.

CHAPTER 2

LITERATURE REVIEW

1. INTRODUCTION

Methods of food preservation have been an important part of food technology since antiquity. It is designed to prevent the adverse chemical and quality changes caused by the natural spoilage flora present on any food. Early traditional procedures involved drying, smoking, salting, pickling and combinations of these procedures. Public acceptance of salting and pickling dates back to the Babylonians some 3000 years B.C. Heat sterilisation and meat dehydration are comparatively recent technologies introduced in the 19th century. Cold-air cooling, cold pickling and deep-freezing came to the fore at the beginning of the 20th century and were followed by the use of chemical preservatives, irradiation and the disinfection of storage and manufacturing materials. Apart from the changes in the organoleptic and physical properties of the food, all these processes achieved their objective in inhibiting or interfering with microbial growth (Elias, 1987).

By any criterion, fresh meat is considered one of the more perishable foods. Preservation methods involve application of measures to delay or prevent certain changes which make meat unusable as a food or which downgrade some quality aspect of it. The pathways by which such deterioration can occur are diverse and include microbial, chemical, and physical processes (Urbain & Campbell, 1987).

Preservatives are regarded as a category of food additives. The other food additive categories include nutritional additives, flavouring agents, colouring agents, texturing agents and miscellaneous additives (Branen, 1989). A definition for additives, according to the Food Protection Committee of the Food and Nutrition Board (U.S.) is: “a substance or mixture of substances, other than basic foodstuff, which is present in a food as a result of any aspect of production, processing, storage, or packaging. The term does not include chance contaminants" (Branen, 1989).

Preservatives are used to prevent or retard both chemical and biological deterioration of foods. Those preservatives used to prevent chemical deterioration include antioxidants (prevent autoxidation of pigments, flavours, lipids, and vitamins); antibrowning compounds (prevent enzymatic and non enzymatic browning); and antistaling compounds (prevent texture changes). The primary additives used to prevent biological deterioration, are the antimicrobials (prevent food poisoning from various bacteria and moulds) (Branen, 1989; 1993).

Antimicrobials continue to be some of the most important food preservatives. According to Dillon & Board (1994) and Sökmen *et al.* (2004) current research is on synthetic, natural occurring, and biologically derived antimicrobials. Research is, however, especially needed on application of naturally occurring and biologically derived antimicrobials in food systems. One reason for this is that consumers are rejecting the use of chemical preservatives, but still demand foods with an acceptable shelf-life (Dillon & Board, 1994). The trend is, therefore, towards less heavily preserved foods (less chemical preservatives, salt and sugar, also with less impact on the environment – a trend of "green consumerism") that are not severely damaged by heat processing or freezing and do not contain artificial additives (Dillon & Board, 1994; Burt, 2004). There are many natural antimicrobial systems, but only a few have been exploited (Dillon & Board, 1994). There is, therefore, scope for new methods of making food safe which have a natural or "green" image. One such possibility is the use of essential oils as antibacterial additives (Burt, 2004).

The empirical observation that salting would preserve meat without refrigeration was made several thousand years ago. By 1000 B.C., salted and smoked meats were available (Lawrie, 1979). Today, many chemical and natural preservatives are used in the preservation of meat. Major concerns have, however, arisen over the health implications of meat preservation (Elias, 1987). Even as early as 1908 there was concern on the use of preservatives in meat (Anonymous, 1908).

In South Africa, boerewors is a fresh sausage which gets consumed in large quantities, between 54–100 tons per month (Lehohla, 2003). Although a few chemical and natural preservatives are used in this product, new and safe methods of preservation of boerewors should also be investigated in order to improve the spoilage potential of this product.

The aim of this review will be to give an overview of boerewors, a traditional South African fresh sausage, to discuss SO_2 as a conventional preservative used in boerewors manufacture and to discuss the different types of natural preservatives available in the food sector.

2. SOUTH AFRICAN BOEREWORS

2.1. Background and history

"Sausage" is deducted from the Latin word *salsus* which means salted, or meat preserved by salt. Sausage has been known as a food since 900 B.C., and preferred by the Romans. During the Middle-ages each country developed its own type of sausage according to their national tastes and climate. The Italian Bologna sausage, the French Lyons sausage and the German Bruwurst are well known examples (Steyn, 1989).

Boerewors was first made on farms in South Africa. It contained a mixture of beef and pork, with cubes of speck infused. The meat mixture was flavoured with salt, pepper and various spices, especially coriander. Originally the finely cut / minced meat was stuffed through a cattle horn into cleaned pork, beef or sheep intestine (casing). Fresh boerewors was used as a "braai" (fried) dish for breakfast and supper. Boerewors could also be dried by wind for later use as “droëwors” (dry wors / dried sausage). The first printed Afrikaans cookbook (1891) contained a recipe for boerewors (Steyn, 1989).

According to law, boerewors is defined as being the clean, healthy and wholesome muscle and fat of beef, mutton or pork or a mixture of two or more. It must contain at least 90% meat and maximum 30% fat. It may contain rusk, spices, harmless flavourants, vinegar and allowed preservatives (sulphur dioxide: 450 mg/kg) (Department of Health [DoH] of South Africa, 1990).

Legal requirements for Boerewors are as follows (Department of Health of South Africa, 1990):

Raw boerewors shall be manufactured from the meat of an animal of the bovine, ovine, porcine or main caprine species or from a mixture of the meat 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;
(d) May contain a maximum of 0.02 g of calcium per 100 g of the product mass

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

2.2. Types of micro-organisms and spoilage

After slaughter the meat surface usually becomes contaminated with microbes from the ground, air, water and intestines of the animal. The bacterial load of boerewors will therefore, be a reflection of the original contamination of the fresh meat plus cross contamination and contamination during processing. By mincing the meat, the micro-organisms are mixed into the meat and a larger surface and more moisture is released for the growth of microbes (Steyn, 1989).

Carcass meat that is processed hygienically contains to a large degree saprophytes, mainly micrococci (*Micrococcus* and *Staphylococcus* spp.) and Gram-negative bacteria. Faecal streptococci (enterococci), lactic acid bacteria, *Brochothrix thermosphacta* and many *Bacillus* spp. are present in small amounts. Gram-negatives consist of *Acinetobacter, Aeromonas, Alcaligenes, Flavobacterium, Moraxella, Pseudomonas,* and *Enterobacteriaceae*. Of the yeasts, *Debaryomyces hansenii* is found the most, followed by *Candida zenylanoides*, and *Pichia membranaefaciens.* Moulds can also be present. The influence of the different types of micro-organisms on carcass meat quality is influenced by further processing. Manufacturing of fresh sausage leads to the partial expulsion of air which causes *Brochothrix thermosphacta* and lactobacilli to become dominant (Steyn, 1989).

Fresh mince contains mainly *Micrococcaceae* as well as lactobacilli, *Pseudomonas* and *Enterobacteriaceae*. Mince processed according to Good Manufacturing Practices (GMP), may contain a total count of 10^6 cfu/g at 22 ºC. Small amounts of coliforms, *Escherichia coli* and enterococci are also present. Pathogens which have been isolated include *Staph. aureus, Clostridium, E. coli*, *Salmonella* and *Listeria.* Temperature is an important control mechanism for mince products - within 24 hours the total count can increase to 10^8 cfu/g and off smells may develop (Steyn, 1989).

When the minced meat particles are filled into a casing, they are forced against each other, and an anaerobic atmosphere is created in the meat mass, which prevent the growth of aerobes like *Pseudomonas*. On the surface, under the casing, aerobic spoilage continues in the partial aerobic atmosphere (Steyn, 1989).

The microbial quality of boerewors is influenced by many factors. Aerobic spoilage is fast in raw meat products with a pH of higher than 6. *Pseudomonas* and *Brochothrix thermosphacta* grow optimally at pH 5.5–7. The hygienic quality of boerewors is determined by the hygienic quality of the meat processed. Carcass meat usually has a microbial load of 10^3–10^5 aerobic mesophiles/cm^2. The psychrotroph population makes out 0.1–10% of the mesophile population. The mincing process increases the distribution of carcass flora and leads to the release of meat juices which increases microbial growth. Butchery hygiene further influences the hygienic quality as expressed by microbial counts and types of microbes found in fresh sausages (Steyn, 1989).

2.3. Shelf-life and preservation

During the mincing and filling process the red myoglobin meat pigments are cut off from oxygen in the air and after 24 h a brown-grey colour of metmyoglobin develop under the casing in the filled boerewors. Under this surface layer of metmyoglobin some oxygen are included and may maintain the red myoglobin pigment. The included oxygen may also react with the fatty tissue of the meat and cause rancidity or fat oxidation. The age of the meat used in processing of the boerewors will determine how soon this rancidity will become sensorialy noticeable. Free fatty acids originating from spices may contribute to this rancidity. For optimal flavour and taste development the boerewors should always have a stabilisation time of 24 h at 4 ºC (Steyn, 1989).

Although spices may contribute to the microbe population (up to 10^6 cfu/g), nutmeg, black pepper and clove act as anti-oxidants. Citric acid and sodium glutamate increase the anti-oxidative action of spices. Ascorbic acid not only improves the colour of boerewors, but also delays oxidation of fat. To lower the microbe population, it is recommended that irradiated spices or spice oils and spice oleoresins should be used (Steyn, 1989).

The shelf-life of boerewors is largely determined by the initial bacterial load of the meat, plus bacteria that are included into the boerewors due to contamination during processing (casings, processing areas, equipment, band saws, mincers, water, and human contact). Preservatives like sodium chloride (14–16 g/kg), sulphur dioxide (450 mg/kg) and benzoic acid (750 mg/kg) contribute to increase the shelf-life of the boerewors (Steyn, 1989).

Meat products may contain 450 mg/kg sulphur dioxide. Sulphur dioxide is a strong antimicrobial, especially against bacteria. In fresh sausage with a pH of 5.8–6.8 sulphite ionizes by forming $S_2O_5^{2-}$ and H_2O (Davidson & Juneja, 1989; Ough, 1993). Sulphite promotes the growth of yeasts at 4–10 ºC. These yeasts produce acetaldehyde, which binds with free sulphite; this reduces the inhibiting action of sulphite on lactobacilli, *Brochothrix* and *Pseudomonas* (Steyn, 1989).

The allowed 750 mg/kg benzoic acid has little influence on bacteria in meat products. Benzoic acid inhibits primarily yeasts and moulds, and is often used in combination with sulphite salts for fungistatic effect (Steyn, 1989).

Traditionally boerewors contains 5% vinegar which reduces the pH to 4.9–5.0. Vinegar influences the colour and flavour of boerewors and results in more moisture release. If sodium ascorbate is used in conjunction with vinegar, the meat colour is improved. It was suggested to use vinegar in addition to sulphite, salt and or benzoic acid for optimal bacteriostatic and fungistatic effects (Steyn, 1989).

Shelf-life is determined by the amount of moisture available for the micro-organisms. Fresh meat has a water activity (a_w) of 0.99, and fresh sausage an a_w of 0.97–0.98, which limits the shelf-life. An addition of 20 g/kg sodium chloride does not lower the a_w enough to inhibit microbial growth. The combined activity of sodium chloride and sodium nitrite (or sulphur dioxide) improves the shelf-life of fresh sausage due to the lowered a_w (Steyn, 1989).

Temperature is generally seen as one of the most important parameters with which the shelf-life of meat products may be extended. Meat products with a pH of 5.5–5.8 with an a_w of 0.99 are easily spoiled, and the only way to extend the shelf-life is storage at 0–2 ºC, or by the lowering of the pH and a_w (Steyn, 1989).

Spoilage microflora determines the microbial and organoleptic shelf-life of fresh sausage. *Pseudomonas* grows aerobically and causes off-smells (sulphite smell spoilage) due to the breakdown of protein. *Brochothrix thermosphacta* causes aerobic and anaerobic spoilage. Under anaerobic conditions this microbe grows a lot slower, so that shelf-life of vacuum packed fresh sausage is increased (Steyn, 1989).

Lactobacilli are responsible for the sour-cheesy smell that develops during anaerobic spoilage of meat. There is a correlation between the presence of lactic acid producing bacteria and organoleptic spoilage of sausage. Under anaerobic conditions the lactobacilli over grow all aerobic proteolytic microflora and causes microbial spoilage of fresh sausage. Lactic acid spoilage is more acceptable to the consumer than aerobic proteolytic spoilage (Steyn, 1989).

A study by Steyn (1989) found that an arbitrary shelf-life “end point” of 10^7 cfu/g should be used. The study found the spoilage organisms present at the end point as follows: lactobacilli: 19.8%, *Pseudomonas*: 14.2% and *B. thermosphacta*: 12.1%. When these ratios are converted to counts, it meant that lactobacilli were present in amounts on average of 2 x 10^6 cfu/g, and *Pseudomonas* and *B. thermosphacta* in counts of 1 x 10^6 cfu/g. If any of the organisms should reach a count of 10^7 cfu/g, it would be associated with signs of spoilage. It was suggested that the mentioned counts should not be exceeded for the sausage to remain unspoiled. Lactococci were the dominant microflora in the boerewors and were regarded to be harmless (Steyn, 1989).

3. CONVENTIONAL SO_2 PRESERVATION OF BOEREWORS

Sulphur dioxide and its various salts claim a long history of use, dating back to the times of the ancient Greeks. They have been used extensively as antimicrobials and to prevent enzymatic and non-enzymatic discolouration in a variety of foods (Davidson & Juneja, 1989). It is an effective antioxidant in sausages as well as other comminuted fat – protein – water emulsions and it is a reducing agent that preserves the red colour of meat. It is used as a common preservative of some fresh meat, poultry and game products that are produced using comminuted meats. These products include raw unprocessed sausage and sausage meat, mortadella, chicken and turkey loaves, frankfurters, luncheon meats, Polish salami, devon and hamburgers. It increases the bacterial lag phase, selects against spoilage bacteria and is effective against *Salmonella* spp. and many other *Enterobacteriaceae*, *Pseudomonas* spp., *Lactobacillus* and some yeast species (Charimba *et al*., 2010).

Until recently, the use of SO_2 has had GRAS (Generally Regarded as Safe) status. Investigations have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites (Ough, 1993; Roller *et al.*, 2002). It may also cause symptoms of an allergic response in sulphite sensitive people (Charimba *et al.,* 2010). This has caused a great deal of research in all areas concerning sulphites and SO_2. Other materials can act as antimicrobial agents, but none has been found to replace the antioxidant capabilities of SO_2 (Ough, 1993). Comments have been requested by Codex on sulphite use due to concerns about the intake of these compounds and their inclusion levels in jams and jellies, fruit desserts and soups and broths (Heller, 2007).

3.1. Form and solubility

In water solutions the sulphur dioxide can be written to show equilibrium (Davidson & Juneja, 1989; Ough, 1993):

$SO_2 + H_2O \leftrightarrow [H_2SO_3]$
$[H_2SO_3] \leftrightarrow HSO_3^- + H^+$
$HSO_3^- + H^+ \leftrightarrow SO_3^{2-} + 2H^+$

As the pH decreases, the proportion of sulphur dioxide increases at the expense of bisulphite ions (HSO_3^-). It is useful to have the sulphur dioxide in a salt form. The dry salts are easier to store and less of a problem to handle than gaseous or liquid sulphur dioxide. The metabisulphite is the anhydride of the acid sulphite:

$2HSO_3^- \leftrightarrow S_2O_5^{2-} + H_2O$

Two of the most common used sulphites are sodium sulphite (Na_2SO_3, theoretical % yield of SO_2 is 50.8%) and sodium metabisulphite ($Na_2S_2O_5$, theoretical % yield of SO_2 is 67.4%) (Davidson & Juneja, 1989; Ough, 1993).

3.2. Antimicrobial activity

The growth-inhibiting or lethal effects of sulphurous acids are most intense when the acid is in the un-ionized form. It was also noted that the bacteria were much more sensitive to sulphur dioxide than were yeasts and moulds. Bisulphites had lower activity than sulphur dioxide against yeasts, and sulphites had none (Ough, 993).

There is a specific reaction is between bisulphite and disulphide bonds:

$R1 - S - S - R2 + HSO_3^- \leftrightarrow R1SH + R2 - S - SO_3^-$

This reaction can cause conformational changes to enzymes. Thiamine pyrophosphate, a required co-factor for many enzymatic reactions, can be destroyed by the action of bisulphite. One type of activity of the sulphite against the yeast cell is its reaction with cellular ATP and / or its blocking of the cystine disulphide linkages. ATP reaction is also reduced with lactic acid bacteria with the addition of SO_2. There is an antimicrobial effect of SO_2 associated with activity at the surface of the cell. Further activities possible with SO_2 include blockage of transport, inhibition of glycolysis, nutrient destruction, and inhibition of general metabolism (interaction with structural proteins, enzymes, co-factors, vitamins, nucleic acids, and lipids) (Davidson & Juneja, 1989; Ough, 1993).

Sulphur dioxide is more effective against the growth of Gram-negative rods, such as *E. coli* and *Pseudomonas*, than in inhibiting Gram-positive rods, such as *Lactobacillus.* In *E. coli*, NAD-dependent formation of oxaloacetate from malate is inhibited (Davidson & Juneja, 1989; Ough, 1993).

A study found that the addition of 100 mg/kg of SO_2 as sodium metabisulphite to canned pork inoculated with *Clostridium botulinum* spores delayed cell growth. The delay was proportional to the concentration of the bisulphite addition (Ough, 1993).

Banks *et al.* (1985) reviewed the use of sulphite as an additive to control microbiological changes occurring in minced meat products. It was noted that many sulphite binding materials are present in meats. The main bacterial components of minced meats are *Pseudomonas* sp*., Brochothrix thermosphacta, Enterococcus* sp., lactobacilli, and members of the *Enterobacteriaceae*. In addition, oxidative yeasts are present. The *Salmonella* serovars and *E. coli* are inhibited to a greater extent than the other bacteria listed (Ough, 1993). Differences in other contaminants (yeasts) can affect the role of sulphites by producing binding compounds (e.g. acetaldehyde) (Ough, 1993; Roller *et al.*, 2002).

In a study in 1987, it was found that minced goat meat could be preserved up to 11–13 days if held at 7 ºC with 450 mg/liter of sulphur dioxide added. The effect was inhibition of growth of the flora. Sensory tests showed no adverse results. The shelf-life of ground beef was effectively increased from 1.8 days at 7 ºC storage with no treatment to 12.6 days at 0 ºC with the addition of 250 mg/kg of sulphur dioxide. The packaging used was a gas-permeable wrapping that allowed oxidative conditions (von Holy *et al.*, 1988; Ough, 1993).

In another study in 1987, it was found that vacuum packaging and a good oxygen barrier film decreased the spoilage in sulphite treated sausage. This was due to the lack of oxygen and the production of sulphite binding substances. Thus the free sulphite, which inhibited growth, was maintained for a longer period (Ough, 1993).

3.3. Antioxidant activity

The preservation of the colour and odour of meats are improved by sulphite treatment. Although slowing or prevention of growth of surface bacteria is probably important, the main effect in meat appears to be the antioxidant properties (Davidson & Juneja, 1989; Ough, 1993).

3.4. Toxicology

It appears from all the published reports that humans are reasonably tolerant to sulphur dioxide and, unless damaging doses are given, can recover unaffected. In the last years, however, cases concerning the sensitivity of asthmatics to sulphur dioxide have been reported. Some of these were life threatening or fatal. It may cause headaches, nausea, and diarrhea in some humans; the toxic effect is variable in humans and persons may tolerate different levels (Davidson & Juneja, 1989; Ough, 1993).

In the USA, sulphating agents are not permitted in meats or in foods recognised as a source of Vitamin B_1 and since 1986 have been banned from use in fresh fruit and vegetables. Consumers regard the deliberate addition of sulphites and / or any other chemical preservatives to foods as a form of adulteration. Yet, a low level of sulphite is required to give meat products (e.g. pork sausage) the “bloom” that is attractive to the purchaser. There is thus a clear need to develop novel preservation systems (Roller *et al.*, 2002).

4. NATURAL PRESERVATION METHODS

Over the years thoughts of natural preservative systems have been directed at the diverse range of natural antimicrobial systems that have evolved over millions of years to protect animals and plants from microbial attack. There has also been much effort in the search for antimicrobial agents, such as nisin of microbial origin (Dillon & Board, 1994).

A study of antimicrobial agents in plants and animals reveals that few, if any, act alone; they almost invariably act in concert with each other. This trend in evolution must not be overlooked by those who seek natural systems for food preservation. Successful exploitation will most probably stem from a combination of natural and established systems with hopefully a reduction in the levels of the latter (Dillon & Board, 1994).

For a natural antimicrobial compound to be used as a biopreservative in food systems, it needs to be produced economically on a large scale, it must not cause unacceptable organoleptic changes and it must be toxicologically safe.

The future of natural antimicrobial agents is most likely to be in combination with other preservative systems or with physical treatments, such as heating or freezing processes (Dillon & Board, 1994).

Selection of the proper antimicrobial is dependent upon several factors, including the chemical and antimicrobial properties; the properties and composition of the food product in question; the type of preservation system, other than chemicals, used in the food product; the type, characteristics, and the number of micro-organisms; the safety of the antimicrobial; and the cost effectiveness of the antimicrobial (Branen, 1993).

Due to the economical impacts of spoiled foods and the consumer's concerns over the safety of foods containing synthetic chemicals, a lot of attention has been paid to naturally derived compounds or natural products (Sökmen *et al.*, 2004). Myriad compounds in nature have the ability to inhibit micro-organisms. As a consequence, it is recognised that antimicrobials can occur as natural compounds of some foods. The recent increased demand for minimally processed, extended shelf-life foods have renewed interest in exploitation of these natural antimicrobials for food preservation uses (Conner, 1993).

4.1. Sorbic acid and Sorbates

Sorbic acid inhibits the growth of moulds, yeast, and some highly aerobic bacteria (Urbain & Campbell, 1987). In the United States it may be used in any food product that allows generally recognised as safe (GRASS) food additives and in about 80 more food products that have federal standards of identity. As food preservatives, sorbates have found wide application in various foods (including certain meat products). Amounts of sorbate used in foods are in the range of 0.02–0.3%. These concentrations have no major impact on food quality, but higher levels may cause undesirable changes in the taste of most foods. In general, amounts of 0.1–0.3% is tolerated, but levels as low as 0.1% may be detectable in some foods (Sofos & Busta, 1993).

The only approved use of sorbates in meat products in the United States is to suppress mould growth on the surface of dry sausages during the drying period (and in certain acid meat foods, especially in the fermented sausages where a mould problem may exist). For this purpose a solution of up to 10% potassium sorbate may be used to protect unrefrigerated dry sausages. These compounds are more common meat preservatives in other countries, however, such as Japan and Korea (Urbain & Campbell, 1987; Davidson & Juneja, 1989; Sofos & Busta, 1993). It is used to preserve Biltong (dried meat) in South Africa (Anonymous, 2009).

Studies in the 1950's and 1960's indicated its potential as a preservative in meat products. It was shown that sorbate retarded the growth of *Salmonella* serovars and *Staph. aureus* and delayed growth and toxin production by *Clostridium botulinum.* Extensive studies published in the 1970's and 1980's established the antibotulinal and overall antimicrobial activity of sorbates in various cured and uncured meat and poultry products. The antimicrobial activity of sorbate was demonstrated in bacon, comminuted pork products, beef, poultry, soy protein and pork frankfurters, poultry emulsions, pork slurries, uncured cooked sausage, sliced bologna, raw and cooked pork chops, beef steaks and poultry products and carcasses (Davidson & Juneja, 1989; Sofos & Busta, 1993).

In addition to *C. botulinum,* other pathogenic and spoilage bacteria inhibited by sorbate in various meat products include *Clostridium perfringens, E. coli, Yersinia enterocolitica, Brochothrix thermosphacta, Serratia liquefaciens, Lactobacillus, Clostridium sporogenes, Bacillus cereus, Bacillus licheniformis, Pseudomonas,* mesophiles, psychotrophs, and lipolytic organisms (Sofos & Busta, 1993). The antimicrobial activity of sorbate in meat products has been enhanced when combined with nitrite, sodium chloride (3.5%), phosphates, antioxidants, acids, low pH (less than 6.0), low storage temperature, low oxygen, and increased carbon dioxide atmospheres (“hurdle” concept) (Sofos & Busta, 1993). Sorbates in meat products (< 0.3%) had no major adverse effects on sensory qualities, such as colour and flavour (Davidson & Juneja, 1989; Sofos & Busta, 1993). Sorbate was also proposed as a means of reducing nitrite and nitrosamine levels in bacon while maintaining antimicrobial activity and inhibition of *C. botulinum* spores during temperature abuse of the product (Sofos & Busta, 1993).

The antimicrobial activity of sorbic acid is greatest when the compound is in the undissociated state (Davidson & Juneja, 1989). Sorbate inhibits cell growth and multiplication as well as germination and outgrowth of spore-forming bacteria. This may partially be due to its suggested effect on enzymes: inhibit dehydrogenases in fatty acid oxidation, inhibit sulph-hydryl enzyme, and interfere with enolase, proteinase, and catalase or inhibit respiration by competitive action with acetate in acetyl CoA formation (Davidson & Juneja, 1989; Sofos & Busta, 1993).

Davidson & Juneja, 1989) suggested that lipophilic acids, such as sorbic acid, interfere with transport across the cytoplasmic membrane (eliminate the ΔpH component of the proton motive force).

4.2. Organic Acids

One effective means of limiting microbial growth is to increase the acidity of a food, thereby creating an unfavourable environment. Adding an acidulant to the food or enhancing natural fermentation to develop acidity, changes the pH of the food. These actions tend to be microstatic rather than microcidal (Doores, 1993).

The incorporation of acids into a food can shorten sterilization times for heat treatment owing to the lowered heat resistance of micro-organisms in acid foods. The continued presence of acid can effectively inhibit germination and out-growth of spores that may survive the thermal process. Salt, sugar, and curing agents in conjunction with acids serve to decrease the process times further. Not only will this interaction ensure the commercial sterility of the food, but the decreased processing time would aid in preserving the palatability of the product (Doores, 1993).

The inhibitory effect of the acids has been compared on the basis of pH, concentration, chain length, type, and degree of branching to inhibit or kill a wide variety of micro-organisms (Doores, 1993). The undissociated portion of the molecule is believed to be responsible for the antimicrobial effect. It would be advantageous to use the acids near their pKa values. This primary concern limits the use of acidulants to those with pH values of less than 5.0. Since this value lies at the lower limits of growth for many bacteria, organic acids are usually more effective as antimycotic agents (Doores, 1993).

The toxicology and safety of the acids must be considered when selecting an acid to use. Most of the acids appear safe for use in food products and all acids are metabolized (Doores, 1993).

4.2.1. Acetic acid

Acetic acid (pKa = 4.75) and its related salts are widely used as acidulants and antimicrobials (Davidson & Juneja, 1989). Acetic acid is a monocarboxylic acid with a pungent colour and taste, which limits its use. It is the principal component of vinegars and as such is primarily used for its flavouring abilities. It is highly soluble in water. It is used in condiments (mustard, catsup, salad dressings, and mayonnaise), pickled products and sausages. Acetic acid, and its sodium and calcium acetates, has GRAS status for miscellaneous and general-purpose usage (Davidson & Juneja, 1989; Doores, 1993).

Acetic acid usually possesses rather weak bacteriostatic properties, and therefore reasonably high concentrations must be employed in foods to preserve them effectively at room temperature. A minimum concentration of 3.6% acetic acid in the water phase is necessary for preservation of vinegar pickled sausages (Urbain & Campbell, 1987).

Acetic acid is more effective against yeasts and bacteria than against moulds. Only acetic, lactic, and butyric acid bacteria are markedly tolerant to acetic acid. The activity of acetic acid varies with food product, environment, and micro-organism (Davidson & Juneja, 1989).

Bacillus spp. and Gram-negative bacteria are more inhibited than lactic acid bacteria, *Clostridium,* Gram-positive bacteria, yeasts and moulds at pH 4–6. At below pH 4 the latter 5 groups are similarly affected. On equal acidity basis, acetic acid is more effective than lactic acid. Therefore, pH is not a reliable indicator of preservative value (Doores, 1993).

Acetic has been used to increase the shelf-life of poultry when added to cut-up chicken parts in cold water at pH 2.5. Addition of acetic acid at 0.1% to scald tank water used in poultry processing decreased the D_{50} of *Salmonella* Newport*,* *Salmonella* Typhimurium and *Campylobacter jejuni* five- to ten-fold. Increasing the acid to 1.0% caused instantaneous death of all three genera (Davidson & Juneja, 1989).

At 1.2% as a 10 seconds dip for beef, acetic acid reduced micro flora such as *Salmonella* Typhimurium*, Shigella sonnei, Y. enterocolitica, E. coli, Pseudomonas aeruginosa,* and *Streptococcus faecalis* by an average of 65% (Davidson & Juneja, 1989).

4.2.2. Lactic acid

Lactic acid is one of the primary preservatives in many fermented products. It is used for pH control and flavouring (Davidson & Juneja, 1989, Zhou *et al.,* 2010). Lactic acid sprays have been effective in limiting microbial growth on meat carcasses under a variety of storage conditions. Lactic acid inhibits *Enterobacteriaceae.* Mixtures of lactic, acetic, citric and ascorbic acids and heat treatment (or vacuum packaging) lower the aerobic bacterial counts (*Salmonella* Typhimurium*, Enterobacteriaceae* and *Lactobacillaceae).* It was shown to also inhibit *Pseudomonas* (Davidson & Juneja, 1989; Doores, 1993).

The antimicrobial effects of lactates are due to their ability to lower water activity and the direct inhibitory effect of the lactate ion (Zhou *et al*., 2010). Sodium lactate can inhibit *C. botulinum* and *C. sporogenes.* A 100 mM concentration of sodium lactate buffered at pH 5.5 inhibits anaerobic growth of e.g. *Y. enterocolitica.* A 2.5% concentration is organoleptically still acceptable (Doores, 1993). Lactic acid as well as calcium, potassium and sodium lactates have GRAS status for miscellaneous or general-purpose usage (Doores, 1993).

The mode of action of organic acids in inhibiting microbial growth appears to be related to maintenance of acid-base equilibrium, proton donation, and the production of energy by the cells. Undissociated acids of short chain length can penetrate the cell more easily because they possess the ability to approach the cell membrane from the aqueous medium and easily, without requiring energy, penetrate the membrane lipid bilayers. The mode of action of the short chain lypophilic acids destroyed the proton-motive force, thereby limiting substrate transport. It is further speculated that acids that possess both lipoidal and aqueous solubilities are the most effective antimicrobial agents and that some sort of membrane attachment of the acid is involved (Doores, 1993).

4.3. Phenolic compounds

Phenolic compounds have been used as antimicrobial or antiseptic compounds since 1867 with the introduction of “carbolic acid” by Lister to sanitize equipment and in surgery. The use of phenol has declined steadily over the years because of its high toxicity and low antimicrobial activity, but other phenolic compounds have been introduced for use as antimicrobials in foods, pharmaceuticals, and cosmetics. Phenolic compounds may be categorized as: those currently approved for use in foods (alkyl esters of *p*-hydroxybenzoic acid); those currently approved for use other than as antimicrobials (phenolic antioxidants); and those that occur naturally in foods or are added to foods through processing (phenol to complex polyphenolics) (Davidson, 1993).

4.4. Spices, Essential Oils, and Oleoresins

Spices are added to foods primarily as flavouring agents. The functional properties (i.e. major flavour and aroma compounds and antimicrobial factors) of a spice reside in its essential oil (Conner, 1993). As a rule, spice essential oils are obtained commercially through steam distillation processes (Conner, 1993; Burt, 2004). Extraction by means of liquid carbon dioxide under low temperature and high pressure produces a more natural organoleptic profile but is much more expensive (Burt, 2004). Oleoresins, crude spice extracts with or without an organic carrier, are also commercially available and generally contain a higher concentration of essential oil than the spice itself. Spices could be a potential source of high levels of micro-organisms (Conner, 1993).

In addition to contributing flavour to foods, many spices also exhibit antimicrobial activity. In many instances, concentrations of compounds in spices and herbs necessary for inhibiting micro-organisms exceed those resulting form normal usage levels in foods. Nevertheless, the preservation effects of these seasoning agents should not be discounted (Dillon & Board, 1994).

Herb and spice compounds have been used in foods for a long time as flavour enhancers and would, therefore, not cause too much concern with consumers, regulatory agents or the food industry. Despite widespread use of often complex spice mixtures, very little is known about their antimicrobial properties, particularly possible synergistic effects. Additionally there are only a few studies that have looked at the antimicrobial effect of spice or herb extracts in foods. More research needs to be done on their antimicrobial effects in foods before their usefulness can be assessed. Additionally, spice and herb extracts need to be evaluated in combination with other established preservatives so that synergistic effects can be noted. Their main role in the future would, therefore, probably be a combination system, where their presence would permit a reduced amount of another preservative to be used. As with all the natural antimicrobial compounds, they lack adequate research, particularly in predictable mathematical modelling systems which would assess their performance in conjunction with other compounds (Dillon & Board, 1994).

Spices could also be used in conjunction with lactic acid bacteria. The presence of manganese, for example, in spices was shown to stimulate the rate of acid production by lactic acid bacteria in fermented sausages. Hence, a more effective preservative system can be set up using lactic acid bacteria and spices (Dillon & Board, 1994).

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are usually obtained by steam or hydro-distillation. Known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgestic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies (Bakkali *et al.,* 2008).

Essential oils can comprise more than sixty individual components. Major components can constitute up to 85% of the essential oil, whereas other components are present only as traces. There is some evidence that minor components have a critical part to play in antibacterial activity, possibly by producing a synergistic effect between other components (Burt, 2004; Bakkali *et al.,* 2008). The composition of the essential oil from a particular species of plant can differ between harvesting seasons and between geographical sources. The composition of essential oils from different parts of the same plant can also differ widely (Burt, 2004).

There has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing micro-organisms in foods (Sökmen *et al.*, 2004).

The means by which micro-organisms are inhibited by essential oils seems to involve different modes of action (Ouattara *et al.*, 1997). Because of the great number of constituents, essential oils seem to have no specific cellular targets (Bakkali *et al.,* 2008). As typical lipophiles, they pass through the cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids and permeabilize them, causing a leakage of vital intracellular constituents (metabolites and ions), impairment of bacterial enzyme systems, degradation of the cell wall, coagulation of cytoplasm and depletion of the proton motive force (Ouattara *et al.*, 1997; Burt, 2004, Bakkali *et al.,* 2008).

Undesirable organoleptic effects can be limited by careful selection of essential oils according to the type of food. Gram-positive organisms are generally more sensitive to essential oils than Gram-negative organisms (Burt, 2004).
Environmental conditions play a role in expression of antimicrobial activity (Conner, 1993). pH greatly affects the ability of thymol and carvacol to inhibit the mycelial growth of eight toxigenic aspergilli (Lueck, 1980). More work is needed

to define the effect of environmental conditions on antimicrobial activity of spices, essential oils, and oleoresins, which in turn can provide additional insight into probable models of action (Conner, 1993).

High-fat-content of food materials has effects on the application of essential oils (EOs). It may be because of the lipid solubility of EOs compared to aqueous parts of food. Combination of 1% cloves and oregano in broth culture showed inhibitory effect against *Listeria monocytogenes*, however, the same concentration was not effective in meat slurry. However encapsulated rosemary EOs showed better antimicrobial effect compared to standard rosemary EOs against *L. monocytogenes* in pork liver sausage (Tajkarimi *et al.*, 2010).

4.4.1. Cinnamon, allspice and clove

Cinnamon and clove exhibit a strong inhibitory effect, and allspice exhibits a medium inhibitory effect toward selected meat spoilage bacteria. The antimicrobial activity of cinnamon, allspice and clove is attributed to eugenol (2-methoxy-4-allyl phenol) and cinnamic aldehyde (Conner, 1993; Ouattara *et al.*, 1997; Zhou *et al*., 2010). Cinnamic aldehyde (non-phenolic compound) inhibits mould growth and mycotoxin production (Beuchat, 1994; Ouattara *et al.*, 1997).

Tests found cloves to inhibit *Bacillus subtilis* at 1:100 and *Staph. aureus* at 1:800 dilutions. The inhibition was dependant upon Gram-type and species. Cinnamon has a strong inhibitory effect on moulds like *Aspergillus* spp. and yeasts like *Saccharomyces cerevisiae.* An oleoresin of cinnamon was found to be inhibitory against 8 yeasts (Conner, 1993; Beuchat, 1994).

Cinnamic aldehyde has been shown to possess antibacterial properties by inhibiting amino acid decarboxylase activity. Allylhydroxycinnamates, which are quite similar to cinnamic aldehyde, inhibited *Pseudomonas fluorescens* by a specific mode of action related to cellular energy depletion (Ouattara *et al.*, 1997).

Eugenol is more bactericidal against *E. coli*, *Enterobacter sakazakii,* and *Klebsiella pneumoniae* than several antibiotics including ampicillin, erythromycin, and sulphamethizole (Ouattara *et al.*, 1997).

4.4.2. Oregano and thyme

These spices have been reported to have substantial antimicrobial activity towards selected meat spoilage bacteria. It has also been classified as exhibiting medium to high inhibitory activity. The terpenes carvacol, *p*-cymene and thymol are the major volatile compounds of oregano and thyme, and likely account for the antimicrobial activity (Conner, 1993; Beuchat, 1994; Ouattara *et al.*, 1997).

The bacteria *B. subtilis, Salmonella* Enteritidis*, Staph. aureus, Pseudomonas aeruginosa, Proteus morganii,* and *E. coli* are inhibited by carvacol and thymol dilutions of > 1:2000. Mesophilic, aerobic, and facultatively anaerobic micro-organisms were not inhibited by oregano. Some micro-organisms grow again after time at optimal incubation temperature. This indicates that either the active fraction was volatilised or the organism became resistant. Oregano can inhibit lactic acid production and growth of meat starter cultures (Conner, 1993).

In a study by Tepe *et al*. (2004), it was found that oregano not only exhibited antioxidant activity, but also antimicrobial activity against *Candida albicans* and *Candida krusei*, followed by *Mycobacterium smegmatis, Strep. pneumoniae, Acinetobacter lwoffi* and *Clostridium perfringens.* Low activity was shown against *Staph. aureus, Enterobacter aerogenes, E. coli, Proteus mirabilis* and *Moraxella catarrhalis.* Lowest activity was against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

It has been demonstrated that oregano oil, alone or in combination with sodium nitrite, could prevent botulinal out-growth in a broth medium. The two additives acted synergistically. This effect was explainable by their modes of action. The oregano oil affects germination and vegetative growth; sodium nitrite affects out-growth and vegetative growth. Unfortunately, oregano oil was not as effective when tested in pork. This was thought to be due to the high soluble oil components absorbed into the lipid fraction of the meat. At the lowest spore levels tested, there was a consistent trend, although statistically insignificant, towards increasing inhibition with increasing level of oregano oil in three of the four sets of nitrite levels. Perhaps the results would be more favourable at lower botulinal spore levels (Lueck, 1980).

Thyme oil is highly active against both Gram-negative and Gram-positive bacteria. Research indicated minimum inhibitory concentrations (mg/ml) of 0.75–1.25 and 0.125–0.5 for Gram-negative and Gram-positive bacteria respectively (Conner, 1993).

Oregano and thyme are reported to be quite antifungal in nature, and was shown to inhibit the growth of seven mycotoxigenic moulds. Yeast growth, as measured by biomass production, was reported to be inhibited by these oils. When added to growth medium, each essential oil inhibited the growth of seven yeast species. These oils and oleoresins were shown to impair pseudo-mycelium formation, sporulation, and respiration of several yeasts and reduced recovery of heat-stressed yeast cells (Conner, 1993; Beuchat, 1994).

Carvacol and thymol containing oils may have low activities in some instances due to insolubility in aqueous media, pH of the medium, or seasonal and intraspecific variation of essential oil composition (Ouattara *et al.*, 1997).

4.4.3. Rosemary, sage, clove, cassia bark and liquorice

Other spices, including rosemary, sage, and turmeric, possess antimicrobial activities. Rosemary spice extract at a 0.1% level substantially inhibited the growth of *Salmonella* Typhimurium and *Staph. aureus* in culture media. A concentration of 0.3% of either sage or rosemary in culture media inhibited the growth of 20 food-borne Gram-positive organisms, whereas a level of 0.5% was considered bactericidal for these bacteria. The inhibitory effect of rosemary and sage were attributed to their terpene fraction, which was comprised of borneol, cineole, pinene, camphene, camphor (all rosemary), and thujone (sage) (Conner, 1993).

A trial by Ouattara *et al.* (1997), found rosemary oil to be as inhibitory as cinnamon and clove oils. Yet, rosemary does not contain cinnamic aldehyde or eugenol, and all the other background components were present in small amounts. Camphor (0.10%) was the only component which was present in rosemary oil in concentrations higher than in the other oils under study. Therefore, the antimicrobial efficacy of rosemary oil could be at least partly related to the presence of camphor. This supports the idea that in some essences, minor compounds could have a huge antibacterial impact. The small amount of the total identified components (0.15%) in rosemary oils suggests that some other components may have contributed to its high antibacterial action (Ouattara *et al.*, 1997). Alcoholic extracts of rosemary were demonstrated to inhibit germination, growth, and toxin production by *C. botulinum* at levels of 500 ppm (Conner, 1993).

A three-way study was done by Zhang *et al.* (2009). In the first experiment the antimicrobial activity of 14 spice extracts was screened against four common meat spoilage and pathogenic bacteria (*L. monocytogenes, E. coli, Pseudomonas fluorescens* and *Lactobacillus sake*) in culture media. The results showed that individual extracts of clove, rosemary, cassia bark and liquorice contained strong antimicrobial activity, but the mixture of rosemary and liquorice extracts was the best inhibitor against all four types of microbes. Subsequently, mixed rosemary / liquorice extracts were spray-applied to inoculated fresh pork in modified atmosphere packaging (experiment 2) and to inoculated ham slices in vacuum packaging (experiment 3). The meat samples were stored at 4 ºC over a 28-day period and microbial growth was monitored regularly. The *L. monocytogenes* population on fresh pork by day 28 decreased by 2.9, 3.1 and 3.6 logs; the mesophilic aerobic bacteria (MAB) decreased by 2.7, 2.9 and 3.1 logs; the *Pseudomonas* spp. count decreased by 1.6, 2.1 and 2.6 logs and the total coliform count decreased by 0.6, 0.8 and 1.2 logs, corresponding to 2.5, 5.0 and 10 mg/ml op spray, respectively, when compared to the control ($p < 0.05$). The number of *L. monocytogenes* on ham slices decreased by 2.5, 2.6 and 3.0 logs; the MAB plate counts decreased by 2.9, 3.0 and 3.2 logs and the lactic acid bacteria (LAB) counts decreased by 2.4, 2.6 and 2.8 logs ($p < 0.05$), respectively, after 28 days, by the same levels of mixed rosemary / liquorice extract treatments. The results demonstrated strong potential of mixed rosemary and liquorice extracts as a natural preservative in fresh pork and ham products.

Hayouni *et al.* (2008) did a study on sage and peppertree. The essential oils (EOs) extracted from the aerial parts of cultivated *Salvia officinalis* L. (sage) and the berries of *Schinus molle* L. (peppertree) were analysed by gas chromatography-mass spectrometry (GC-MS) and 68 and 67 constituents were identified, respectively. The major constituents were 1,8-cineole (33.27%), β-thujone (18.40%), α-thujone (13.45%), borneol (7.39%) in *S. officinalis* oil and α-phellandrene (35.86%), β-phellandrene (29.3%), β-pinene (15.68%), p-cymene (5.43%) and α-pinene (5.22%) in *S. molle* oil.

The inhibitory effect of these EOs were evaluated against two food borne pathogens belonging to the *Salmonella* genus, experimentally inoculated (10^3 cfu/g) into minced beef meat, which was mixed with different concentrations of the EO and stored at 4 to 7 ºC for 15 days. Although the antibacterial activities of both EOs in minced beef meat were clearly evident, their addition had notable effects on the flavour and taste of the meat at concentrations of more than 2% for *Schinus molle* and 1.5% for *Salvia officinalis*. One solution to this problem may be the use of combinations of different food preservation systems. In this context, each of the EOs has been used along with low water activity (addition of NaCl) in addition to low refrigeration temperatures. Results on the *Salmonella* growth showed that some combinations could be recommended to eliminate bacteria from minced raw beef. By using this method, a stable and, from a microbiological point of view, safe meat can be produced without substantial loss in sensory quality. Results obtained herein, may suggest that the EOs of *S. officinalis* and *S. molle* possess antimicrobial activity, and therefore, they can be used in biotechnological fields as natural preservative ingredients in food industries (Hayouni *et al.,* 2008).

4.4.4. Allyl Isothiocyanate

Commercial allyl isothiocyanate (AIT) was examined by Nadarajah *et al.* (2005), for its ability to reduce numbers of *E. coli* O157:H7 inoculated in fresh ground beef packaged under nitrogen and stored refrigerated or frozen. A five-strain cocktail of *E. coli* O157:H7 containing 3 or 6 log_{10} cfu/g was inoculated into 100 g ground beef and formed into 10 x 1 cm patties. A 10 cm diameter filter paper disk treated with AIT suspended in sterile corn oil was placed on top of a single patty. One patty and paper disk was placed in a bag of Nylon / EVOH / PE with an O_2 permeability of 2.3 cm^3/m^2 24h atm at 23 ºC. The bags were back-flushed with 100% nitrogen, heat sealed and stored for 8, 21 or 35 days, respectively. During storage, the AIT levels in the package headspaces were determined by gas liquid chromatography, and mesophilic bacteria and *E. coli* O157:H7 were counted.

The mesophilic aerobic bacteria in ground beef patties were largely unaffected by the addition of AIT. At an initial population of 3 log_{10} cfu/g, *E. coli* was reduced by AIT to undetectable levels after 18 days at 4 ºC or 10 days at -18 ºC. In samples inoculated with 6 log_{10} cfu/g, a higher than 3 log_{10} reduction of *E. coli* O157:H7 was observed after 21 days at 4 ºC, while a 1 log_{10} reduction was observed after 8 and 35 days at 10 and -18 ºC, respectively (Nadarajah *et al.,* 2005).

The final AIT concentrations in the headspace after storage at 10, 4 and -18 ºC were 444, 456 and 112 µg/ml at 8, 21 and 35 days, respectively. Results showed that AIT can substantially reduce numbers of *E. coli* O157:H7 in fresh ground beef during refrigerated or frozen storage. AIT is one of many natural antimicrobials that are found in the seeds, stem, leaves, and roots of cruciferous plants, including horseradish and black and brown mustard (Nadarajah *et al.,* 2005).

4.4.5. Garlic, Onion, and other Allium species

Garlic and onion have been extensively studied for their antimicrobial properties. Investigations have shown that extracts from *Allium* bulbs inhibit growth and respiration of pathogenic fungi and bacteria. Aqueous extracts from fresh garlic bulbs al levels of 3%, 5% and 10% inhibited the growth of *Bacillus cereus* on nutrient agar plates by 31.3%, 58.2% and 100% respectively. A 5% garlic extract concentration was found to have a germicidal effect on *Staph. aureus,* whereas concentrations of ≥ 2% had a clear inhibitory effect and concentrations < 1% were not considered inhibitory (Conner, 1993).

An investigation of the effect of garlic and onion oils on toxin production of *C. botulinum* in meat slurry indicated that these oils, when used in the proportion of 1500 µg/g meat slurry, inhibited toxin production by *C. botulinum* type A. However, the inhibition was incomplete and toxin production by *C. botulinum* type B and type E was not inhibited (Conner, 1993; Beuchat, 1994).

Garlic sap inhibits the growth of several Gram-negative food spoilage and pathogenic bacteria, including *Enterobacter, Escherichia, Klebsiella, Proteus,* *Pseudomonas, Salmonella, Serratia,* and *Shigella.* It was shown that garlic juice was a very potent antimicrobial agent, both to *Staph. aureus* and the pathogenic yeast *Candida albicans.* Growth of other yeasts is also inhibited by extracts of *Allium* spp. (e.g. *Candida, Cryptococcus, Rhodotorula, Torulopsis,* and *Trichosporon*) (Beuchat, 1994).

Measurable bactericidal activity against *Salmonella* Typhimurium and *E. coli* was observed in freshly reconstituted dehydrated onion and garlic at concentrations of 1 and 5% (w/v), respectively, whereas maximal death rates occurred with concentrations of 5 and 10% (Conner, 1993). Aqueous extracts of garlic and various extracts of onion inhibit the growth of many moulds such as *Aspergillus flavus* and *Aspergillus parasiticus* (Beuchat, 1994).

The major antimicrobial constituent of garlic and onion is allicin, along with several other sulphur-containing compounds (sulphides) in the essential oils of garlic and onion. It is isolated from bulbs by steam distillation or ethanolic extracts. Allicin is extremely pungent and characterise the principle odour and taste of garlic and onion (Conner, 1993; Beuchat, 1994).

Allicin (0.0005 M) was found to inhibit several sulph-hydryl metabolic enzymes but few non-sulph-hydryl enzymes. Some enzymes were inhibited by 0.00005 M allicin. Allicin disrupts microbial cell metabolism primarily by inactivation of SH-containing proteins by oxidation of thiols to disulphides; competitive inhibition of the activity of sulph-hydryl components, such as cysteine and glutathione, by binding with them and / or by non-competitive inhibition of the enzyme functions by oxidation of the binding of SH-groups to allosteric sites. It could also interfere with cell metabolism and cell division (Conner, 1993; Beuchat, 1994).

Even though some compounds might show consistent antibacterial activities against meat spoilage bacteria, the extrapolation of these results to meat systems must be done with caution. Bacteria present on meat surfaces may attach firmly resulting in reduced exposure to essential oils or fatty acids. Proteins and lipid components of meat can also interact with the active components of antibacterial compounds (Ouattara *et al.*, 1997).

4.4.6. Fruits and other plants

Shan *et al.* (2009) did a study to find natural spice and herb extracts with antibacterial and antioxidant capacities that could potentially be used as natural preservatives in raw pork. The inhibitory effects of cinnamon stick, oregano, pomegranate peel and grape seed extracts on *L. monocytogenes, Staph. aureus* and *Salmonella* Enterica were evaluated in raw pork at room temperature (~ 20 ºC). The influences of these extracts on lipid oxidation in the meat were also investigated. The pH, colour parameters and TBARS (thiobarbituric acid-reactive substances) values were tested periodically. The results showed that all five natural extracts, especially clove, were effective against the bacteria. During storage the colour parameters of the extract-treated pork samples changed slightly, in comparison with significant changes in the control. Treatments with these extracts increased the stability of raw pork against lipid oxidation. Clove was the most effective for retarding lipid oxidation and presented the highest activity in raw pork. The conclusion was made that the tested extracts, especially clove, have potential as natural preservatives to reduce the numbers of pathogenic bacteria, colour degradation and lipid oxidation in raw pork.

The possible use of cranberry concentrate (CC) as a natural preservative was studied by examining its antimicrobial effect on the growth of *Escherichia coli* O157:H7 inoculated in ground beef, its organoleptic effect on beef patties, and its antimicrobial mechanism on the gene regulation level. Inoculated ground beef, to which CC was added, was stored at 4 ºC for 5 days. Bacteria were detected on days 0, 1, 3 and 5. Cranberry concentrate (2.5%, 5% and 7.5% w/w) reduced total aerobic bacteria by 1.5 log, 2.1 log, and 2.7 log cfu/g and *E. coli* O157:H7 by

0.4 log, 0.7 log, and 2.4 log cfu/g, respectively, when compared to the control on day 5 (Wu *et al.,* 2009).

Fifty panellists evaluated the burgers supplemented with CC. No difference in appearance, flavour, and taste were found among burgers with 0%, 2.5% and 5% CC. Flavour and overall acceptability ratings of the burgers with 7.5% cranberry concentrate were lower than the control burgers ($p < 0.05$). Burgers with 7.5% cranberry concentrate were neither liked nor disliked by the panellists probably because the flavour was a little sour (Wu *et al.*, 2009).

4.4.7. Other

Other spices and spice oils have also been shown to have some antimicrobial activity. Studies reported antimicrobial activity by spice oils and extracts of sweet marjoram, laurel, pimento (Chilli), coriander, anise, carvone, peppermint, caraway, cardamom, cumin, fennel, celery, dill, and mustard. Many of these preparations seem to be active against only one species, and some are even active in one study but not in another. The normal inhibitory concentrations used in these studies are very high, indicating little activity (Conner, 1993).

Black pepper and nutmeg stimulated growth and lactic acid production by starter cultures even at 12 g/liter. Inhibition, or the lack thereof, is a function of the test conditions and the micro-organisms tested, which means that studies with the same spice may yield different results (Conner, 1993).

4.5. Usage in Meat and Meat Products

Certain oils stand out as better antibacterials than others for meat applications (Burt, 2004). Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 µl/g in inhibiting *L. monocytogenes, Aeromonas hydrophila* and autochthonous spoilage flora in meat products, sometimes causing a marked initial reduction in the number of recoverable cells (Menon & Garg, 2001; Burt, 2004).

Mustard, cilantro, mint and sage oils are less effective or ineffective. A high fat content appears to markedly reduce the action of essential oils in meat products. For example, mint and cilantro essential oils were not effective in products with a high level of fat, such as pâté (which generally contains 30–45% fat) and a coating for ham containing canola oil. Immobilising cilantro essential oil in a gelatine gel, however, improved the antibacterial activity against *L. monocytogenes* in ham (Burt, 2004). One study found that encapsulated rosemary oil was much more effective than standard rosemary essential oil against *L. monocytogenes* in pork liver sausage (Burt, 2004).

In view of the published data on essential oils in foods, the following approximate general ranking (in order of decreasing antibacterial activity) can be made: oregano / clove / coriander / cinnamon > thyme > mint > rosemary > mustard > cilantro/sage. An approximate general ranking of the essential oil components is as follows (in order of decreasing antibacterial activity): eugenol > carvacol / cinnamic acid > basil methyl chavicol > cinnamic aldehyde > citral / geraniol (Burt, 2004).

4.6. Combinations of Chemical and Natural Preservatives

4.6.1. Chitosan and sulphite

Roller *et al*. (2002) did a study to develop a novel preservation system for fresh pork sausages based on a combination of chitosan (polymeric β-1,4-N-acetylglucosamine) and low concentrations of sulphite. Two pilot scale trials showed that 0.6% chitosan combined with low sulphite levels (170 ppm) retarded the growth of spoilage organisms more effectively (3–4 log cfu/g) than high levels

(340 ppm) of sulphite alone at 4 ºC for up to 24 days. Microbial counts for frozen sausages showed that the preservative efficacy of the chitosan / sulphite combination was maintained following frozen storage. Sulphite was degraded rapidly within the first 3 days of storage that contained only this preservative, but levels decreased less rapidly and persisted for longer in the presence of chitosan. Quantitative Descriptive Analysis using 31 trained panellists judged the sausages containing chitosan and sulphite to be more acceptable for a longer period than sausages containing only sulphite, which means the sausages containing chitosan and sulphite deteriorated less rapidly.

4.6.2. Chitosan and mint

Kanatt *et al.* (2008) noted that mint extract alone has good antioxidant activity but poor antimicrobial activity, while chitosan alone showed poor antioxidant activity with excellent antimicrobial properties. Therefore, the potential of chitosan and mint mixture (CM), as a preservative for meat and meat products, was investigated. Addition of chitosan to mint extract did not interfere with the antioxidant activity of mint. The antimicrobial activities of CM and chitosan were comparable against the common food spoilage and pathogenic bacteria, the minimum inhibitory concentration being 0.05%. CM was more effective against Gram-positive bacteria. The shelf life of pork cocktail salami, as determined by total bacterial count and oxidative rancidity, was enhanced in CM-treated samples at 0–3ºC (Kanatt *et al.*, 2008).

4.6.3. Tarisol Fresh (essential spice oil & food acids)

Tarisol Fresh is a commercially available combination of food acids and a specific essential spice oil with highly effective anti-bacterial and anti-oxidizing properties. The product is suitable for all types of sausages. The recommended dosage is 0.1–0.15% Tarisol Fresh in the final product (1–1.5 g Tarisol Fresh per kg final product). The ingredients are water, food acids (lactic acid, tartaric acid, citric acid, acetic acid), and essences of spices (Anonymous, 1999).

The properties of Tarisol Fresh are (Anonymous, 1999):

- Retains an attractive, fresh odour for longer
- Improvement in the taste of fresh and cooked sausages
- Assures an intensive, stable curing colour
- Extension of shelf-life due to improved hygienic-bacteriological conditions / retardation of bacterial growth
- Delayed rancidity of fat in meat products

4.6.4. Citrus extract and organic acids

A commercial mixture has become available under the name “Citrox”. Citrox comprises a range of phyto-alexins derived from the pith and rind of green Bergamont oranges and fruit acids (Neall, 2006).

The Bergamont orange (*Citrus bergamia*) appeared in the south of Italy before 1700. It is by some authors defined as a hybrid of *Citrus aurantium* (the bitter orange) and *Citrus limon* L. (the lemon) for some authors and by other authors of *Citrus aurantium* and *Citrus aurantiflora.* The growing, unripe fruit synthesizes high-molecular structures such as proteins, polysaccharides, lipids and flavonoids by carbohydrate metabolism initiated by photosynthesis in the leaves (Moufida *et al.,* 2003).

The anti-viral, -bacterial, -fungal, -mould and -yeast effect of Citrox is due to a synergistic activity of bioflavonoid and organic acid compounds. It offers the following benefits (Neall, 2006):

- Is a totally natural organic compound

- Has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative), viruses, moulds, yeast and fungi
- Is non-mutagenic, non-carcinogenic, non-toxic, non-corrosive, non-tainting, and non-volatile
- Has extended action (residual effect), but does not possess a knock down (shock action)
- Has the ability to break down biofilm
- Is effective even in the presence of organic and debris matter
- Its mechanism of action is by the destruction of the cell wall

This technology dates back to the 1930's and the work on vitamins and bioflavonoids by Albert Szent-Györgi, the Hungarian physiologist who won the Nobel Prize for medicine in 1937 for his research on the effects of these substances on human and plant health, but the outbreak of the war and the advent of antibiotics diverted attention from his discoveries. Consequently, this technology was not developed (Neall, 2006).

In the 1990's, his work was taken further by a UK biochemist, Ian Ripley who, recognising the increasing problems being encountered with bacteria-resistant antibiotics, founded the Citrox Limited Company. Ripley's hypothesis was that if specific bioflavonoids from fruit sources were combined in certain ratios with natural occurring acids, not only would an effective anti-pathogenic synergy be achieved, but also the products would have minimal toxicity. Furthermore, since bacteria and other pathogens had not previously encountered this form of attack, they would be exceptionally sensitive to this new method of challenge (Neall, 2006).

Additionally, he hypothesised that the pathogens would be unable to mutate successfully to form resistance, due to the high efficacy of kill rate within a short contact period. Five years of intensive research followed, and in 2001 a major technical and commercial breakthrough was achieved when Citrox formulations passed the stringent standards of the BSEN 1276 challenge and suspension test against more than 40 of the most commonly occurring bacteria, fungi, moulds and yeasts (Neall, 2006).

All the R & D work of Ripley's team has been independently assessed in a host of trials / tests around the world and Citrox products have found new application in commercial farming, animal health, plant propagation, food decontamination and sanitisation, water treatment, household products and, more recently, in human medical applications, including against methicillin-resistant *Staphylococcus aureus* (MRSA) that is such a major problem in hospitals around the world (Neall, 2006).

According to the supplier in South Africa, Citrox is being used to decontaminate and sanitise meat, chicken and fish after slaughter / catch and processing. Various Citrox products control pathogens in pigs, poultry and fish (Neall, 2006).

4.6.5. Ascorbate, green tea and grape seed extracts & low sulphite

A study was done by Banon *et al.* (2007), where they proposed green tea (GTE) and grape seed (GSE) extracts as preservatives for increasing the shelf-life of low sulphite raw beef patties. The antioxidant and antimicrobial activities of both extracts were compared with an ascorbate treatment. Five groups were established for the patties: Control (with no additives), S (100 mg/kg SO_2), SA (100 mg/kg SO_2 + 400 mg/kg sodium ascorbate), ST (100 mg/kg SO_2 + 300 mg/kg GTE) and SG (100 mg/kg SO_2 + 300 mg/kg GSE). Patties were stored at 4ºC in aerobic packaging for 0, 3, 6, or 9 days under retail display conditions.

Meat spoilage (total viable and coliform counts, pH, lightness, chroma, hue angle, metmyoglobin and TBARS) was determined. The sensory contribution of the extracts to cooked patties was evaluated (colour, odour, flavour and texture).

The results pointed to the possibility of using low SO_2-vegetable extract combinations to preserve raw meat products. ST, SG and SA delayed microbial spoilage, redness loss and lipid oxidation, thus increasing the shelf-life of the raw sulphite beef patties by 3 days. ST, SG and SA also delayed the onset of rancid flavours in cooked patties. No anomalous sensory traits were caused by either extract. Ascorbate, GTE and GSE improved the preservative effects of SO_2 on beef patties, especially against meat oxidation. This suggested that the quantity of SO_2 added can be reduced to obtain healthier raw meat products (Banon *et al.*, 2007).

5. CONCLUSIONS

Sulphur containing compounds, mainly sodium metabisulphite, has been used for a long time as a preservative in fresh processed meat products. Apart from it being a preservative, it is a strong anti-oxidant that preserves the fresh red colour of these products. Some people have asthmatic reactions to sulphite compounds.

Customers are demanding more natural food products (and preservatives) and are beginning to resist foods containing chemical preservatives. Research has been done on natural preservatives and results were varying. Sources researched included organic acids and phenolic compounds (e.g. spices, essential oils and oleoresins).

A single compound has not yet been found to fulfil all the requirements as a single natural preservative. Using the "Hurdle" concept a combination of ingredients and factors are currently needed to sustain shelf-life. This project is aimed at testing the viability of the use of a natural preservative, Citrox 14W - derived from a specific type of orange and containing food acids, in a traditional fresh processed South African sausage (Boerewors).

CHAPTER 3

MATERIALS AND METHODS

3.1. Preparation of Boerewors Models

Boerewors models were manufactured following typical industrial procedures (Hugo *et al.*, 1993) and in compliance with the South African regulations for boerewors (Department of Health South Africa, 1990). Table 3.1 shows the formulation used for the manufacture. Fresh meat [beef (70/30) and pork (50/50)], was purchased from a butchery in Bloemfontein. The models consisted of 90% total meat content formulated to contain 30% fat and 60% lean meat. Beef and pork meat were minced through a 13 mm steel plate.

The necessary SO_2 level and/or Citrox level was obtained by including the appropriate amount of sodium metabisulphite and/or Citrox in the spice mixture. A spice mixture (405 g) was made up consisting of coriander (98.6985 g), monosodium glutamate (26.325 g), black pepper (24.3 g), nutmeg (20.25 g), cloves (16.2 g), thyme (4.05 g), sodium chloride (188.527 g), sodium metabisulphite (10.6515 g), ascorbic acid (9.315 g) and dextrose (15.066 g). The rusk (commercially dried yeastless and sugarless bread crumbs), Worcester sauce, white vinegar and spice mixture containing sodium metabisulphite and/or Citrox were mixed in 360 g ice water and left to stand for 5 minutes to allow for hydration. 67.5 g of the spice mixture was added to each 2.5 kg batch. This was then thoroughly mixed with the meat and minced through a 4.5 mm plate.

The different models were then stuffed in 28/32 hog casings. The boerewors models were cut into 60–80 g pieces and placed in polyester trays and overwrapped with oxygen permeable polyethylene film. Six trays were done for each model. The models were stored at 4 °C under fluorescent light for six days.

Table 3.1. Ingredients used for manufacturing of the seven boerewors model systems used in this study.

	No preservative	450 mg/kg SO_2	100 mg/kg SO_2	1% Citrox	2% Citrox	100 mg/kg SO_2 + 1% Citrox	100 mg/kg SO_2 + 2% Citrox
Ingredient	**(%) w/w**	**(%) w/w**	**(%) w/w**	**(%) w/w**	**(%) w/w**	**(%) w/w**	**(%) w/w**
Beef 70/30	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Pork 50/50	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
Water	3.4	3.4	3.4	1.4	1.4	1.4	1.4
Rusk	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
Worcester sauce	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Citrox	0.0	0.0	0.0	1.0	2.0	1.0	2.0
SO_2 (mg/kg)	0.0	450.0	100.0	0.0	0.0	100.0	100.0
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Models were sampled on days 0, 2, 4 and 6. Four samples per model were used for microbiological and chemical analysis. The whole experiment was repeated three times with three different batches of meat purchased in different weeks.

3.2. Microbial Analysis

The effect of the preservative (sodium metabisulphite and/or Citrox) against a wide spectrum of micro-organisms, namely total bacteria, 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, 2, 4, and 6, on all seven 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 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*, 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).

The pour plate method was also used for determination of yeast and mould counts using rose bengal chloramphenicol agar (RBC; Oxoid CM0549) and plates were incubated at 25 °C for 4–5 days (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).

3.3. Colour Stability

On days 0, 2, 4 and 6 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.

3.4. Lipid Stability

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 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.

3.5. Sensory Analysis

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 (Figure 3.1), 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?"

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

Figure 3.1. Nine-point hedonic scale used in this study for sensory analysis (Lawless & Heymann, 1998; Stone & Sidel, 2004).

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.

3.6. Statistical analysis

The microbial counts obtained of total bacteria, yeasts and moulds, coliforms and *Staph. aureus* 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 interaction between preservation type and storage time (preservative x time) were further investigated by means of the Tukey-Kramer multiple comparison test (NCSS, 2007).

All the sensory analysis data was collected in spread sheets using Microsoft Excell 2007 and all 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% ($p \leq 0.05$). If the main effect was significant, the Tukey-Kramer multiple comparison test was applied to determine the direction of the differences between mean values.

CHAPTER 4

RESULTS AND DISCUSSION

The analysis of variance of the treatments of preservative, storage time and batch and the interactions among these variables in relation to total aerobic plate count, yeast and mould counts, coliform counts, *Staph. aureus* counts, colour L*, colour a*, colour b* and TBARS values, are given in Table 4.1.

The type of preservative, storage time and batch had a significant ($p < 0.001$ – $p < 0.01$) effect on all the parameters tested. The significant effect of preservative type and storage time was to be expected. The significant effect of batch may be ascribed to variation in quality of meat purchased on different days. The following interactions were not significant against the tested parameters: (Preservative type x Storage time) on colour L*; (Preservative type x Storage time x Batch) on coliform counts and *Staph. aureus* counts.

The following interactions had a significant ($p < 0.01$) effect on the following parameters: (Preservative x Batch) on colour a* and colour b*. The following interactions had a significant ($p < 0.05$) effect on the following parameters: (Preservative x Storage time) on *Staph. aureus* counts; (Storage time x Batch) on coliform counts; (Preservative x Storage time x Batch) on colour L*; (Preservative x Storage time x Batch) on colour b*.

For the purpose of this study the very important interaction between Preservative type and Storage time were further explored with a Tukey-Kramer multiple comparison test.

Table 4.1. Analysis of variance for various treatments and their interactions.

Treatment	**TAPC**	**Y & M count**	**Coliform count**	***Staph. aureus***	**Colour L***	**Colour a***	**Colour b***	**TBARS**
Preservative	***	***	***	***	***	***	***	***
Storage Time	***	***	***	***	**	***	***	***
Batch	***	***	***	***	***	***	***	***
Interactions								
Preservative x Storage Time	***	***	***	*	NS	***	***	***
Preservative x Batch	***	***	***	***	***	***	**	***
Storage Time x Batch	***	***	*	***	***	**	**	***
Preservative x Storage Time x Batch	***	***	NS	NS	*	***	*	***

*, $p < 0.05$

**, $p < 0.01$

***, $p < 0.001$

NS, not significant

TAPC, total aerobic plate count

Y&M, yeast and mould count

TBARS, Thiobarbituric Acid Reactive Substance

4.1. Microbial analysis

4.1.1. Total aerobic plate count

Figure 4.1 indicates the total aerobic plate counts for the seven boerewors model systems over the 6 day storage period at 4 ºC. For the control (no preservative) there was a gradual increase in counts from day 0 to day 6, the counts at day 6 showed a significant ($p < 0.001$) increase from the counts on day 0. It reached a value of over 10^7 cfu/g after 6 days storage; this is the arbitrary shelf life “end point” where signs associated with spoilage are found (Steyn, 1989). At day 0 (day of manufacturing) the control had significantly ($p < 0.001$) higher counts than the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox models, this may be indicative of an immediate effect of 2% Citrox. There were no significant differences between the control and the rest of the treatments.

After 2 days of storage the control still had significantly ($p < 0.001$) higher counts than the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox. Although all the rest of the treatments had lower counts than the control, the differences were not significant. The treatments of 450mg SO_2, 100 mg/kg SO_2, 1% Citrox and 100 mg/kg SO_2 + 1% Citrox had the same counts. There were no significant differences between the counts of 2% Citrox and 100 mg/kg SO_2 + 2% Citrox.

At day 4 the control counts were significantly ($p < 0.001$) higher than those for all the different preservatives and different preservative combinations. There were no significant differences in the inhibitory effect on the total aerobic plate counts for the different preservatives and different preservative combinations. Although not significant, the treatments of 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox had lower counts than the rest of the treatments.

After the 6 days storage period, the control had significantly ($p < 0.001$) higher

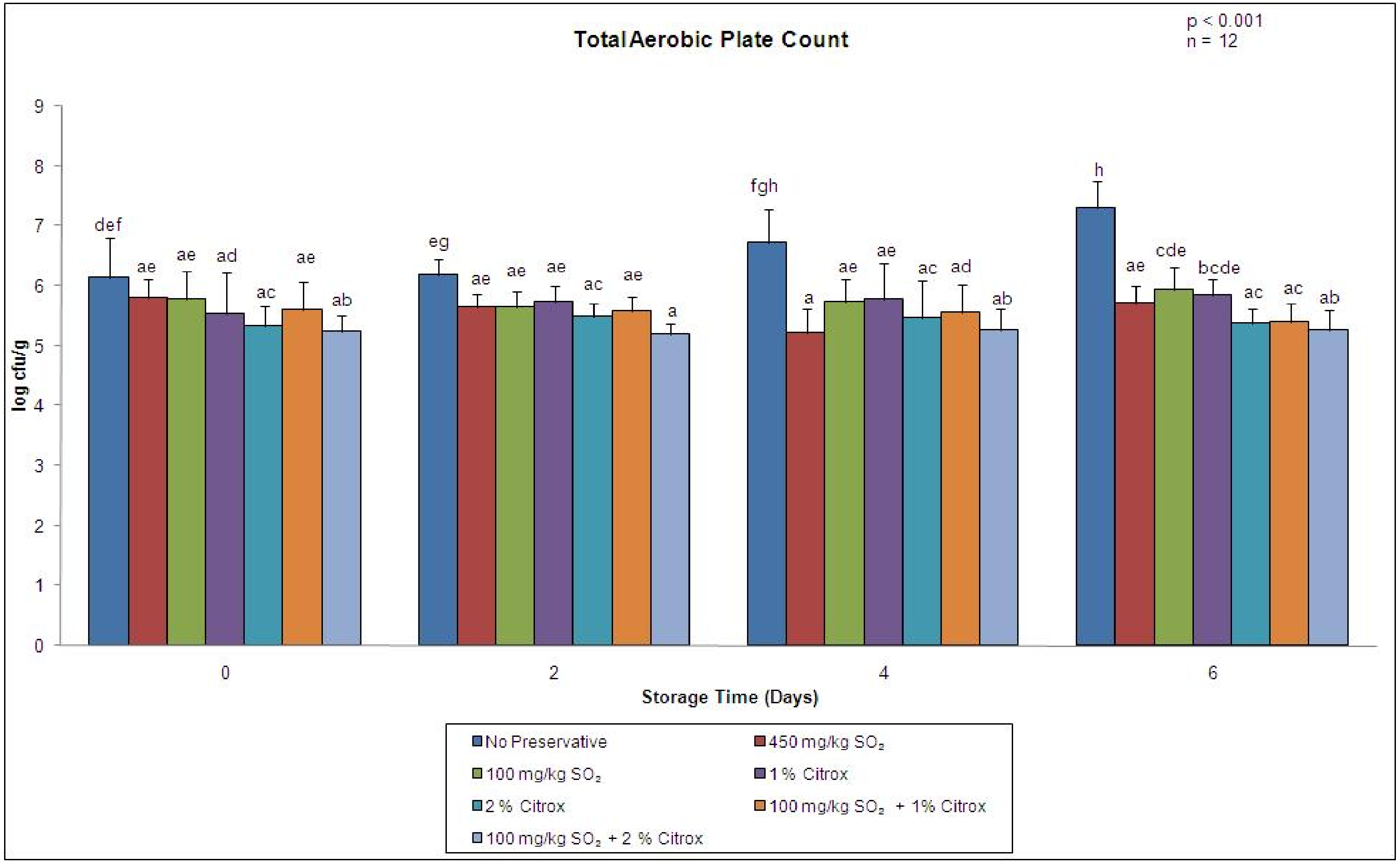

Figure 4.1. Aerobic plate counts of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

counts than the rest of the treatments. The 100 mg/kg SO_2 + 2% Citrox treatment had a significantly (p < 0.001) better inhibitory effect than the 100 mg/kg SO_2 treatment. Although not significant, the 100 mg/kg SO_2 + 2% Citrox, the 2% Citrox treatment and the 100mg/kg SO_2 + 2% Citrox treatment were also more efficient in inhibiting TAPC than 450 mg/kg SO_2. The 100 mg/kg SO_2 and 1% Citrox were slightly (not significant) less efficient in inhibiting TAPC. For the 6 day period there were no significant increases in counts for any of the treatments.

The use of sulphur dioxide as preservative has been widely documented. The legal amount of SO_2 allowable in minced fresh processed products like boerewors, is 450 mg/kg SO_2 (Department of Health, 2001). Sulphur dioxide is more effective against the growth of Gram-negative rods, such as *E. coli* and *Pseudomonas*, than in inhibiting Gram-positive rods, such as *Lactobacillus* (Davidson & Juneja, 1989; Ough, 1993).

The Citrox (1% and 2%) treatments, on its own and in combination with 100mg/kg SO_2, were as efficient as 450 mg/kg SO_2 in keeping TAPC below the 10^7 cfu/g limit over the 6 day storage period. It has been documented that Citrox has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative). Its activity is due to a synergistic activity of bioflavonoid and organic acid compounds (Neall, 2006).

A similar study was done, using cranberry concentrate (CC) as a natural preservative. The cranberry concentrate was added to beef patties inoculated with *E. coli* O157:H7, the patties were then stored at 4ºC for 5 days. Bacteria were detected on day 0, 1, 3, and 5 on CC levels of 2.5%, 5% and 7.5% w/w. The total aerobic bacteria were reduced by 1.5 log, 2.1 log and 2.7 log cfu/g when compared to the control on day 5 (Wu *et al.*, 2009).

In another study Kanatt *et al*. (2008) used a mixture of chitosan and mint (CM) at 0.1% as a preservative in meat and meat products. In the commercial preparation of pork cocktail sausages, CM was incorporated in the normal formulation that is added during its preparation. The total bacterial counts of both the samples (treated and untreated) increased on chilled storage. Control salamis spoiled in less than 2 weeks (log cfu/g > 6), while salamis containing CM displayed a shelf life of three weeks.

Other researchers also used reduced levels of sulphite in combination with natural preservatives. A study was done to develop a novel preservation system for fresh pork sausages based on a combination of chitosan and low concentrations of sulphite. Two pilot scale trials showed that 0.6% chitosan combined with low sulphite levels (170 ppm) retarded the growth of spoilage organisms more effectively (3–4 log cfu/g) than high levels (340 ppm) of sulphite alone at 4 ºC for up to 24 days. Microbial counts for frozen sausages showed that the preservative efficacy of the chitosan / sulphite combination was maintained following frozen storage. Sulphite was degraded rapidly within the first 3 days of storage that only contained this preservative, but levels decreased less rapidly and persisted for longer in the presence of chitosan (Roller *et al.,* 2002).

Similar results were achieved by a study done by Banon *et al.* (2007) which proposed green tea (GTE) and grape seed (GSE) extracts as preservatives for increasing the shelf life of low sulphite raw beef patties. The antioxidant and antimicrobial activities of both extracts were compared to ascorbate. They found that ascorbate, GTE and GSE improved the preservative effects of low (100 mg/kg SO_2) doses; microbial growth was inhibited and the shelf life of the patties was increased by 3 days – although the trends indicated only marginal improvement in microbial quality as a result of the addition of ascorbate, GTE and GSE as compared to 100 mg/kg SO_2 alone. The addition of 100 mg/kg SO_2 was not sufficient to delay the growth of total aerobic bacteria and total coliforms in raw beef patties packaged in air and stored for 9 days under retail display conditions (4 ºC, display cabinet, white fluorescent light). Reducing agents like ascorbate or polyphenols could protect SO_2 against oxidation, enabling SO_2 to act for more time. It is suggested that tea and grape polyphenols also have certain antibacterial activity *in vitro*, although no specific studies on meat products have been made. GSE would be mainly effective against Gram-positive bacteria, with gallic acid as the main active component. GTE would inhibit *E. coli, Staph. aureus, Staphylococcus epidermidis* and *Streptococcus mutans.* They concluded that their results suggested that tea and grape seed extracts would probably be more effective preservatives in precooked or cooked meat products, but further study is merited.

4.1.2. Coliform count

Figure 4.2 shows the coliform counts for the seven boerewors model systems over the 6 day storage period at 4 ºC. At day 0 there were no significant difference in the counts of the control and all the treatments, although, similar as was the case with the TAPC counts, the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox had slightly lower (not significant) counts than the 450 mg/kg SO_2. No significant change in counts occurred for the control over the 6 day storage period. At day 0 and day 2, there were no significant differences in the counts of all the treatments, although 100 mg/kg SO_2 + 2% had the lowest counts. There was a reduction of counts from day 0 to day 2 for all the treatments, the reduction was not significant for all the treatments.

At days 4 and 6 the 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox treatments had significant ($p < 0.001$) lower counts than the control. The other treatments had a slight, but insignificant, decrease in counts, suggesting that these treatments had a bacteriostatic effect whereas the 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% had bactericidal effects. Over the 6 day period, the 100 mg/kg SO_2 + 2% Citrox treatment had the same inhibitory effect as the 450 mg/kg SO_2 treatment. The rest of the treatments had slightly higher counts, however the differences were not significant when compared to the counts of 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox.

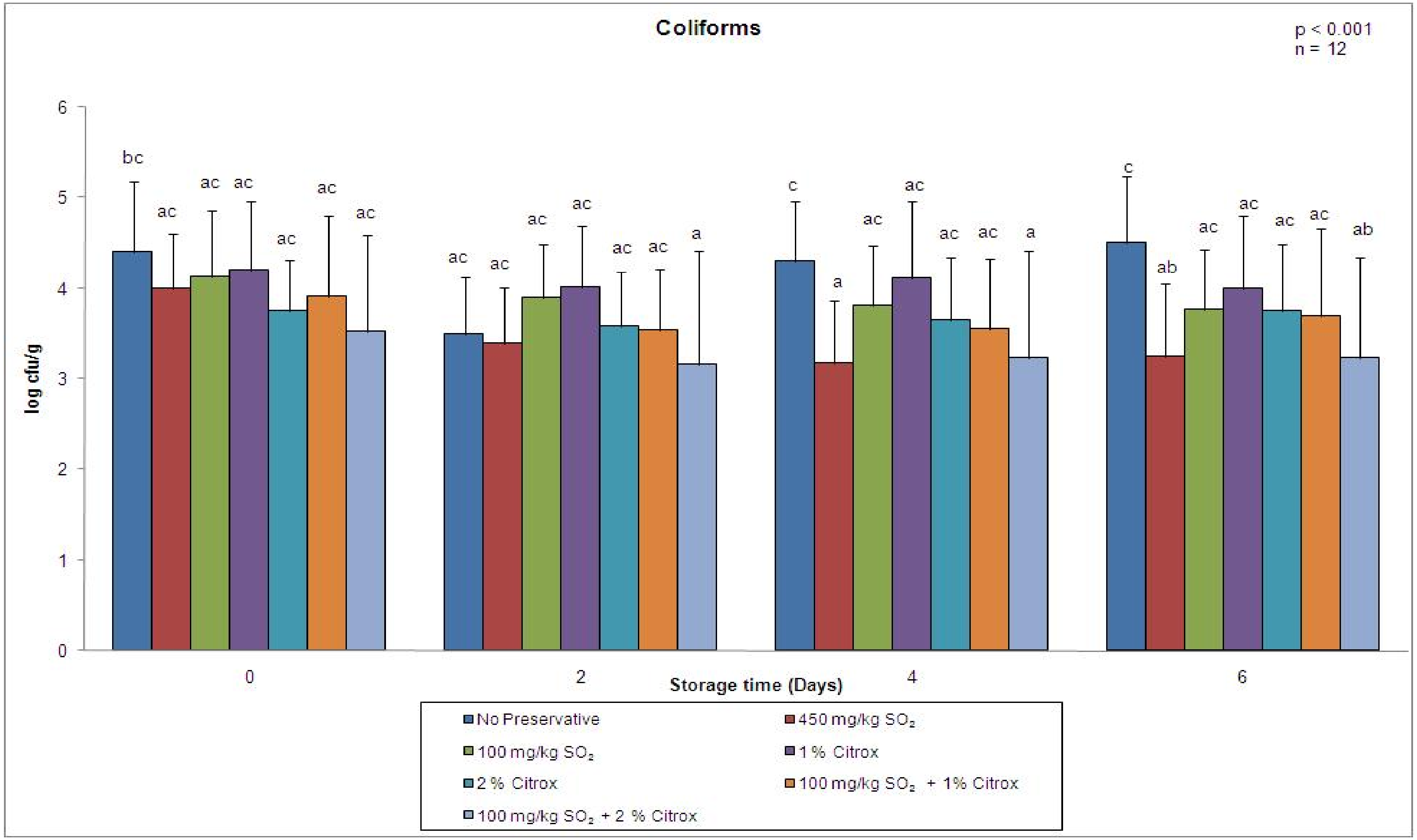

Figure 4.2. Coliform counts of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

Over the 6 day period as a whole there was a reduction of counts for all the treatments, however the reductions in counts were not significant. Citrox treatments (1% and 2%) in combination with 100 mg/kg SO_2 had lower, although not significantly lower, counts than the average of 100 mg/kg SO_2 or Citrox treatments on their own. Especially the bactericidal effects of the 100 mg/kg SO_2 + 2% Citrox on coliforms, could suggest a synergistic working between these preservatives.

The reductions in counts that were seen in this part of the study could also be attributed to the microbial hurdles incorporated in the boerewors models. The hurdle technology uses a combination of suboptimal growth conditions, sometimes in combination with chemical preservatives, in which each factor on its own is insufficient to prevent the growth of spoilage organisms or pathogens but the combination gives effective control (Garbutt, 1997). Notable microbial hurdles in the boerewors in the control treatment (no preservative) were vinegar (1.5%), ascorbic acid (62.31 mg/kg) and sodium chloride (1.26%) leading to a reduced pH and water activity (Charimba *et al*., 2010).

Although the presence of *E. coli* was investigated in this study, it could not be detected in any of the samples. This could be ascribed to good manufacture hygiene not only of the minced meat used for the seven boerewors models in this study, but also during the manufacture of the seven models. A study by Charimba *et al*. (2010) found a reduction in *E. coli* counts after 2 days of storage at 4ºC both in the presence and absence of a preservative (450 mg/kg SO_2) at both a high and low inoculum of *E. coli* into a same boerewors model as used for this study.

It could also be a similar case as the study done by Roller *et al.* (2002): they did a study to develop a novel preservation system for fresh pork sausages based on a combination of chitosan and low concentrations of sulphite. Their results suggested that selective inactivation and inhibition of Gram-negative bacteria had occurred. They did not do counts on *E. coli*, but only on Gram-negative bacteria as a group. They explained the efficacy of the chitosan/sulphite combination on the basis that chitosan protected sulphite from breakdown. However, Neall (2006) reported that Citrox has a broad spectrum antimicrobial activity, which works against most bacteria (Gram-positive and Gram-negative).

Sulphur dioxide is more effective against the growth of Gram-negative rods, such as *E. coli*, than in inhibiting Gram-positive rods. In *E. coli*, NAD-dependent formation of oxaloacetate from malate is inhibited (Davidson & Juneja, 1989, Ough, 1993).

Until recently, the use of SO_2 has had GRAS status. Investigations have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites (Ough, 1993; Roller *et al*., 2002). This has caused a great deal of research in all areas concerning sulphites and SO_2 (Ough, 1993).

Nadarajah *et al.* (2005) examined allyl isothiocyanate (AIT) for its ability to reduce numbers of *E. coli* O157:H7 inoculated in fresh ground beef packaged under nitrogen and stored refrigerated or frozen. Mesophilic aerobic bacteria in ground beef patties were largely unaffected by the addition of AIT. An initial population of 3 $\log_{10}$ cfu/g *E. coli* was reduced by AIT to undetectable levels after 18 days at 4ºC or 10 days at -18ºC. Samples inoculated with 6 $\log_{10}$ cfu/g had a higher than 3 $\log_{10}$ reduction of *E. coli* O157:H7 after 21 days at 4 ºC, and a 1 $\log_{10}$ reduction after 8 days at 10 ºC and 35 days at -18 ºC.

Wu *et al.* (2009) studied the possible use of cranberry concentrate (CC) as a natural preservative by examining its antimicrobial effect on the growth of *E. coli* O157:H7 inoculated in ground beef. Cranberry concentrates of 2.5%, 5% and 7.5% w/w reduced *E. coli* O157:H7 counts by 0.4 $\log_{10}$ cfu/g, 0.7 $\log_{10}$ cfu/g and 2.4 $\log_{10}$ cfu/g respectively when compared to the control after 5 days of storage at 4 ºC.

4.1.3. Yeast and mould counts

Figure 4.3 shows the yeast and mould counts for the seven boerewors model systems over the 6 day period stored at 4 ºC. The control had a significant ($p < 0.001$) increase in counts from day 0 to day 2; then again from day 2 to day 4, from day 4 to day 6 the counts increased again, although the increase was not significant increase. At day 4 the counts were already at 10^6 and therefore a contributing factor in bringing the total counts close to 10^7 where spoilage takes place (Steyn, 1989). The environment of the boerewors (low pH, high salt) might select for these organisms.

On day 0 the 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox treatments had significantly ($p < 0.001$) lower counts than the control. The rest of the treatments did not differ significantly from the control or from each other. On day 2 the 450 mg/kg SO_2, 2% Citrox and 100 mg/kg SO_2 + 2% Citrox treatments had significantly lower counts than the control. The 100 mg/kg SO_2 + 2% Citrox treatment had significantly lower counts than the 2% Citrox treatment. There was no significant difference between the counts of the 450 mg/kg SO_2 and the 100 mg/kg SO_2 + 2% Citrox, nor between the 450 mg/kg SO_2 and 2% Citrox treatments. There was no significant difference between the control and other treatments.

On day 4 the 450 mg/kg SO_2, 2% Citrox, 100 mg/kg SO_2 + 1% Citrox and 100 mg/kg + 2% Citrox treatments had significantly ($p < 0.001$) lower counts than the control. There was no significant difference between 2% Citrox and 100 mg/kg SO2 + 1% Citrox, both did have significantly ($p < 0.001$) higher counts than the 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox treatments. 450 mg/kg SO_2 had significantly ($p < 0.001$) higher counts than the 100 mg/kg SO_2 treatment. 100 mg/kg SO_2 + 2% Citrox had significantly ($p < 0.001$) lower counts than all the other treatments.

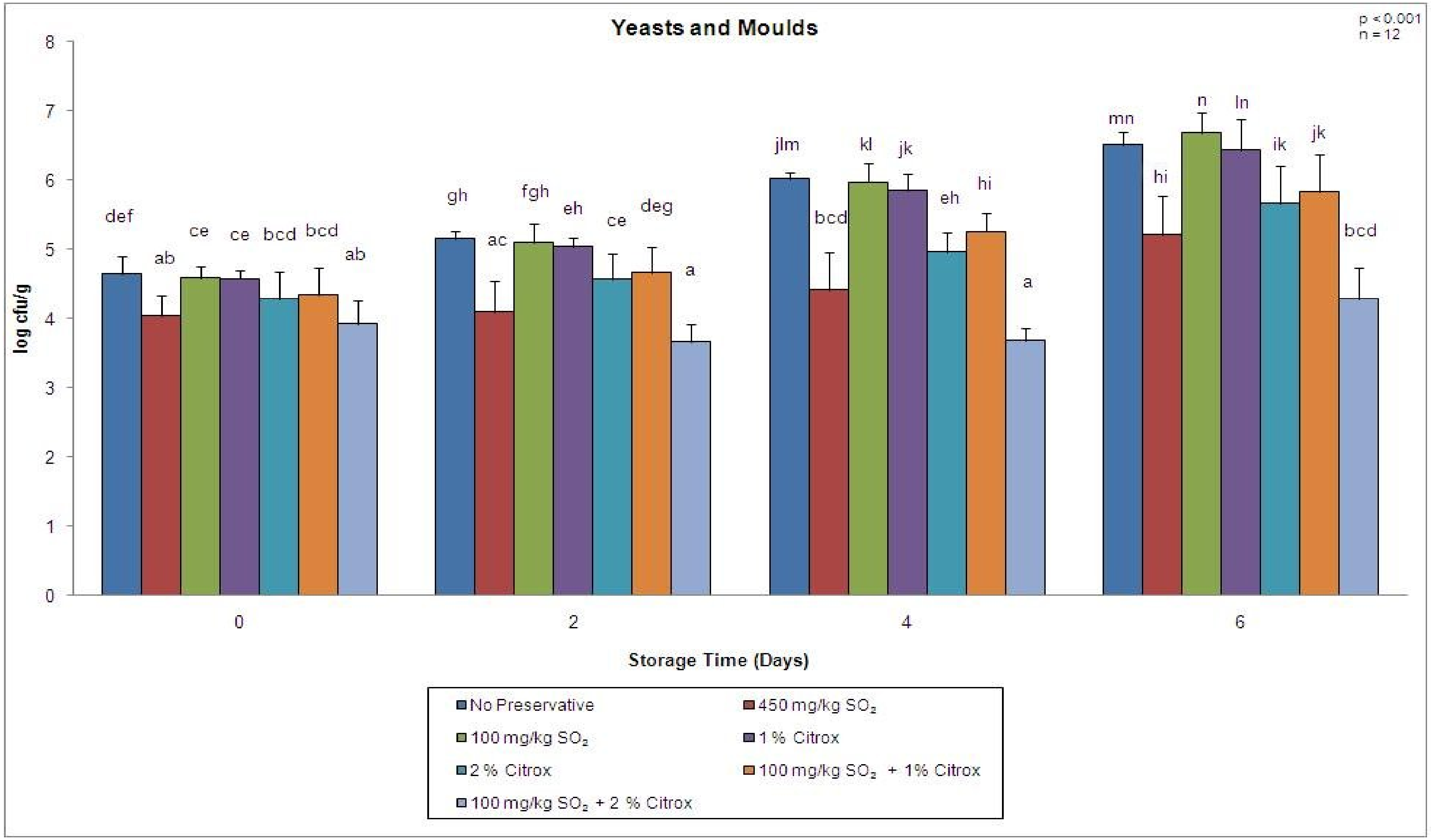

Figure 4.3. Yeast and mould counts of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

On day 6 the 450 mg/kg SO_2, 100 mg/kg SO_2 +1% Citrox, 100 mg/kg SO_2 + 2% Citrox and 2% Citrox treatments had significantly ($p < 0.001$) lower counts than the control.

There was no significant difference between 2% Citrox and 100 mg/kg SO_2 + 1% Citrox counts. The 100 mg/kg SO_2 + 2% Citrox had significantly ($p < 0.001$) lower counts than all the treatments, including the 450 mg/kg SO_2 treatment.

Over the 6-day period, there was a significant ($p < 0.001$) increase in the counts for the control and all the treatments, except for the 100 mg/kg SO_2 + 2% Citrox, which did not have a significant increase in counts. The combination treatment of 100 mg/kg SO2 + 2% Citrox, therefore, had the best preservative effect in terms of yeasts and moulds. It has been reported that Citrox has a broad spectrum antimicrobial activity against moulds and yeasts (Neall, 2006).

Ough (1993) and Roller *et al.* (2002) found that yeasts can affect the role of sulphites in minced meat products due to the production of binding compounds (e.g. acetaldehyde). There are also many sulphite binding materials present in meats (Banks *et al.*, 1985). In another study in 1987, it was found that vacuum packaging and a good oxygen barrier film decreased the spoilage in sulphite treated sausage. This was due to the lack of oxygen, delaying yeast growth and the production of sulphite binding substances. Thus the free sulphite, which inhibited growth, was maintained for a longer period (Ough, 1993). This effect can explain why there was no difference between the control and the 100 mg/kg SO_2 addition in this study. Both the 1% and 2% Citrox treatments had an inhibitory effect on yeasts and moulds. It could be postulated that when combined with SO_2 the Citrox (1% and 2%) inhibited the growth of yeasts and moulds, therefore reducing the production of binding compounds and leaving more SO_2 free to inhibit growth further – or in the case of the 2% Citrox + 100 mg/kg SO_2 achieve a significant reduction in counts when compared to all the other treatments.

The study done by Roller *et al.* (2002) on the combination of chitosan and low concentrations of sulphite as a novel preservation system for fresh pork sausage raised the possibility that the mechanism of preservation by the combination may be two-fold. Firstly, chitosan may have acted as a “slow-release” agent for sulphite, particularly free sulphite, thereby preventing its premature degradation or irreversible binding with other ingredients in the sausage. Secondly, any unbound chitosan may have selectively inhibited the yeast flora, thereby preventing acetaldehyde production and the consequent inactivation of sulphite by binding. It is pity that mould and yeast counts were not done in the study. However their results is comparable to Citrox (1% and 2%) in that the natural preservative had an inhibitory effect on the yeasts and moulds, therefore reducing the sulphur binding compounds and allowing more free SO_2 for more inhibitory effects.

4.1.4. Staphylococcus aureus count

Figure 4.4 shows the *Staphylococcus aureus* counts for the seven boerewors model systems over the 6 day period stored at 4 ºC. On day 0 there were no significant differences between the counts of the control or any of the treatments. From day 0 to day 2 there was an increase in the counts of the control and all the treatments. The increase in counts was not significant; there was also no significant difference between the counts of the control and all the treatments, although the control had the highest counts, and the 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox had the lowest counts.

From day 2 to day 4 there was a decrease in the counts of the control and all the treatments, the decrease in counts was not significant. The control still had the highest counts; however the 100 mg/kg SO_2 + 1% Citrox and the 100 mg/kg SO_2 + 2% Citrox now had the lowest counts – lower than 450 mg/kg SO_2. The 450 mg/kg SO_2 and 2% Citrox had the same counts.

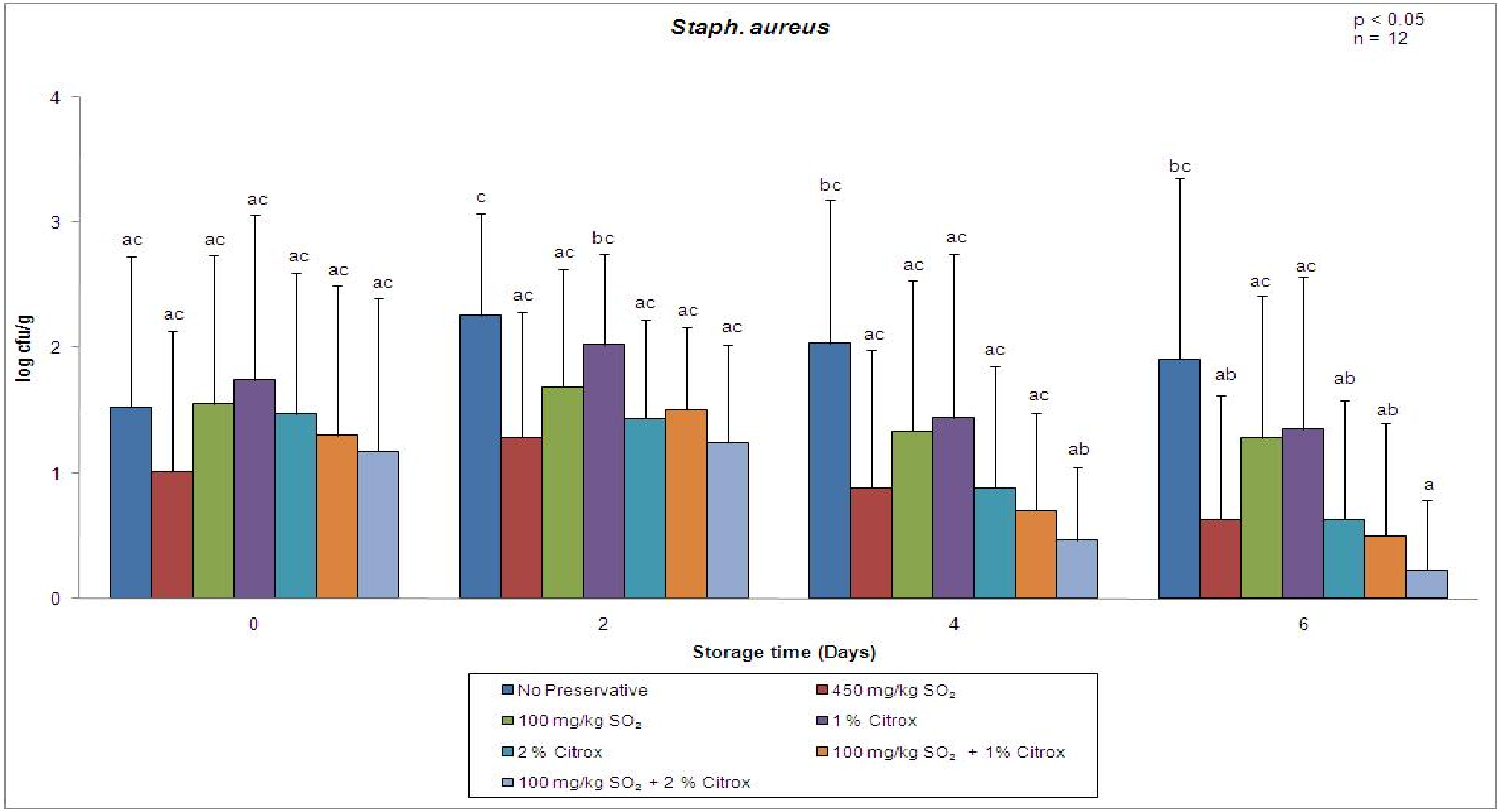

Figure 4.4. ***Staphylococcus aureus*** **counts of the seven boerewors models stored for 6 days at 4 °C.** Results with different superscripts are significantly different. Bars represent average values and standard deviations.

From day 4 to day 6 there was no significant change in the counts for the control. The 450 mg/kg SO_2, 100 mg/kg SO_2 and 2% Citrox treatments had slight, but insignificant, reductions in counts. On day 6 the 100 mg/kg SO_2 + 2% Citrox treatment had significantly ($p < 0.05$) lower counts than the control. There were no significant differences between the counts of the rest of the treatments. This could be an indication that there was a synergistic working between these 2 preservatives. This is also supported by the reduction in counts found with the 100 mg/kg SO_2 + 1% Citrox. Throughout the trial, 2% Citrox addition had similar reduction in counts than 450 mg/kg SO_2. Therefore it could be concluded that SO_2 + Citrox could be a good combination in the prevention of Gram-positive bacteria in this type of meat product.

Other studies on natural preservatives to inhibit *Staph. aureus* have been done, e.g. Shan *et al.* (2009) did a study to find natural spice and herb extracts with antibacterial and antioxidant capacities that could potentially be used as natural preservatives in raw pork. The inhibitory effects of cinnamon stick, oregano, pomegranate peel and grape seed extracts on *Listeria monocytogenes, Staph. aureus* and *Salmonella* Enterica were evaluated in raw pork at room temperature (~ 20 ºC). The results showed that all five natural extracts, especially clove, were effective against the bacteria. The conclusion was made that the tested extracts, especially clove, have potential as natural preservatives to reduce the numbers of a pathogenic bacteria like *Staph. aureus*. In the study by Banon *et al.* (2007), they reported that grape seed extracts would be mainly effective against Gram-positive bacteria, with gallic acid as the main active component.

Neall (2006) reported that the Citrox products have found application in human medical applications, including against methicillin-resistant *Staph. aureus* (MRSA) that is such a major problem in hospitals around the world. It was also reported that Citrox has a broad spectrum antimicrobial activity, which works against most bacteria, including Gram-positive bacteria. It seems that in this study the Citrox did not have a big influence by itself on the counts, but when used in combination with lowered levels of SO_2, it showed the best results of all the preservatives tested.

4.2. Colour and lipid stabilities

4.2.1. Colour a-values (redness)*

Figure 4.5 shows the colour a* (stability of the red colour) values for the 7 boerewors model systems over the 6 day period stored at 4 ºC. Studies in meat colour on instrumentally measured meat colour often focus on the a* value (redness), because the redness of the meat is an important component of visual appeal to customers. Several authors have studied the colour of meat and meat products, and reported that the meat oxidation caused a decrease in a* value (Banon, 2007; Shan *et al.*, 2009) which is normally unacceptable for consumers.

On day 0 the 450 mg/kg SO_2 treatment had significantly ($p < 0.001$) higher values than the control and all the other treatments, except the 100 mg/kg SO_2 treatment. The 100 mg/kg SO_2 treatment did not differ significantly from the control and 450 mg/kg SO_2 treatments, but was significantly ($p < 0.001$) higher than all the other treatments. The 1% Citrox and 100 mg/kg SO_2 + 1% Citrox treatments' values did not differ significantly, but had significantly ($p < 0.001$) higher values than the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox treatments. There was no significant difference between the values of the treatments containing 2% Citrox and 100 mg/kg SO_2 + 2% Citrox.

On day 2 the 450 mg/kg SO_2 had significantly ($p < 0.001$) higher values than all the other treatments. The 100 mg/kg SO_2 treatment had significantly ($p < 0.001$) lower values than 450 mg/kg SO_2, but significantly ($p < 0.001$) higher than the control and the rest of the treatments. All the treatments containing Citrox had significantly ($p < 0.001$) lower values than the control. Treatments with 1% Citrox had significantly ($p < 0.001$) higher values than treatments with 2% Citrox. There

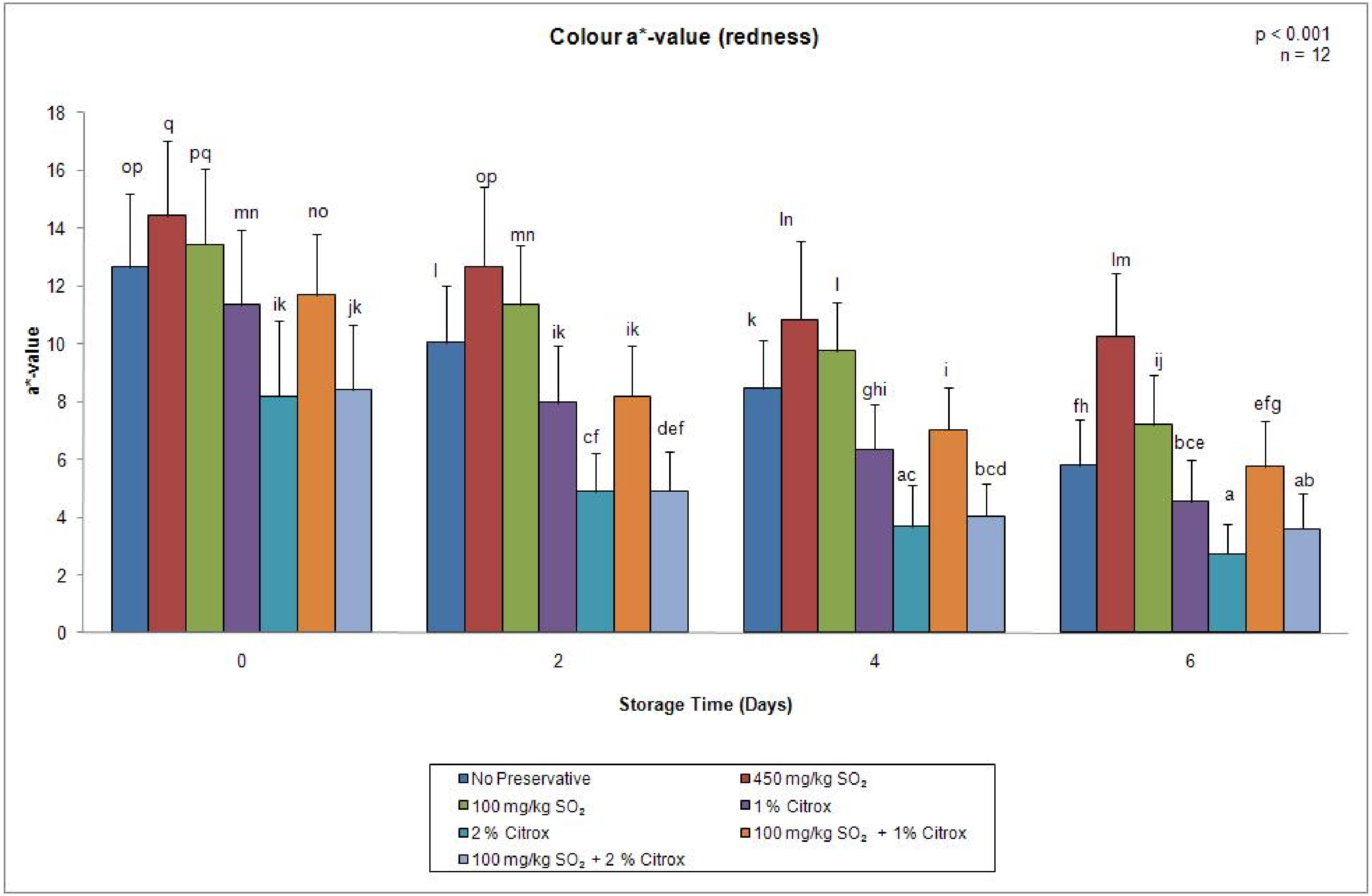

Figure 4.5. Colour a*-values (redness) of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

was no significant difference between treatments containing 1% Citrox, or with treatments containing 2% Citrox. From day 0 to day 2, there was a significant ($p < 0.001$) reduction in values for the control and all the treatments. The values at day 2 for 100 mg/kg SO_2 was the same as for 1 % Citrox at day O; the values for 450 mg/kg SO_2 did not differ significantly from the values for 100 mg/kg SO_2 at day 0.

From day 2 to day 4 there was a significant ($p < 0.001$) reduction in the value for control, 450 mg/kg SO_2 and100 mg/kg SO_2. The reduction in the values for all the treatments containing Citrox was not significant. The values for 100 mg/kg SO_2 on day 4 was the same as for the control on day 2. The values for the 450 mg/kg SO_2 treatment did not differ from the values for 100 mg/kg SO_2 at day 2.

On day 4 the values of the 450 mg/kg SO_2 and 100 mg/kg SO_2 treatments did not differ significantly and was significantly higher than the control and all the other treatments' values. The values of all the treatments containing Citrox were significantly ($p < 0.001$) lower than the control as well as the 450 mg/kg SO_2 and 100 mg/kg SO_2 treatments. The values of the treatments containing 1% Citrox were significantly ($p < 0.001$) higher than treatments containing 2% Citrox. There was no significant difference between the values of treatments containing 1% Citrox, nor with treatments containing 2% Citrox. On day 6 the results were the same as on day 4.

On day 6 there was a significant ($p < 0.001$) reduction in the values for the control, 100 mg/kg SO_2 and treatments containing 1% Citrox. There was no significant reduction in the values of 450 mg/kg SO_2 treatment and the 2% Citrox treatments. The values for 100 mg/kg SO_2 at day 6 did not differ significantly from the values of 100 mg/kg SO_2 at day 4. The Citrox treatments gave the product a grey – brown colour. In some way the Citrox reacts with the myoglobin in the meat, to form a colour that makes the product look "old".

Sulphur dioxide is well known as an oxidant. Other materials can act as antimicrobial agents, but none has been found to replace the antioxidant capabilities of SO_2 (Ough, 1993). The preservation of the colour and odour of meats are improved by sulphite treatment. Although slowing or prevention of growth of surface bacteria is probably important, the main effect in meat appears to be the antioxidant properties (Davidson & Juneja, 1989; Ough, 1993).

In the study done by Shan *et al.* (2009) to find natural spice and herb extracts with antibacterial and antioxidant capacities that could potentially be used as natural preservatives in raw pork, they found that during storage the colour parameters of the extract-treated pork samples changed slightly, in comparison with significant changes in the control. They reported that the spice and herb extracts had an immediate effect on the colour parameters of the pork samples after treatment in comparison with the control. The a* values of the pork samples treated with all extracts except cinnamon stick were lower than the control. However during 9 days of storage, most of the colour parameters of the treated pork samples were basically steady (slightly changed), in comparison with a significant decrease in a* values of the control.

The study done by Shan *et al.* (2009) to some extent revealed the protective effects of spice and herb extracts against the decrease in colour a* value in raw pork during storage. This, unfortunately, is in contrast to Citrox that had a significant reduction in the colour a* value of boerewors in this study. It is speculated that the Citrox must contain an ingredient that oxidizes meat colour which in turn will have a negative effect on consumer acceptability.

In their study of green tea and grape seed extracts as preservatives for increasing the shelf life of low sulphite raw beef patties, Banon *et al.* (2007) used different measurements for colour determination. With the addition of the extracts to the patties (stored at 4 ºC, in aerobic packaging for 0, 3, 6, or 9 days; retail display conditions), they found a gradual increase in H* (“hue angle”) and MM (relative metmyoglobin percentage) while C* ("chroma") values fell, colour changes that are normally associated with loss of redness in meat. Control (no additives) and S (100 mg/kg $SO_{2)}$ showed a noticeable reduction in C* values throughout storage, compared to SA (100 mg/kg SO_2 + 400 mg/kg sodium ascorbate), SG (100 mg/kg SO_2 + 300 mg/kg grape seed extract) and ST (100 mg/kg SO_2 + 300 mg/kg green tea extract) patties. There was significant differences ($p < 0.05$) in mean C* value between Control and ST – SG (days 3, 6, & 9), between Control and SA (days 3 and 9), between Control and S (day 3) and S and SA – SG – ST (day 9). Mean C* was similar in SA, SG and ST on any storage day.

4.2.2. Colour L-values (lightness)*

Figure 4.6 shows the colour L* (lightness) values for the seven boerewors model systems over the 6 day period stored at 4 ºC. The L* value was done to determine the "lightness" of the colour of the product: the higher the value, the lighter the product, a value of 100 = white and a value of 0 = black (Shan *et al.*, 2009).

On day 0 there was no significant difference between the control and 450 mg/kg SO_2 models. The control did have significantly ($p < 0.001$) lower values than 100 mg/kg SO_2, 2% Citrox, 100 mg/kg SO_2 + 1% Citrox and 100 mg/kg SO_2 + 2% Citrox. The rest of the treatments did not differ significantly, although they did have an immediate effect on the colour of the boerewors, their values were higher than the control (the control had a darker colour).

On day 2 the control did not differ significantly with 450 mg/kg SO_2, but had significantly ($p < 0.001$) lower values than all the rest of the treatments. On day 4 there was no significant difference between the control, 450 mg/kg SO_2 and 100 mg/kg SO_2. The control had significantly lower values than all treatments

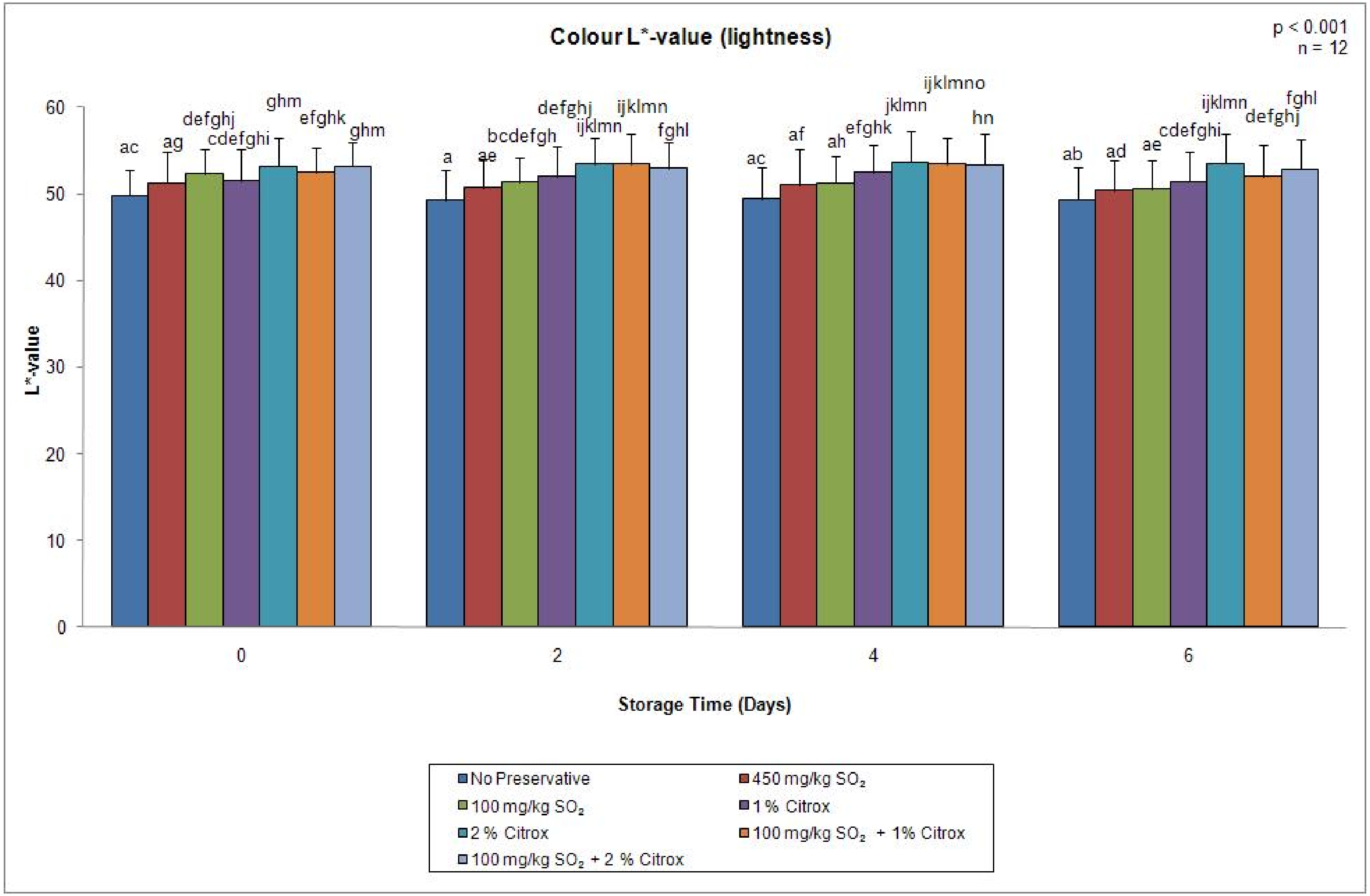

Figure 4.6. Colour L*-values (lightness) of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

containing Citrox. There was no significant difference between 450 mg/kg SO_2 and 1% Citrox; there was no significant difference between 100 mg/kg SO_2, 1% Citrox and 100 mg/kg SO_2 + 2% Citrox. On day 6 there was no significant difference between the control, 450 mg/kg SO_2 and 100 mg/kg SO_2. There was no significant difference between 450 mg/kg SO_2 and 1% Citrox. There was no significant difference between 100 mg/kg SO_2 and 1% Citrox.

Over the 6 day period there was no significant change in the *L value for all the treatments. All the treatments containing Citrox had significantly ($p < 0.001$) higher values than the control. The 2% Citrox treatments had significantly ($p < 0.001$) higher values than 450 mg/kg SO_2, except for day 0. The 2% Citrox treatment had the highest values; the control had the lowest values (although not significantly lower than 450 mg/kg SO_2). There was no significant difference between 100 mg/kg SO_2 and 1% Citrox. There was no significant difference between 2% Citrox and 100 mg/kg + 2% Citrox. It could be concluded that the L* value was relatively stable in all the treatments.

Similar results were found in the mentioned study of Banon *et al.* (2007) on green tea (GTE) and grape seed (GSE) extracts as preservatives of low sulphite raw beef patties, they found the L* value was quite stable throughout storage in all patty groups. The extract addition did not affect L*, differences in mean L* between treatments were not significant ($p < 0.05$).

The study done by Shan *et al.* (2009) on the antimicrobial and antioxidant effects of five spice and herb extracts on raw pork, found that the spice and herb extracts had an immediate effect on the colour parameters of the pork samples after treatment in comparison with the control. In contrast to our study on boerewors, the treatments on the raw pork's L* values were lower at the initial stage (day 0) for cinnamon stick, oregano, clove and grape seed; the samples were darker. Pomegranate peel was lower than the control. Over the 9 day storage period, most of the colour parameters of the treated pork samples were basically steady (slightly changed), in comparison with a significant decrease in L* value.

4.2.3. Colour b-values (yellowness)*

Figure 4.7 shows the colour b* (yellowness) values for the seven boerewors model systems over the 6 day period stored at 4 ºC. The b* value is indicative of the yellowness of the colour of the product, a lower value is preferred, so that it does not effect the redness of the product.

On day 0 there was no significant difference between any of the treatments. From day 0 to day 2, there was a significant (p < 0.001) reduction in the values for the control, 450 mg/kg SO_2, 100 mg/kg SO_2, 2% Citrox and 100 mg/kg SO_2 + 2% Citrox. There was no significant change in values for 1% Citrox and 100 mg/kg SO_2 +1% Citrox. On day 2 the control had significantly (p < 0.001) lower values than 100 mg/kg SO_2, 2% Citrox and 100 mg/kg SO_2 + 1% Citrox. There were no significant differences in the values for the control, 450 mg/kg SO_2, 100 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox.

On day 4 there was no significant difference between the control, 1% Citrox, 2% Citrox, 100 mg/kg SO_2 + 1% Citrox and 100 mg/kg SO_2 + 2% Citrox. There was a significant lower value for 450 mg/kg SO_2 when compared to 1% Citrox and 2% Citrox. There was no significant difference between the treatments containing Citrox. On day 6 the 1% Citrox treatment had a significantly higher value than the control, 450 mg/kg SO_2, 100 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox; there was no significant difference between the rest of the treatments.

Over the 6 day period the control had a significant (p < 0.001) reduction in the b* value from day 0 to day 2 and from thereon there was no significant change to day 6. The value for 450 mg/kg was reduced significantly (p < 0.001) from day 0

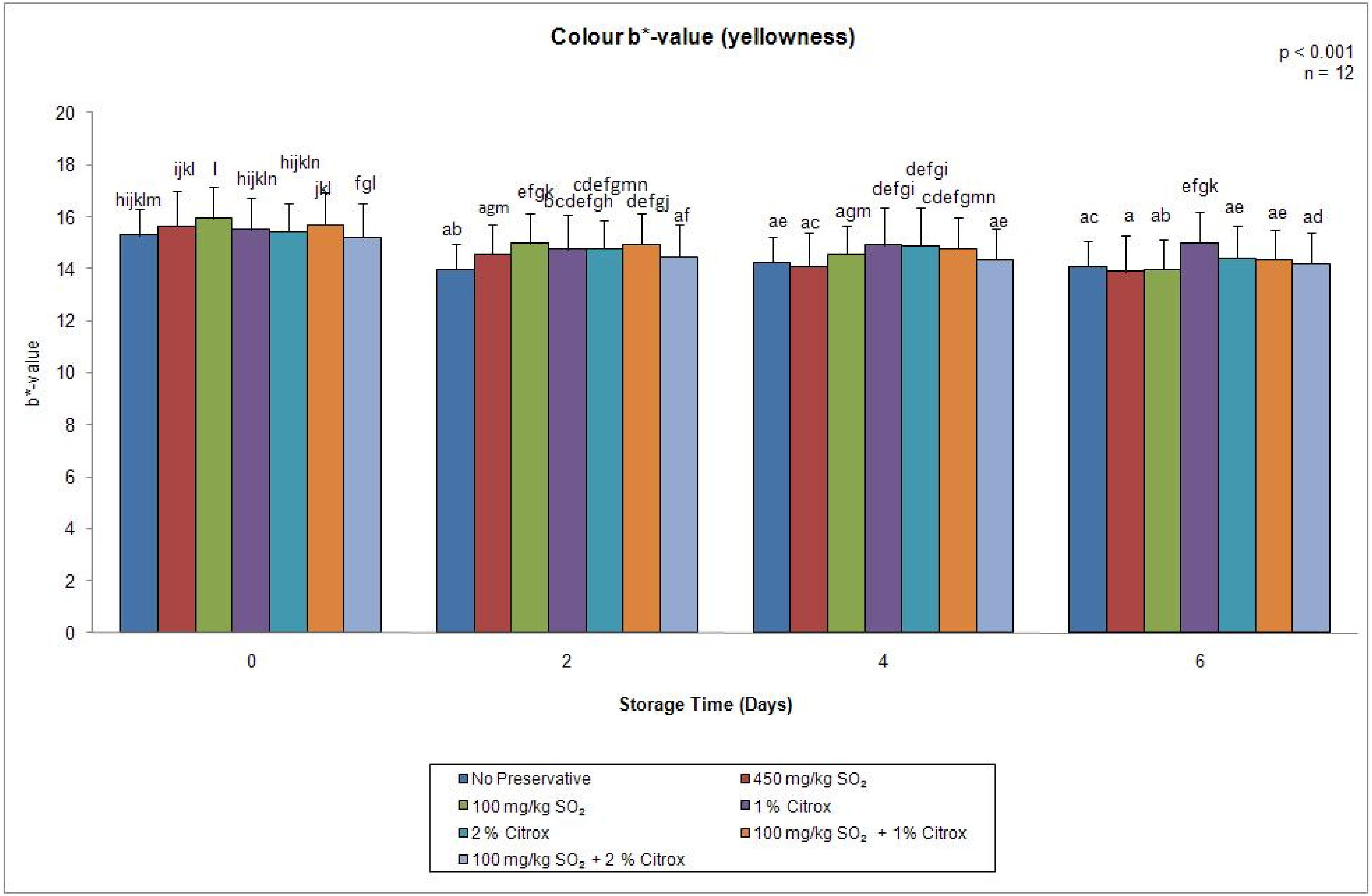

Figure 4.7. Colour b*-values (yellowness) of the seven boerewors models stored for 6 days at 4 °C. Results with different superscripts are significantly different. Bars represent average values and standard deviations.

to day 2, from thereon there was no significant change to day 6. For 100 mg/kg SO_2 the value was reduced significantly from day 0 to day 2; from day 2 to day 4 there was no significant change. From day 4 to day 6 there was no significant change, however the value on day 6 was significantly ($p < 0.001$) lower than day 2. There was no significant change from day 0 to day 2, or from day 2 to day 4 and neither from day 4 to day 6 for 1% Citrox. For 2% Citrox and 100 mg/kg SO_2 + 1% Citrox there was a significant ($p < 0.001$) reduction in the b* value from day 0 to day 2, thereafter there was no significant change to day 6. There was no significant change in the values for 100 mg/kg + 2% Citrox from day 0 to day 2; there was no significant change in the values from day 2 to day 6, however there was significant reduction in the values from day 0 to day 4 and from day 0 to day 6. On day 6 there were no significant difference between the values of the control or any of the treatments, except for 1% Citrox that had a significantly higher value than the control and all the other treatments. Overall the values were quite constant.

This is in contrast to the study done by Shan *et al.* (2009) on the antioxidant effects of cinnamon stick, oregano, clove, pomegranate peel and grape seed extract on raw pork, where there was an increase in the b* value of all treatments. During the 9 days of storage most of the colour parameters of the treated pork samples were basically steady (slightly changed), in comparison with a significant increase in the b* value of the control.

4.2.4. Lipid stability (TBARS values)

Figure 4.8 shows the lipid stability (TBARS) values for the seven boerewors model systems over the 6 day period stored at 4 ºC. Boerewors does contain some fat that could be oxidized and lead to rancid off – flavours, especially when frozen. The higher the TBARS value is, the higher the rancidity of the product will be.

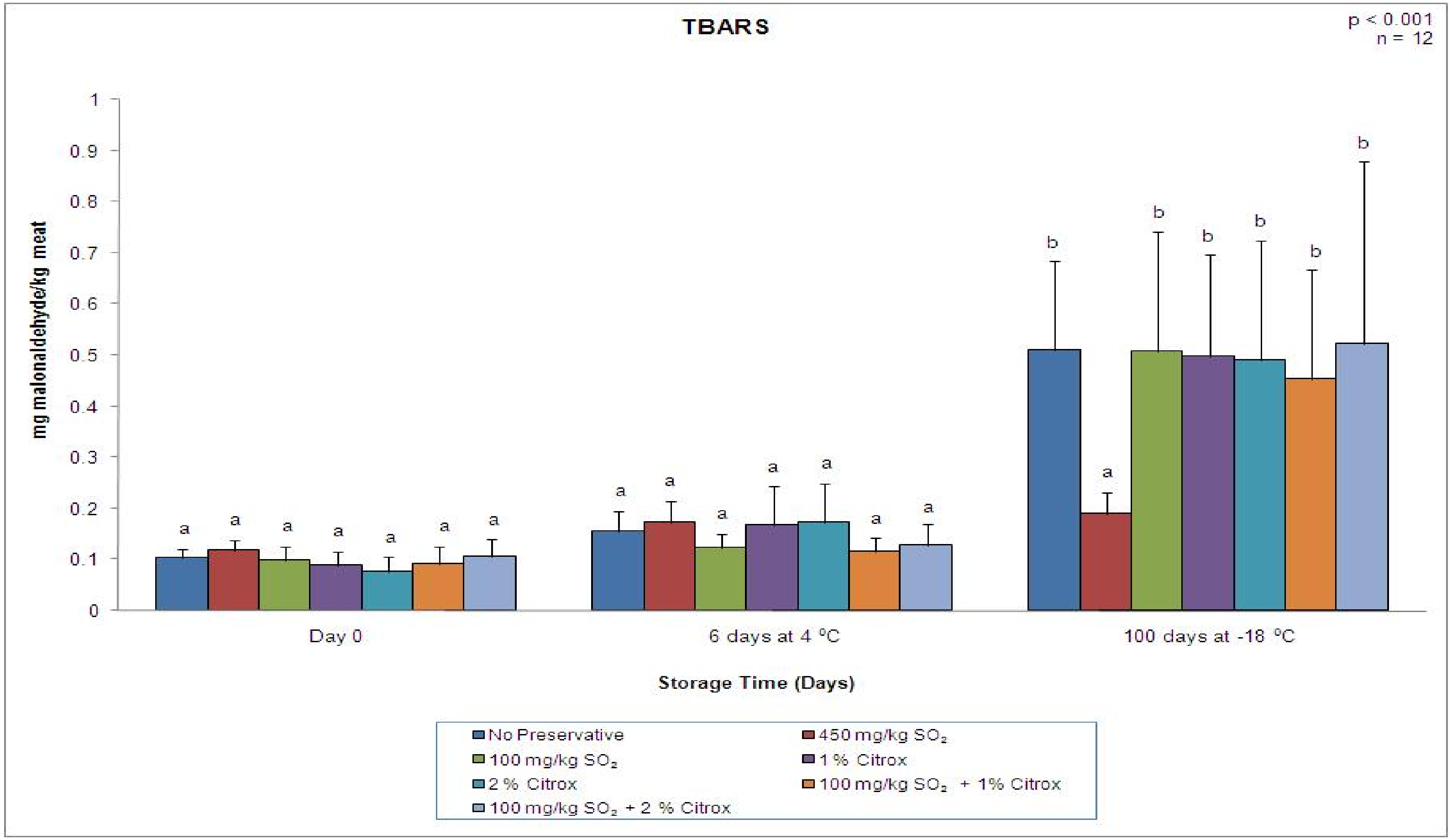

Figure 4.8. TBARS-values (lipid stability) of the seven boerewors models stored for 6 days at 4 °C. Results with differ superscripts are significantly different. Bars represent average values and standard deviations.

On day 0 and day 6 there were no significant difference between the TBARS values for all the treatments, although there was a slight increase in the values from day 0 to day 6. On day 100 the values for the 450 mg/kg SO_2 treatment was significantly ($p < 0.001$) lower than all the other treatments. There was no significant difference between the values of the rest of the treatments; all the values were far below 1 where rancid off-flavours become detectable by taste panels (Buckley & Connolly, 1980; Gray & Pearson, 1987; Boles & Parrish, 1990).

The study done by Banon *et al.* (2007) on green tea (GTE) and grape seed (GTE) extracts as preservatives for increasing the shelf life of low sulphite raw beef patties found a noticeable degree of lipid oxidation at day 0 for all treatments. Significant differences were found in mean TBARS values between control and SA (100 mg/kg SO_2 + 400 mg/kg sodium ascorbate), ST (100 mg/kg SO_2 + 300 mg/kg green tea) and SG (100 mg/kg SO_2 + 300 mg/kg grape seed) on days 3, 6 and 9; and between control and S (100 mg/kg SO_2). SA, SG and ST had similar mean TBARS values on any day of storage. TBARS increased strongly throughout storage in control and S, but stabilized in SA, SG and ST. A final significant increase in S compared with control was found on day 9.

Kanatt *et al*. (2008) did a study on a mixture of chitosan and mint (CM) as a preservative for meat and meat products. Mint extract on its own has poor antimicrobial activity, but good antioxidant activity. Chitosan on its own has poor antioxidant activity, but good antimicrobial activity. They did trials on lipid oxidation in terms of TBARS of pork cocktail salami containing CM (0.1%) during chilled storage. The data indicated that the TBA values were significantly affected ($p < 0.05$) by both storage period and the CM treatment. Salamis with CM incorporated were relatively resistant to lipid oxidation throughout the storage period (3 weeks).

In the study done by Shan *et al.* (2009) on natural spice and herb extracts with antioxidant capacities on raw pork they found that the initial concentrations of TBARS were between 0.71 and 0.72 mg malondialdehyde/kg and not significantly different. The TBARS values for all treatments increased by 21–28% after 9 days of storage. During the same period the TBARS value of the control increased by more than 3000%. At 3 days, all treatments showed significantly lower TBARS values than the control. The concentrations of TBARS in all treatments were considerably lower than in the control indicating that the extracts effectively protected against lipid oxidation in raw pork. Clove extract was particularly effective.

4.3. Sensory Analysis

The taste preference of a 75-member panel of the seven boerewors samples manufactured with different preservatives, are given in Table 4.2.

Table 4.2. Mean values for the taste preference of boerewors samples manufactured with different preservatives. 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.

Treatment	Sensory Score
No Preservative (control)	6.8^{ac}
450 mg/kg SO_2	7.3^{c}
1% Citrox	6.7^{ac}
2% Citrox	6.1^{a}
100 mg/kg SO_2	7.2^{c}
100 mg/kg SO_2 + 1% Citrox	7.0^{bc}
100 mg/kg SO_2 + 2% Citrox	6.3^{ab}
p	< 0.001

The 450 mg/kg SO_2 treatment had the highest score, the 100 mg/kg SO_2 treatment had a slightly lower score, followed by 100 mg/kg SO_2 + 1% Citrox treatment. This is indicative that SO_2 has flavour enhancing properties. The differences in flavour between the control, 450 mg/kg SO_2, 1% Citrox, 100 mg/kg SO_2, and 100 mg/kg SO_2 + 1% Citrox were not significant. The scores for the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox were significantly ($p < 0.001$) lower than the score for 450 mg/kg SO_2, but not significantly lower than the control (no preservative).

One of the general remarks was that the samples containing Citrox had a bit of a sour flavour, this was expressed in the lower scores that the Citrox treatments received. The combination of vinegar in the formulation and fruit acids in the Citrox probably produced the sour flavour.

Although the 450 mg/kg SO_2 treatment was preferred by the panel with an average score of 7.3 (between like moderately and like very much) the least preferred 2% Citrox treatment had an average score of 6.1 (between like slightly and like moderately). This indicate that the Citrox treated Boerewors was still considered as acceptably by the panel. This can be best explained by Figure 4.9.

Figure 4.9 can be divided into two parts: i.) bars pointing upwards, indicating the positive or “like” side of the hedonic scale, ranging from “like extremely” to “neither like nor dislike”; and ii) bars pointing downwards, indication the negative side of the hedonic scale, categorized as between “dislike slightly” to “dislike extremely”. Although a very small part of the scale was used (6.1–7.3), it is clear that the positive indicators were in general used more frequently than the negative ones. Even the samples from the Citrox treated sausages were on the positive side of the scale with scores between 6 and 7 (between like slightly and like moderately).

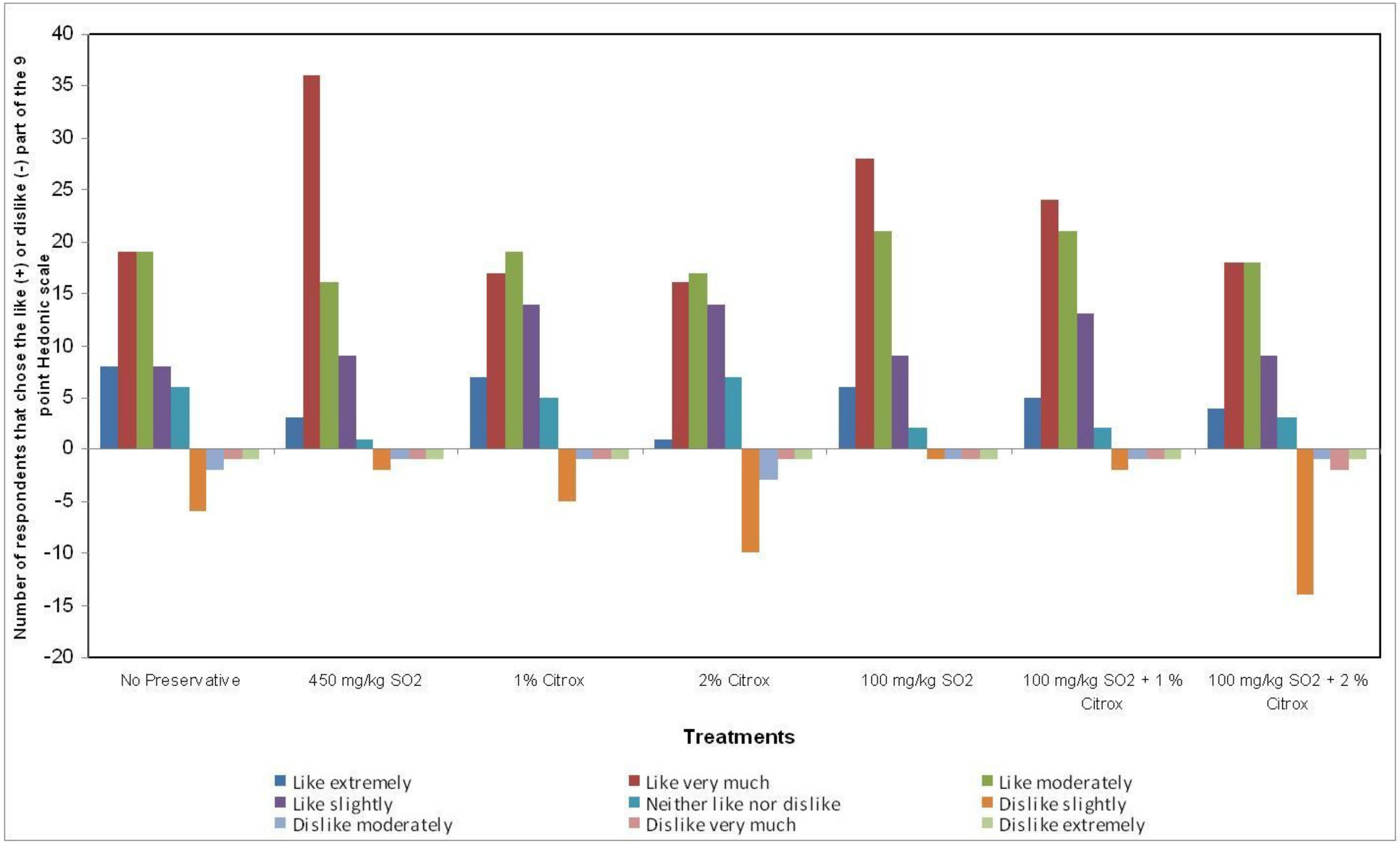

Figure 4.9. Frequency of respondent's ratings per treatment on the nine-point hedonic scale. Bars pointing upwards indicates the positive (+) or "like" side of the hedonic scale while bars pointing downwards, indicates the negative (-) or "dislike" side of the hedonic scale.

Roller *et al.* (2002) found with their study on the combination of chitosan and sulphite on the preservation of chilled pork sausages that the sausages containing the combination (0.6% chitosan + 170 ppm SO_2) deteriorated less rapidly than the control, the sausages containing high sulphite (340 ppm SO_2), low sulphite (170 ppm SO_2) and chitosan (0.6%) by itself. The combination was judged more acceptable by a sensory panel than all the other treatments.

Banon *et al.* (2007) found that green tea extract (GTE) and grape seed extract (GSE) in combination with low sulphite concentrations did not produce appreciable odour, flavour or texture in cooked beef patties. The100 g/kg SO_2 treatment on its own did not prevent rancidity in beef patties, packaged in air and stored up to 6 days under retail display conditions and later cooked. However, the low SO_2-extract combinations delayed rancidity.

In their study on cranberry concentrate to control *E. coli* O157:H7, Wu *et al.* (2009) found no significant difference in flavour among 0%, 2.5% and 5% addition of cranberry concentrate in beef burgers. Flavour and overall acceptability ratings of burgers with 7.5% cranberry concentrate were lower than the control burgers ($p < 0.05$). Burgers with 7.5% cranberry concentrate were neither liked nor disliked by the panellists, probably because the flavour was a little sour. For overall acceptability, the burgers with 2.5% cranberry concentrate had the highest score among all four kinds of burgers. Burgers with 5% cranberry concentrate were not significantly different from control burgers (0%) for overall acceptability.

Kanatt *et al.* (2008) found that at a 0.1% addition of a chitosan and mint (CM) mixture in pork cocktail salamis, the initial sensory analysis showed that sensorialy there was no significant ($p < 0.05$) difference between the treated and untreated samples. With respect to colour, flavour, taste and texture, the CM-treated and control samples were similar.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Boerewors is a South-African fresh sausage made from beef and / or pork. it is flavoured with salt, pepper and various spices, especially coriander. It is minced and filled into pork, beef or sheep intestine (casings) (Steyn, 1989).

According to law, boerewors is defined as being the clean, healthy and wholesome muscle and fat of beef, mutton or pork or a mixture of two or more. It must contain at least 90% meat and maximum 30% fat. It may contain rusk, spices, harmless flavourants, vinegar and allowed preservatives (sulphur dioxide, 450 mg/kg) (Department of Health of South Africa, 1990).

Sulphur dioxide and its various salts claim a long history of use, dating back to the times of the ancient Greeks. It has been used extensively as antimicrobials and to prevent enzymatic and non-enzymatic discolouration in a variety of foods (Davidson & Juneja, 1989).

Until recently, the use of SO_2 has had GRAS (Generally Regarded as Safe) status. Investigations, however, have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites (Ough, 1993; Roller *et al.*, 2002). This has caused a great deal of research in all areas concerning sulphites and SO_2. Other materials can act as antimicrobial agents, but none has yet been found to replace the antioxidant capabilities of SO_2 (Ough, 1993).

Research is needed on the application of naturally occurring and biologically derived antimicrobials in food systems. One reason for this is that consumers are more health conscience, rejecting the use of chemical preservatives, but still demand foods with an acceptable shelf-life (Dillon & Board, 1994). The future of natural antimicrobial agents is most likely to be in combination with other preservative systems or with physical treatments, such as heating or freezing processes (Dillon & Board, 1994).

In this study a natural occurring compound, called Citrox, was evaluated as a potential natural preservative. Citrox is a commercial mixture that comprises of a range of phyto-alexins derived from the pith and rind of green Bergamont oranges and fruit acids. Its anti-viral, -bacterial, -fungal, -mould and -yeast effect is due to a synergistic activity of these bioflavonoid and organic acid compounds (Neall, 2006). The aim of this study was to evaluate the effect of two dosage levels of Citrox (1% and 2%) as sole preservative and in combination with a reduced amount of SO_2 (100mg/kg) over a 6-day storage period (normal shelf-life) on the microbial, chemical and sensory quality of boerewors.

Observations as presented in Chapter 4 revealed that Citrox did have a preservative effect on total aerobic counts. The 2% Citrox addition had similar results than 1% Citrox + 100mg/kg SO_2. Over a 6-day storage period, the 2% Citrox + 100 mg/kg SO_2 had the lowest total aerobic counts, however the counts were not significantly ($p < 0.001$) lower than those of the 450 mg/kg SO_2 treatment. It could be concluded that there is a synergistic working between SO_2 and Citrox.

Citrox on its own did not achieve a reduction in coliform (Gram-negative organisms) counts; however there was also not an increase in counts and the indication that citrox has a bacteriostatic effect on coliforms. The same effect was found with 1% Citrox in addition with a low dosage (100 mg/kg) of SO_2. It was only with the addition of 100 mg/kg SO_2 with 2% Citrox that a significant ($p < 0.001$) reduction in counts were found compared to the control. The 2% Citrox + 100 mg/kg SO_2 had the same effect as 450 mg/kg SO_2. This is a further indicator of the synergistic working between SO_2 and Citrox.

The best synergistic working was shown on the yeasts and mould counts. Low dosages of SO_2 had a significant increase in yeast and mould counts. This could by explained due to yeasts that can affect the role of sulphites by producing binding compounds (e.g. acetaldehyde) (Ough, 1993; Roller *et al.*, 2002). The 1% Citrox had the same effect as 100 mg/kg SO_2. The 2% Citrox as well as the 100 mg/kg SO_2 + 1% Citrox had significantly ($p < 0.001$) lower counts than the control. The 2% Citrox had similar results to 450 mg/kg SO_2. The best results were achieved by 2% Citrox + 100 mg/kg SO_2, the counts were significantly ($p < 0.001$) lower after 6 days, than any of the other treatments. The 2% Citrox was probably the ideal concentration to protect the SO_2 against the sulphite binding compounds, and the 100 mg/kg SO_2 was sufficient to reduce the yeast and mould counts.

Over the 6-day period there were no significant ($p < 0.001$) differences between counts of the treatments and control against *Staph. aureus* (Gram-positive organism), the only exception being 2% Citrox + 100 mg/kg SO_2 which had significantly ($p < 0.001$) lower counts than the control. Here too, a synergistic working was observed between Citrox and SO_2.

In this study, Citrox at a 2% addition proved to have a good preservative effect on the microbial counts. In most cases the counts on all tested micro-organisms were similar to those of 450 mg/kg SO_2. Although not always significant ($p < 0.001$), the combination of 2% Citrox + 100 mg/kg SO_2 overall had the lowest counts of all the treatments. The combination of 2% Citrox + 100 mg/kg SO_2 therefore could have value as a preservative combination since the 100 mg/kg SO_2 is a 77.8% reduction in SO_2.

The evaluation of Citrox on the colour of the boerewors, showed, however, a negative effect, especially on the colour a* (redness) value. Both Citrox additions, 1% and 2%, on its own showed significantly ($p < 0.001$) lower a* values than 450 mg/kg SO_2 and the control, indicating the boerewors lost its red colour. The addition of 100 mg/kg SO_2 + 1% Citrox did give a higher value. It could be concluded that Citrox has no anti-oxidant effect on muscle pigment and probably reacts with the myoglobin in the meat to create an “old meat” grey-brown colour. Interestingly, the colour L* (lightness) value was the highest for the 2% Citrox and 2% + 100 mg/kg SO_2 treatments; indicating that as the colour of the meat changed, it also lightened. Over the 6-day storage period there was a significant (p < 0.001) reduction in the colour b* (yellowness) value for all treatments and the control.

This study also showed that Citrox does not have any anti-oxidant effect on meat fats. TBARS values were the same for the control and treatments at day 0 and day 6. However, after 100 days storage, the 450 mg/kg SO_2 treatment had a significantly (p < 0.001) lower value than the control and all the other treatments. The control and the rest of the treatments had similar values (no significant differences at p < 0.001), so it could be concluded that although the Citrox did not protect the fat against lipid oxidation, it also did not take part in oxidation of the fat.

Citrox had a slight negative effect on the flavour of Boerewors. Respondents on the sensory evaluation panel remarked that the treatments containing Citrox had a bit of a sour flavour. This could be ascribed to the fruit acids combined with the vinegar which created enough acidity for it to be tasted by the respondents. This effect could be overcome by reducing the amount of vinegar in the formulation. Even with the sour flavour of the Citrox treatments, the Citrox treatments were still considered as acceptable by the panel.

The results of this study could lead to the conclusion that Citrox at 2%, by itself, has some value as a natural preservative against TAPC, Gram-negative bacteria, Gram-positive bacteria and yeasts and moulds. For better results against these micro-organisms, it could be combined with 100 mg/kg SO_2, which is a 77.8% reduction in SO_2. Of concern is its negative effect on the colour of Boerewors, and on its lack of protection against lipid-oxidation. In this regard, the 450 mg/kg SO_2 still outperforms Citrox. The anti-oxidant capabilities of SO_2 have been reported before (Ough, 1993).

Other similar recent studies have demonstrated the use of other natural preservatives, on its own, or in combination with reduced amounts of SO_2, e.g. spice and herb extracts (Shan *et al.*, 2009), cranberry concentrate (Wu *et al.,* 2009), chitosan and sulphite (Roller *et al.*, 2002), ascorbate, green tea and grape seed extracts and low sulphite (Banon *et al.*, 2007). It could be beneficial to evaluate some of these against Citrox.

Further research may include:

- The use of a natural anti-oxidant (maybe ascorbate as was used by Banon *et al.*, 2007) in combination with 2% Citrox and 100 mg/kg SO_2 to protect the meat colour and add some protection against lipid oxidation.
- Replacing some of the spices in the Boerewors formulation with spice oils and oleoresins (as was done by Shan *et al.*, 2009). In this case spice oils and oleoresins should be used that is typical for Boerewors flavour (pepper, clove, nutmeg and coriander).
- A comparison between 2% Citrox + 100 mg/kg SO_2 vs the other studies that also used natural preservatives and reduced levels of sulphite (e.g. chitosan, green tea extract and grape seed extract).

Although Citrox had a preservative effect similar to SO_2 in Boerewors model systems, it had a slight negative effect on colour (redness) and flavour. The modern. health conscious consumer may, however, be willing to sacrifice some colour and flavour attributes in the Boerewors knowing that the product is free of chemical preservatives.

CHAPTER 6

REFERENCES

Anonymous. 1908. Preservatives in meat foods. *Lancet* 172, 101.

Anonymous. 1999. Tarisol Fresh Data Sheet. BK Giulini GmbH, Dr. Albert Reimann Strasse 2, D – 68526, Ladenburg.

Anonymous, 2009. Biltong. http://www.ejozi.co.za/south-african-cuisine/biltong. html. Retrieved on 01 May 2010.

Bakkali, F., Averbeck, S., Averbeck D. & Idaomar, M. 2008. Biological effects of essential oils – A review. *Food and Chemical Toxicology* 46, 446-475.

Banks, J.G., Dalton, H.K., Nychas, G.J. & Board, R.G. 1985. Review – sulphite, an elective agent in the microbiological and chemical changes occurring in uncooked comminuted meat products. *Journal of Applied Bacteriology* 7, 161-179.

Banon, S., Diaz, P., Rodriguez, M., Garrido, M.D. & Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Science* 77, 626-633.

Beuchat, L.R. 1994. Antimicrobial properties of spices and their essential oils. In *Natural Antimicrobial Systems and Food Preservation,* Dillon V.M. & Board R.G. (Ed.), pp 167-172. CAB International: Wallingford.

Boles, J.A. & Parrish, F.C. Jr. 1990. Sensory and chemical characteristics of precooked microwave-reheatable pork roasts. *Journal of Food Science* 55(3), 618-620.

Branen, A.L. 1989. Preface and introduction to food additives. In *Food Additives,* Branen, A.L., Davidson P.M. & Salmien S. (Ed.), pp iii-iv, 1-8. Marcel Dekker, Inc.: New York.

Branen, A.L. 1993. Preface and introduction to use of antimicrobials. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp i-iv, 1-8. Marcel Dekker, Inc.: New York.

Buckley, J. & Connolly, J.F. 1980. Influence of alpha-tocopherol (vitamin E) on storage stability of raw pork and bacon. *Journal of Food Protection* 43(4), 265-267.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology* 94, 223-253.

Campbell-Platt, G. 1995. Fermented meats – a world perspective. In *Fermented Meats*, Campbell-Platt, G. & Cook, P.E. (Ed.), pp 39-51. Blackie Academic & Professional: London.

Campbell-Platt, G. & Cook, P.E. 1995. Preface. In *Fermented Meats,* Campbell-Platt, G. & Cook, P.E. (Ed.), p iii. Blackie Academic & Professional: London.

Charimba, G., Hugo, C.J. &, Hugo, A. 2010. The growth, survival and thermal inactivation of *Escherichia coli* O157:H7 in a traditional South African sausage. *Meat Science*, 85, 89-95.

Conner, D.E. 1993. Naturally occurring compounds. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 441-450. Marcel Dekker, Inc.: New York.

Davidson, P.M. 1993. Parabens and phenolic compounds. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 263-264. Marcel Dekker, Inc.: New York.

Davidson, P.M. & Juneja, V.K. 1989. Antimicrobial agents. In *Food Additives,* Branen, A.L., Davidson, P.M. & Salmien, S. (Ed.), pp 83-116. Marcel Dekker, Inc.: New York.

Department of Health (DoH) of South Africa. 1990. Regulation No R2718, 23 November 1990: Regulations governing the composition and labelling of raw boerewors, raw species sausage and raw mixed-species sausage. Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act 54 of 1972), pp 285-286.

Department of Health (DoH) of South Africa. 2001. *Meat Annexure: Fresh and Processed Meat*. http://www.doh.gov.za/docs/factsheets/guidelines/foodser_vice/meat.pdf. Retrieved on 28 February 2008.

Dillon, V.M. & Board, R.G. 1994. *Natural Antimicrobial Systems and Food Preservation,* Dillon, V.M. & Board, R.G. (Ed.), pp xiii, 1-11, 297-303. CAB International: Wallingford.

Dillon, V.M. & Cook, P.E. 1994. Biocontrol of undesirable microorganisms in food. In *Natural Antimicrobial Systems and Food Preservation,* Dillon, V.M. & Board, R.G. (Ed.), pp 255-275. CAB International: Wallingford.

Doores, S. 1993. Organic acids. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 95-124. Marcel Dekker, Inc.: New York.

Elias, P.S. 1987. The public's response to decontamination. In *Elimination of Pathogenic Organisms from Meat and Poultry,* Smulders, F.J.M. (Ed.), pp 345-352. Elsevier: Amsterdam.

Garbutt, J. 1997. Death of microorganisms and microbial populations. In *Essentials of Food Microbiology*. Arnold: London.

Gray, J.I. & Pearson, A.M. 1987. Rancidity and warmed-over flavor. In *Advances in Meat Research*, Vol. 3, A.M. Pearson and T.R. Dutson (Ed.), p. 221-270. Van Nostrand Reinhold Company: New York.

Harrigan, W.F. 1998. *Laboratory Methods in Food Microbiology*. Academic Press: San Diego.

Hayouni, E.A., Chraief, I., Abedrabba, M., Bouix, M., Leveau, J.-Y., Mohammed, H. & Hamdi, M. 2008. Tunisian *Salvia officinalis* L. and *Schinus molle* L. essential oils: Their chemical compositions and their preservative effects against *Salmonella* inoculated in minced beef meat. *International Journal of Food Microbiology* 125, 242-251.

Heller, L. 2007. Codex questions certain sweetener, colour uses. http://www.cee-foodindustry.com/news/ng.asp?id=79098, Retrieved on 16/08/2007.

Hoover, D.G. 1993. Bacteriocins with potential for use in foods. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 409, 419-420, 432. Marcel Dekker, Inc.: New York.

Hugo, A., Roberts, J.J. & Smith, M.S. 1993. Rapid detection of selected non-meat proteins in model boerewors and emulsified meat systems by means of an accelerated ELISA technique. *South African Journal of Food Science and Nutrition* 5(2), 34-40.

Kanatt, S.R., Chander, R. & Sharma, A. 2008. Chitosan and mint mixture: A new preservative for meat and meat products. *Food Chemistry* 107, 845-852.

Lawless, H.T. & Heymann, H. 1998. *Sensory Evaluation of Food: Principles and Practices*, 2nd ed., Chapman & Hall: New York.

Lawrie, R.A. 1979. *Meat Science.* Pergamon Press: Oxford.

Lawrie, R.A. 1995. The structure, composition and preservation of meat. In *Fermented Meats,* Campbell-Platt, G. & Cook, P.E. (Ed.), pp 18-33, Blackie Academic & Professional: London.

Lehohla, P.J. 2003. Manufacturing statistics – Products Manufactured: Food and Beverages; Statistics South-Africa. http://www.statssa.gov.za/PublicationsHTML/P30511August2003/html/P30511August2003_6.html?gInitialPosX=10px&gInitialPosY=10px&gZoomValue=100, p4. Retrieved on 01 May 2010.

Lueck, E. 1980. *Antimicrobial Food Additives*, translated from German by G. F. Edwards. Springer-Verlag: Berlin.

Menon, K.V. & Garg, S.R. 2001. Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. *Food Microbiology* 18, 647-650.

Moufida, S. & Marzouk, B. 2003. Biochemical characterization of blood orange, sweet orange, lemon, bergamont and bitter orange. *Phytochemistry* 62, 1283-1289.

Nadarajah, D., Han, J.H. & Holley, R.A. 2005. Inactivation of *Escherichia coli* O157:H7 in packaged ground beef by allyl isothiocyanate. *International Journal of Food Microbiology* 99, 269-279.

NCSS, 2007. Statistical System for Windows. NCSS Statistical Systems, Kaysville, Utah, USA.

Neall, B. 2006. Citrox rolls out in SA. *Food Review* 33(1), 32-33.

Ouattara, B., Simard, R.E., Holley, R.A., Piette, G.J.-P. & Begin, A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *International Journal of Food Microbiology* 37, 155-162.

Ough, C.S. 1993. Sulfur dioxide and sulphites. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 137-161. Marcel Dekker, Inc.: New York.

Raharjo, E.L., Sofos, J.N.S. & Schmidt, G.R. 1992. Improved speed specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C_{18} method for measuring lipid peroxidation in beef. *Journal of Agricultural and Food Chemistry* 40, 2182-2185.

Roller, S., Sagoo, S., Board, R., O'Mahony, T., Caplice, E., Fitzgerald, G., Fogden, M., Owen, M. & Fletcher, H. 2002. Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. *Meat Science* 62, 165-177.

Shan, B., Cai, Y.-Z., Brooks, D.B. & Corke, H. 2009. Antibacterial and antioxidant effects of five spice and herb extracts as natural preservatives of raw pork. *Journal of the Science of Food and Agriculture* 89, 1879-1885.

Sofos, J.N. & Busta, F.F. 1993. Sorbic acid and Sorbates. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 49-62, 68-71. Marcel Dekker, Inc.: New York.

Sökmen, A., Gulluce, M., Akpulat, H.A., Daferera, D., Tepe, B., Pollissiou, M., Sökmen, M. & Sahin, F. 2004. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius. Food Control* 15, 627-634.

Steyn, M.S. 1989. *Aspekte rakende die higiëne en potensiële rakleeftyd van Suid- Afrikaanse Boerewors.* M.Sc. Thesis. University of the Free State: Bloemfontein, South Africa.

Stone, H. & Sidel, J.L. 2004. *Sensory Evaluation Practices*, 2nd ed. Elsevier Academic Press: London.

Tajkarimi, M.M., Ibrahim, S.A. & Cliver, D.O. 2010. Antimicrobial herb and spice compounds in food. *Food Control* 21, 1199-1218.

Tepe, B., Daferera, D., Sökmen, M., Plissiou, M. & Sökmen, A. 2004. The *in vitro* antioxidant and antimicrobial activities of the essential oil and various extracts of *Origanum syriacum* L. var. *bevanii*. *Journal of the Science of Food and Agriculture* 84(11), 1389-1396.

Tompkin, R.B. 1993. Nitrite. In *Antimicrobials in Foods,* Davidson, P.M. & Branen, A.L. (Ed.), pp 191-192, 223-246. Marcel Dekker, Inc.: New York.

Urbain, W.M. & Campbell, J.F. 1987. Meat preservation. In *The Science of Meat and Meat Products,* 3rd edition*,* Price, J.F. & Schweigert, B.S. (Ed.), pp 371-375, 388-408. Food & Nutrition Press, Inc.: Westport, Connecticut, USA.

Von Holy, A., Cloete, T.E., Groeneveld, H.T. & Holzapfel, W.H. 1988. The effect of storage temperature, packaging and additives on the microbiological shelf-life of ground beef. *South African Journal of Science* 84, 766-769.

Wu, V.C.H., Qiu, X., de los Reyes, B.G., Lin, C.-S. & Pan, Y. 2009. Application of cranberry concentrate (*Vaccinium macrocarpon*) to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the down regulated *slp*, *hdeA* and *cfa*. *Food Microbiology* 26, 32-38.

Yin, L.-J., Wu, C.-W. & Jiang, S.-T. 2003. Bacteriocins from *Pediococcus pentosaceus* L and S from pork meat. *Journal of Agricultural and Food Chemistry* 52, 1071-1076.

Zeuthen, P. 1995. Historical aspects of meat fermentations. In *Fermented Meats,* Campbell-Platt, G. & Cook, P.E. (Ed.), pp 53-66. Blackie Academic & Professional: London.

Zhang, H., Kong, B., Xiong, Y.L. & Sun, X. 2009. Antimicrobial activities of spice extracts against pathogenic and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4ºC. *Meat Science* 81, 686-692.

Zhou, G.H., Xu, X.L. & Liu, Y. 2010. Preservation technologies for fresh meat – a review. *Meat Science* 86, 119-128.

CHAPTER 7

SUMMARY

Boerewors is a South-African fresh sausage, made from beef and pork and flavoured with salt, pepper and various spices, especially coriander. It may contain a maximum of 450 mg/kg SO_2 as preservative. Until recently, the use of SO_2 has had GRAS (Generally Regarded as Safe) status. Investigations have indicated certain asthmatic individuals were placed at risk by relatively small amounts of sulphites. The increased demand for minimally processed, extended shelf-life foods have renewed interest in exploitation of natural antimicrobials for food preservation uses.

The aim of this study was to evaluate the efficacy of Citrox as a natural preservative in Boerewors. Citrox comprises of a range of phyto-alexins derived from the pith and rind of green Bergamont oranges and fruit acids. Seven Boerewors models were made following a typical commercial procedure: the control containing no preservative, current dosage of SO_2 in Boerewors (450 mg/kg), two dosages of Citrox on its own at 1% and 2% addition, a reduced amount of SO_2 (100 mg/kg) on its own and Citrox in combination with reduced amounts of SO_2 (1% Citrox + 100 mg/kg SO_2 and 2% Citrox + 100 mg/kg SO_2). The models were stored at 4 ºC for 6 days under retail display conditions. The models were evaluated every 48 hours for microbiological growth as expressed by total aerobic plate count (TAPC), coliform count, *Staph. aureus* count, yeast and mould count. The models were also evaluated every 48 hours for colour changes as expressed by the colour a* value (redness), colour b* value (yellowness) and colour L* value (lightness). The models were evaluated after 6 days, and again after 100 days storage at -18 ºC, for lipid oxidation as expressed by TBARS (thiobarbituric acid reactive substance). The models were also evaluated organoleptically by a sensory panel.

It was established that all the treatments had significantly lower counts than the control over the 6 day storage period on TAPC. There was no significant difference in the counts of 450 mg/kg SO_2, 2% Citrox, 100 mg/kg SO_2 + 1% Citrox and 100 mg/kg SO_2 + 2% Citrox. The 450 mg/kg SO_2 and 100 mg/kg SO_2 + 2% Citrox had significantly lower coliform counts than the control. Against yeasts and moulds, 100 mg/kg SO_2 + 2% Citrox had significantly lower counts than the control and 450 mg/kg SO_2.

Citrox had a negative effect, on its own and in combination with 100 mg/kg SO_2 on the colour of the Boerewors. The colour a* values were significantly lower than those for the control and 450 mg/kg SO_2. There was very little effect on the colour L* value, most values stayed relatively stable over the 6 days, the 2% Citrox and 100 mg/kg SO_2 + 2% Citrox showed the highest values. For all the models, there were a slight decrease in the colour b* value. At the end of the 6 days, all models had similar values.

Over the 6-day fresh display period there was no significant difference in the TBARS values for all the models. After 100 days storage at -18 ºC, 450 mg/kg SO_2 had significantly lower values than the control and the rest of the models. The control had the same values as the rest of the models, which led to the conclusion that although the Citrox had no anti-oxidant capabilities, it also did not increase lipid oxidation.

The sensory panel found that the models containing 2% Citrox had a slight sour flavour (can be explained by the fruit acids in Citrox in combination with the vinegar added to the Boerewors formulation), however the average scores for these models were still found to be “liked slightly” to “liked moderately”.

Key words: Fresh sausage, Boerewors, natural preservative, Citrox, sulphur dioxide

OPSOMMING

Boerewors is ‘n Suid-Afrikaanse wors wat gemaak word van bees- en varkvleis en dit word gegeur met sout, peper en verskeie speserye, veral koljander. Dit mag ‘n maksimum van 450 mg/kg SO_2 bevat. Tot onlangs het die gebruik van SO_2 GRAS (Generally Regarded as Safe – Algemeen Aanvaar as Veilig) status geniet. Ondersoeke het bevind dat sekere asmatiese individue op risiko geplaas word deur relatiewe klein hoeveelhede sulfiete. Die onlangse vraag na minimaal geprosesseerde voedsels met verlengde rakleeftyd het hernude belangstelling in die gebruik van natuurlike antimikrobiese middels vir voedselpreservering laat ontstaan.

Die doel van die studie was om die doeltreffendheid van Citrox as ‘n natuurlike preserveermiddel in Boerewors te ondersoek. Citrox bestaan uit ‘n reeks fito-aleksiene, verkry uit die pit en skil van groen Bergamont lemoene, en vrugte sure. Sewe Boerewors modelle is gemaak deur die tipiese kommersiële metode te gebruik: die kontrole met geen preserveermiddel, huidige toediening van SO_2 in Boerewors (450 mg/kg), twee toedienings van Citrox teen 1% en 2% op sy eie, ‘n verminderde hoeveelheid van SO_2 (100 mg/kg) op sy eie en Citrox in kombinasie met verminderde hoeveelhede SO_2 (1% Citrox + 100 mg/kg SO_2 en 2% Citrox + 100 mg/kg SO_2). Die modelle is gehou by 4 ºC vir 6 dae onder kleinhandel-uitstal kondisies. Die modelle is elke 48 uur geëvalueer vir mikrobiese groei soos uitgedruk in totale aerobiese plaat tellings (TAPT), koliforme tellings, *Staph. aureus* tellings, gis en skimmel tellings. Die modelle was ook elke 48 uur geëvalueer vir kleur veranderinge soos uitgedruk deur die kleur a* (rooiheid), kleur b* (geelheid) en kleur L* (ligtheid) waarde. Die modelle is na 6 dae, en weer na 100 dae geberg by -18 ºC, geëvalueer vir lipied oksidasie soos uitgedruk deur TBSRS (tiobarbitiensuur reaktiewe stowwe). Die modelle is ook organolepties geëvalueer deur ‘n sensoriese paneel.

Daar is gevind dat al die behandelings betekenisvol laer tellings na die 6 dae opbergings periode gehad het as die kontrole vir TAPT. Daar was geen

betekenisvolle verskil in die tellings vir 450 mg/kg SO_2, 2% Citrox, 100 mg/kg SO_2 + 1% Citrox en 100 mg/kg SO_2 + 2% Citrox nie. Die 450 mg/kg SO_2 en 100 mg/kg SO_2 + 2% Citrox se koliforme tellings was betekenisvol laer as die kontrole s'n. Die 100 mg/kg SO_2 + 2% Citrox het betekenisvol laer tellings vir giste en skimmels gehad as die kontrole en 450 mg/kg SO_2.

Citrox, op sy eie, en in kombinasie met 100 mg/kg SO_2, het 'n negatiewe effek op die kleur van die Boerewors gehad. Die kleur a* waardes was betekenisvol laer as die van die kontrole en 450 mg/kg SO_2. Daar was baie min effek op die kleur L* waarde, meeste van die waardes het stabiel gebly oor die 6 dae periode, die 2% Citrox en 100 mg/kg SO_2 + 2% Citrox het die hoogste waardes gehad. Vir al die modelle was daar 'n effense afname in die kleur b* waarde. Aan die einde van die 6 dae het al die modelle soortgelyke waardes gehad.

Oor die 6 dae vars-vertoon periode was daar geen betekenisvolle verskil in die TBSRS waardes vir al die modelle nie. Na 100 dae opberging by -18 ºC, het 450 mg/kg SO_2 betekenisvol laer waardes gehad as die kontrole en die res van die modelle. Die kontrole het dieselfde waarde gehad as die res van die modelle, wat gelei het tot die gevolgtrekking dat alhoewel Citrox nie anti-oksidant kapasiteit het nie, dit ook nie lipied oksidasie verhoog het nie.

Die proepaneel het bevind dat die modelle wat 2% Citrox bevat het 'n effense suur smaak het (kan verklaar word deur die vrugtesure in Citrox in kombinasie met die asyn wat bygevoeg is in die Boerewors formulasie), hoewel die gemiddelde tellings vir die modelle steeds as “hou effens van” tot “hou redelik van” gevind is.

Sleutel woorde: Vars wors, Boerewors, natuurlike preserveermiddel, Citrox, swael dioksied.