Cape Peninsula
University of Technology

The growth and survival of *Listeria monocytogenes* in polony preserved with chitosan and rosemary extracts

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Food Science and Technology

In the Faculty of Applied Sciences

At the Cape Peninsula University of Technology

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Bellville Campus
August 2024

DECLARATION

I, Zaa-id Rinquest declare that the contents of this thesis represent my own unaided work, and that the thesis/dissertation has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Z. Rinquest** **Date** **21 August 2024**

ABSTRACT

The ubiquitous nature of *Listeria monocytogenes* has caused major concerns in the processing of ready-to-eat (RTE) foods. Their ability to thrive in environments such as refrigerated conditions and high salt concentration allows *L. monocytogenes* to persist in RTE foods. This adaptability leads to its persistence and high resistance to synthetic preservatives used in RTE foods such as sodium nitrite and sorbic acid. These factors in the food chain act as potential drivers for *L. monocytogenes* contamination of RTE foods and listeriosis. Therefore, this study aimed to investigate and evaluate the growth and survival of *L. monocytogenes* in polony models preserved with natural preservatives, Chitosan [(1, 4)-2-Amino-2-deoxy-β-D-glucan] and Rosemary (*Rosmarinus officinalis* L.) extract to inhibit the growth and survival of *L. monocytogenes* in RTE polonies. Four polony models designated P1 (control sample), P2 (chitosan sample), P3 (rosemary sample), P4 (chitosan and rosemary combination) were developed and investigated at 3 different concentration levels (0.5% g/Kg, 1.0% g/Kg and 2.0% g/Kg) and stored at 4 ^{o}C and 10 ^{o}C for preservation against coliforms (SANS 4832), yeasts and moulds (SANS 7954)*, E. coli* (SANS 7251), *S. aureus* (SANS 6888) and *L. monocytogenes* (SANS 11290). Microbiological tests such as total viable counts (TVC) (SANS 4833), coliforms, yeasts and moulds, *E. coli*, *S. aureus* and *L. monocytogenes* were conducted, and all compared to the standard (control) polony model P1. These microbiological tests were also done to ensure that the models were safe for sensory analysis. The growth and survival of *L. monocytogenes* was observed and evaluated after injecting 10^3 log cfu/g of *L. monocytogenes* into the polony models P2, P3 and P4 preserved with chitosan and rosemary extract compared to the control model P1 preserved with salt (NaCl). The antimicrobial activity of chitosan and rosemary extract assisted with inhibition of *S. aureus*, *E. coli* and *L. monocytogenes*. The mean count (cfu/g) for coliforms, TVC and yeasts and moulds were within the acceptable limit and slightly increased on days 5. Polony models inoculated with 10^3 log cfu/g of *L. monocytogenes* indicated a steep decrease from P1 to P2 ($p < 0.05$), indicating less growth in polony models P2 – P4, thus supporting the hypothesis made in this study. For polony models inoculated with *L. monocytogenes* at different concentration levels indicated a significance ($p < 0.05$), indicating that polony models preserved with 1.0% g/Kg showed less growth compared to the models preserved at a 0.5% g/Kg and 2.0% g/Kg concentration. The data obtained for storage temperature indicated that the growth of *L. monocytogenes* in polony models preserved with

chitosan and rosemary extract was higher ($p < 0.05$) at 10 ^{o}C compared to polony models stored at 4 ^{o}C. Summarising that storage temperature influence the rate of *L. monocytogenes*. Little to no growth were found during microbial analysis. Considering Food Safety Management systems (FSMS) polony models used for sensory evaluation were regarded as safe to consume. Sensory analysis showed that the control model P1 and chitosan model P2 were preferred by the participants. In summary, this study found that the use of natural preservatives such as chitosan and rosemary during the production of polony models assisted with the inhibition of *L. monocytogenes.* This provides alternatives to the synthetic preservatives and helps to promote the production and consumption of polonies preserved with natural preservatives.

ACKNOWLEDGEMENTS

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

- Dr G Charimba, my supervisor and Senior Lecturer at the Cape Peninsula University of Technology (CPUT), for his guidance, support, motivation and assistance during the course of my study;
- The Department of Agriculture, for giving me the opportunity to continue my studies and supporting me throughout my tertiary level education. The financial assistance towards this research is acknowledged;
- Mr J Constable, Ms L Thabethe and Ms B Matoti: “thank you for your support, knowledge and guidance”.
- Mr E Achmat and Mr W Manamela, Faculty members in the Department of Mathematical Sciences at CPUT, for their assistance, and guidance pertaining to statistical analysis and inference.

DEDICATION

Dedicated in loving memory of my grandmother Fowzia Harris (†31.08.2010)
For you are the constant I'll always need
I LOVE YOU

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ABBREVIATIONS AND ACRONYMS

a_w	Water activity
SO_2	Sulphur dioxide
$NaHCO_3$	Sodium bicarbonate
RTE	Ready-to-eat
G & S study	Growth and survival studies
NaCl	Sodium chloride
SANS	South African Bureau of Standards
CNS	Central Nervous System
W/W%	Weight concentration
cfu	Colony forming unit

GLOSSARY

Terms	Definition/Explanation
Listeria monocytogenes	Defined as a species of pathogenic bacteria that causes the infection Listeriosis.
Ready to eat food	Food that is readily edible without preparation to achieve food safety, it could be raw or partially cooked food.
Preservative	A substance or chemical added to products such as food, beverages etc. to prevent decomposition by microbial growth or by undesirable chemical changes
Natural preservatives	Defined as ingredients that are found in nature without artificial processing, naturally used to extend shelf life and reduce spoilage.
Persistence	Defined as something that continues to exist. It refers to behaviours or conditions that are constantly recurs
Antimicrobial activity	The ability of a substance to kill or inhibit growth of a microorganism such as bacteria, fungi or virus.
Food safety	Refers to handling, preparing and storing of food, in which the risks of individuals are kept safe from becoming ill from foodborne illnesses.
Organoleptic analysis	Defined as being affected or relating to quality (taste, texture, odour, colour and feel).

CHAPTER 1
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Ready-to-eat (RTE) foods such as polonies stand out as a convenient customer favourite, but continue to face threats from a range of microbial contaminants (Asiegbu et al., 2020:3-15). *Listeria monocytogenes* in particular, is notoriously mentioned amongst these. It has a ubiquitous nature due to its adaptability. *L. monocytogenes* thrives in cold, moist environments where other organisms weaken, thus allowing it to have a persistent and insidious presence in the food production facilities (Okutani et al., 2004:131-134). In 2017, the gravity of this threat became clear during the South Africa's most horrible outbreak, where *L. monocytogenes* claimed over 200 lives, marking it as the country's biggest outbreak to date (Smith et al., 2019:524). Hence the reason why *L. monocytogenes* is a subject of study.

Listeria monocytogenes is pervasive in nature. It has the ability to survive numerous environments and conditions, thus making it highly resistant to different environmental conditions (Anon, 2019:3). *L. monocytogenes* can enter different food-processing facilities through one or more ways such as raw materials, the movement of personnel and equipment that is utilised within the facility. This pathogen is highly adaptive and can survive considerable stressful conditions including, low and high pH, temperature and water activity (a_w) (NicAogáin and O'Byrne, 2016:1). In addition, *L. monocytogenes* is a major health and economic threat to the food industry (Tchatchouang et al., 2020:2).

Listeriosis is defined as an infection of the gastrointestinal tract in humans after the consumption of foods contaminated with *L. monocytogenes* (Leong et al., 2014:1). This food-borne illness generally presents itself with symptoms such as abdominal cramps, diarrhoea, and nausea (Bintsis, 2017:530-531). One of the main sources of contamination arises from delicatessen meats. However, consumers who are immunocompromised can be affected critically compared to those who are not (Hughes, 2008:28). Listeriosis has been reported to be a comparatively rare food-borne disease but can be rather life threatening. *Listeria monocytogenes* has the ability to cause severe infections throughout the body, which makes this food-borne pathogen considerably dangerous (Leong et al., 2014:1).

Ready-to-eat (RTE) food products such as polonies are easily and frequently contaminated with *L. monocytogenes* and can therefore be portrayed as a major safety concern [SA board for people practices (SABPP), 2018:5)]. Some of the essential aspects in RTE food products are storage time and conditions under which the products are processed and prepared. The European Food and Safety Authority (EFSA) guidelines state that storage time should not exceed 15 days for red meat and 3 days for poultry meat at 4 ºC and 7 ºC respectively (Kurpas et al., 2018:50). Ready-to-eat (RTE) food producing plant facilities have various areas, which are classified as high risk such as raw meat warehouses, preparation areas, or low risk such as packaging areas and final product areas. These production areas are often considered as possible sources of cross contamination with *L. monocytogenes* and other microorganisms of which *L. monocytogenes* can spread and cause possible problems with hygiene control (Kurpas et al., 2018:51).

Food preservation has been used for centuries and is important in the development of food products (Gould, 2009:547). Preservatives are used to extend shelf life of food products and maintaining their sensory properties such as colour, odour, texture and taste (Olatunde and Benjakul, 2018:1599). Commonly used preservation methods include artificial and natural preservatives (García-García and Searle, 2015:505). Natural food preservatives are additives used to preserve different food products by reducing the pH level and keeping the redox potential of the food product constant (Sharif et al., 2017:145-147). They are extracted either from plant or animal origins. Natural preservatives are also used to prevent the growth of microorganisms in a food product and avoid the occurrence of food spoilage and foodborne illnesses (Bondi et al., 2017:1). Although artificial preservatives, such as sodium benzoate, sorbates and nitrates are used to extend shelf life, inhibit growth of harmful organisms and prevent food spoilage, they are prone to health risks, resistance and high toxicity levels. These disadvantages highlights the need for research into safer options such as natural alternatives. Therefore, this study aimed at getting insight into the ability of natural preservatives chitosan [(1, 4)-2-Amino-2-deoxy-β-D-glucan] and rosemary (*Rosmarinus officinalis* L.) to inhibit the growth *of L. monocytogenes* in polony and reduce the negative health effects caused by synthetic preservatives. According to (Soultos et al., 2008:1155) a concentration level of 1.0 % (w/w) chitosan was found most effective in “boerewors”. A concentration of 0.026 % (w/w) which is equivalent to 0.26 g/kg for all rosemary models was suggested by Kim (2012:12326) to be an effective antimicrobial and antioxidant agent.

Chitosan is known as an all-natural, non-toxic dietary fibre attained by the deacetylation of chitin which originates from the exoskeleton of aquatic life (Ramawat and Mérillon, 2015:3). Chitosan has a multipurpose advantage to the production of RTE polony since it has both antimicrobial and antioxidant properties which play an important role in the extension of shelf life, quality of a food product and the inhibition of organisms that cause spoilage. As a result, the properties of chitosan support its application in the food industry (Campos et al., 2011:1118). In addition, chitosan is advantageous as it contributes to weight loss and various health effects, including blood pressure, reducing the risk of diabetes and hypertension, and enhancing the digestive system function (Inatani et al., 1983:526).

Plant extracts are used in a variety of food applications to preserve food quality. Rosemary is one of many plant extracts that shows strong antimicrobial and antioxidant effects in preventing microbial contamination (Inatani et al., 1983:526).

In South Africa, the interest in *L. monocytogenes* has grown significantly due to the increased number of outbreaks reported in food facilities and other environments over the past years (Keet and Rip, 2021:42). However, there is limited information on the growth rate and survival of *L. monocytogenes* in foods preserved with natural preservatives (Bucur et al., 2018:1). There is also limited information on the effectiveness of chitosan and rosemary antimicrobial properties on the growth of *L. monocytogenes* (Alvarez et al., 2013:78).

1.2 Problem Statement

In the year 2017, South Africa experienced a major listeriosis outbreak with RTE polonies being the crucial vehicle of transmission (Tchatchouang et al., 2020:1). A total of 1060 cases of listeriosis were reported and 216 of those cases were fatal.

At present, RTE polonies are preserved with the use of synthetic preservatives (Amit et al., 2017:19). One of these preservatives includes the Opti.Form Ace, a powder blend of sodium lactate, sodium acetate and sodium diacetate with total 0.45% sodium (Institute of Food Technologies, 2001). This preservative contributes a significant amount of sodium to the finished product and has been reported to have negative effects such as increased production costs, altering the sensory properties of the product and can cause health problems such as high blood pressure (Sharma et al., 2018:18). In addition, the high amount of sodium in Opti.Form Ace powder increases the growth of *L. monocytogenes* (Ace, 2017:2). Previous studies show that chitosan and rosemary have antimicrobial and antioxidant properties. Therefore, in this study, producing polony with natural preservatives such as chitosan and rosemary could potentially limit and reduce the growth rate *of L. monocytogenes*, microbial spoilage, extend shelf life and retain the product quality.

1.3 Broad Objective of the Study

The aim of the study was to evaluate the effects of chitosan and rosemary extracts on the growth and survival of *L. monocytogenes* in polony models.

1.4 Specific objectives

Specific objectives were to:

1. Develop and investigate four different polony models at three different concentrations, P1 (control sample), P2 (chitosan sample), P3 (rosemary sample), P4 (chitosan and rosemary combination) for preservation against coliforms, yeasts and moulds. *E. coli*, *S. aureus* and *L. monocytogenes*.
2. Conduct microbial contamination analysis such as TVC (total viable counts), coliforms, yeasts and moulds, *E. coli*, *S. aureus* and *L. monocytogenes* on the produced RTE polony.
3. Determine the growth and survival of *L. monocytogenes* in RTE polony preserved with natural chitosan and rosemary extracts.

4. Conduct a preferential sensory evaluation using a consumer panel to determine the most preferred polony model produced in this study.

1.5 Hypotheses

1. It is hypothesized that the polony models preserved with chitosan and rosemary will result in the reduction of the growth and survival of *L. monocytogenes*.
2. Since formulation P4 does not rely on a single dominant preservative, it is hypothesized that it will be the most preferred option as determined by the sensory panel.

1.6 Delineation of the Research

The delineations used in this research were:

1. Only four polony models were produced, with only three preservatives. Two natural preservatives - chitosan (P2) and rosemary extracts (P3) and a combination model consisting of chitosan and rosemary extracts (P4). The fourth model consisted of a control sample (P1) produced with synthetic preservatives (NaCl).
2. The four polony models were produced using only 3 concentration levels, the standard concentration level at 10 g/kg chitosan and 0.26 g/kg rosemary. A doubled concentration level at 20 g/kg chitosan and 0.52 g/kg rosemary and a half of the standard concentration level at 5 g/kg chitosan and a 0.13 g/kg rosemary.
3. Only four models were used to further investigate consumer's preference through sensory evaluation.
4. A concentration level of 10^3 log cfu/g of *L. monocytogenes* was used, with temperatures of 4 and 10 ºC to monitor the growth and survival of *L. monocytogenes* in a RTE polony.
5. Sampling was done six times (days zero, three, six, nine, 12, and 15).

1.7 Significance/Importance of the Study

The significance of this study is to express the importance of *L. monocytogenes* and its ability to resist parameters such as temperature and concentration, as well as to ensure the delivery of safe food to consumers. *L. monocytogenes* is highly resistant, and difficult to remove or get rid of in production facilities. The consumption of polony contaminated with stress-adapted strains of *L. monocytogenes* has the prospect to lead to listeriosis or even death, especially if the consumer is immunocompromised. Therefore, the use of natural preservatives has the potential to limit the survival and reduce the growth of *L. monocytogenes*, leaving less room for contamination by the pathogen and reducing the chances of further outbreaks. Additionally, it will help avoid illnesses caused by artificial preservatives, thereby promoting a safer and healthier food supply.

1.8 Expected Outcomes

This study highlighted the importance of natural preservatives in inhibiting the growth and survival *of L. monocytogenes*. The study also showcased the bio-control properties of natural preservatives to inhibit the growth of *L. monocytogenes*. The success of this study will provide food processors and food producing facilities with healthier preservation alternatives. Finally, the completion of the study will bring awareness and knowledge to consumers and academics and showcase the advantages that natural preservatives have on RTE polonies being produced.

1.9 Thesis Overview

Chapter 1 presents the introduction and motivation of the study. Chapter 2 addresses the literature review with regards to *L. monocytogenes,* including the general properties of the bacterium, the importance of the application of natural preservatives, factors affecting the shelf life, food safety matters, and the safety of the consumer. Chapter 3 includes research based on the selected formulation of the polony models preserved with natural preservatives, anti-microbial activity, microbial analysis on polony models before consumption, as well as sensory analysis. Chapter 4 includes research on growth and survival studies of *L. monocytogenes* on polony models preserved with natural preservatives. Chapter 5 presents the general summary of the thesis and recommendations.

1.10 Ethical Declaration

Polony models preserved with natural preservatives was evaluated by a consumer panel of fifty panellists which were taken from students and staff at the Department of Food Science and Technology at Cape Peninsula University of Technology (CPUT). The panellists were selected based on their availability and product interest. The polony batch packs did not contain any toxic or harmful by-products. All sensory panellists were completely aware of the purpose of the study. All panellists that assisted in the sensory evaluation signed a consent form before proceeding with the sensory analysis. All food safety and ethical principles (222930144/04/2023) were strictly adhered to.

In addition, for sensory analysis, ethical clearance was submitted to the Faculty of Applied Sciences Ethics committee, CPUT. The ethical and professional practices for sensory analysis of foods were followed for the sensory analysis.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Listeria monocytogenes is a dangerous foodborne pathogen and a cause of concern for food processing facilities, food producers, health regulatory officials and consumers (Shamloo et al., 2019:241-243). The world's largest listeriosis outbreak occurred in South Africa in mid-June 2017, where a total of 1060 cases were documented (Smith et al., 2019:524). An investigation of a substantial ready-to-eat (RTE) meat factory in South Africa (SA) under the name of Enterprise Foods was prompted by epidemiological and laboratory findings (Smith et al., 2019:524). *L. monocytogenes* was present in RTE processed meat products including polony, a product resembling bologna sausage produced in the specified factory (Keet and Rip, 2021:42-43; Smith et al., 2019:524-526). Processed RTE foods including polony and sausages, also have a significant salt content. Due to its widespread nature and tenacity, *L. monocytogenes* often adjusts to environments with high salt content (Thomas, 2020:638). The risk of listeriosis has become a major concern globally. Therefore, new methods of food processing must be investigated to stop the spread of *L. monocytogenes*.

2.2 *Listeria monocytogenes*

Foodborne infections continue to pose serious health risks to people all over the world (Todd, 2014:226). *L. monocytogenes* continues to cause sporadic incidences of diseases connected with the intake of certain food products, and is one of the most significant foodborne pathogenic bacteria within the last 30 years (Zhu et al., 2017:1-5). *L. monocytogenes* occurs in a vast variety of habitats, including animals, humans and the environment (Tchatchouang et al., 2020:2). The environment serves as a primary reservoir of this organism. Therefore, it should come as no surprise that any fresh food item derived from plant or animal origin might have *L. monocytogenes* (Manyi-Loh et al., 2023:2). This could be caused by poor fertilisation methods and lack of hygiene practices in agriculture (Gartley et al., 2022:2-6). *Listeria* has the ability to survive, grow and multiply in a variety of foods (Santos et al., 2019:2). Additionally, *L. monocytogenes* is challenging to detect with culturing methods and requires an enrichment step for confirmation (Beumer and Hazeleger, 2003:191-193). Several ecological and environmental variables influence the growth and survival (G&S) of *L.*

monocytogenes in foods (Vivant et al., 2013:1). In other words, the success of this pathogen is influenced by its ability to endure adverse environmental conditions (Gerba, 2015:544-545). This includes temperature, pH, acidity, salt content, water activity and changed atmosphere. Understanding the conditions that affect the G&S of *L. monocytogenes* in foods may help the food sector develop preservation strategies that work and can be adopted in the future (Henney et al., 2010:67).

2.2.1 *General characteristics of Listeria monocytogenes*

L. monocytogenes is derived from the genus *Listeria*. It is characterised as a Gram-positive, non-spore-forming, facultative anaerobic bacterium, which is ubiquitous in nature (Kurpas et al., 2018:49). There are 28 validly published species to date (Manqele et al., 2023:1; Parte et al., 2020:70). This study focuses on *L. monocytogenes*. Selectively, six original members of the genus are shown in Table 2.1; *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii and L. grayi* (Manqele et al., 2023:1), whereby L monocytogenes and L. ivanovii are foodborne pathogens and the rest are important contaminants in industry that are indicators of poor hygiene.

Table 2.1 Scientific classification of *Listeria monocytogenes*

Domain	Bacteria
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	***Listeriaceae***
Genus	*Listeria*
Type species	*L. monocytogenes*^{T}
Other species	• *L. innocua* • *L. welshimeri* • *L. seeligeri* • *L. ivanovii* • *L. grayi*

(Adapted from: Manqele et al., 2023:1)

L. monocytogenes has been implicated globally, in diseases of many humans, and animals (Acheson, 2000:770-773). Even though the organism is abundant in nature, it is frequently found in poorly prepared silage and various environments (Allerberger and Wagner, 2010:770-771).

L. monocytogenes was detected in various food categories (Zhang et al., 2021:1-3). These categories include raw fruits and vegetables, raw ground beef and processed meats, dairy products such as cottage and cheddar cheese with a pH of 5.0, and raw milk and milk that have not been thoroughly pasteurised (Gould et al., 2016:104-144). One of the main sources of contamination arises from delicatessen (deli) meats. These meats are known to be “high risk” and requires special control measures to limit microbial contamination. *L. monocytogenes* contamination of meats is a risk during the different stages of the food production due to its wide distribution and adaptability to various environmental conditions (Okutani et al., 2004:131-134). Meats play an important role in the sustenance of humans and is an important source of protein. This study will focus on RTE polony, since RTE deli meats have a high risk of being contaminated with *L. monocytogenes*.

L. monocytogenes is a major safety concern and is considered to be a more virulent foodborne pathogen in comparison to other common foodborne pathogens (Al-Nabulsi et al., 2015:154-155). *L. monocytogenes* should be controlled before it raises concerns in production and distribution facilities and becomes an epidemic crisis. The widespread distribution of this bacterium in nature increases the chances of contamination making it challenging to protect foods and environments from getting in contact with the organism (Low and Donachie, 1997:9-15). Emphasis should be placed on the reduction of *L. monocytogenes* during production, factors that affect its growth and survival and ability to resist various conditions.

2.2.2 *Infective dose of L. monocytogenes*

The infective dose of *L. monocytogenes* is undetermined and is dependent on various variables, which include, the immune status of the host, the virulence and infectivity of the pathogen, the amount of contaminated products consumed and the type of food consumed (Martin and Fisher, 1999:1236). The infective dose also varies depending on the strain and susceptibility of the host. These variables will affect the dose

response relationship. Low numbers of *L. monocytogenes* such as 10^3 log cfu/g in food products can cause illnesses, especially to immunocompromised consumers.

L. monocytogenes have the tendency to infect mammalians. The infection is initiated with the ingestion of contaminated food products. The pathogen invades the host cell, cross epithelial barriers where it multiplies into the gut, passing through the lymph node. The organism spreads through the organs via the blood stream until it reaches the central nervous system or placenta. In this way it causes severe illnesses especially in immunocompromised people (Figure 2.1).

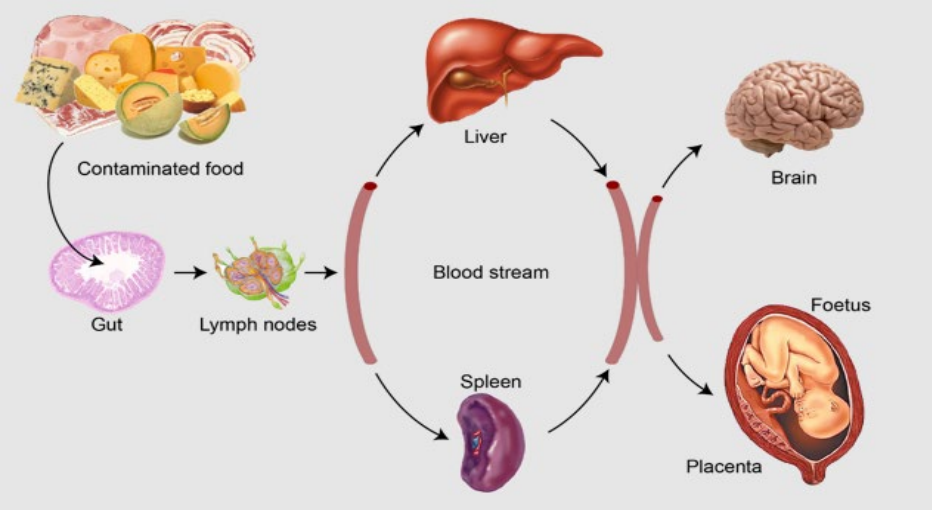

Figure 2.1 The infection cycle of *Listeria monocytogenes* from the source to the central nervous system and placenta (Goeboel et al., 2001:589).

2.2.3 *Pathogenesis and spread of Listeria infection*

In both epidemic and sporadic cases of listeriosis, the major source of infection is contaminated food (Hughes, 2008:7-40). The pathogen which is non-enteric, spread through the bloodstream and targets the organs and tissues affected. Once inside the body, the bacteria multiplies and releases a toxin called listeriosis (Hitchins, 1996:71-82), which contributes to the infections severity by attaching the central nervous system. There are two forms of disease that are caused by the *L. monocytogenes* infection:

1. Non-invasive listeriosis. This is a gastrointestinal illness which resolves in healthy people. It has typical symptoms such as fever, diarrhoea and vomits.

The clinically proven type of infection usually occurs 20 hours after the intake of heavily contaminated food products (Dalton, 1997:1).

2. Invasive listeriosis is known as a much more severe illness. This is due to the severity of symptoms that may lead to septicaemia and meningitis (Maccabe et al., 1961:191-193). The invasive type of illness has a high case-fatality rate (20% - 30%) (Wilking et al., 2021:2485-2487). The infection of this illness tends to be more host dependent. This form of disease affects the more vulnerable consumers (Garrido et al., 2010:1182-1184). The incubation period for this type of illness usually occurs generally longer than non-invasive listeriosis. The incubation period for invasive listeriosis can be between 20 to 30 (Goulet et al., 2013:1-5).

Both non-invasive and invasive forms of listeriosis can affect the CNS, predominantly in the adult patient. Pregnant woman may experience mild flu-like symptoms (feto-maternal neonatal listeriosis) and the infection can invade the foetus via the placenta (Maccabe et al., 1961:199-203). Woman can remain asymptomatic or may show symptoms like chills, tiredness, headaches and muscular pain roughly 2 to 14 days before loss of the foetus/ child (Goulet et al., 2013:4).

2.2.4 *Outbreak of listeriosis in South Africa associated with polony*

South Africa (SA) experienced a deadly outbreak of listeriosis in 2017. According to Thomas et al. (2020:3) an epidemiologic, trace back and environmental investigation was conducted. The study showed a total of 937 cases that were identified as confirmed *Listeria* cases (Thomas et al., 2020:3). 50 % of cases were associated with pregnancy. Others were associated with human immunodeficiency virus (HIV), patients that occurred between the ages of 15 – 49 and patients that have immunocompromised illnesses. The case control analysis of this study showed that patients with *L. monocytogenes* ST6 (*L. monocytogenes* sequence type 6) infections were more likely to have eaten RTE polony. In January 2018, interviews that were conducted with patients, suggested that polony was consumed most commonly amongst persons with *Listeriosis*. According to a study done by Mogomoti and Chinsembu in Windhoek (retail shop1) and Eros (retail shop 2), representing prevalence of *Listeria monocytogenes* in different foods. Polony indicated a much higher levels of *L. monocytogenes* in RTE foods sold at both retail stores. Polony is made from meat, pork and preservatives

which may be tainted by *L. monocytogenes* during the different stages of production (Mogomotsi and Chinsembu, 2012:66-69). Figure 2.2 and Figure 2.3 depicts higher levels of *L. monocytogenes* in polony.

Sporadic outbreaks of *L. monocytogenes* resulted in major food safety concerns, due to its health implications and cost effects (Olanya et al., 2019:231-233). The 2017 – 2018 *Listeriosis* outbreak in SA occurred in different parts of the country. This suggested that contaminated polony could have been consumed and distributed to different regions of SA. According to the International Monetary Fund (IMF) 4000 metric tons of polony related food product cases have been identified and resulted in recalls.

The 2017 – 2018 listeriosis outbreak was the first documented outbreak in South Africa. Since then, cases of listeriosis have been increasing sporadically (Manganye et al., 2018:55-56). Since 5 December 2017, listeriosis have become a notifiable condition in SA. According to the World Health Organisation (WHO, 2016:1-500), for a disease to become notifiable, the outbreak should meet at least two of the five qualifying criteria such as: rapid spread or unusual behaviour. In Addition to the outbreak, the SA Department of Health announced a new policy of making listeriosis notifiable (National Department of Health, 2004:1-48).

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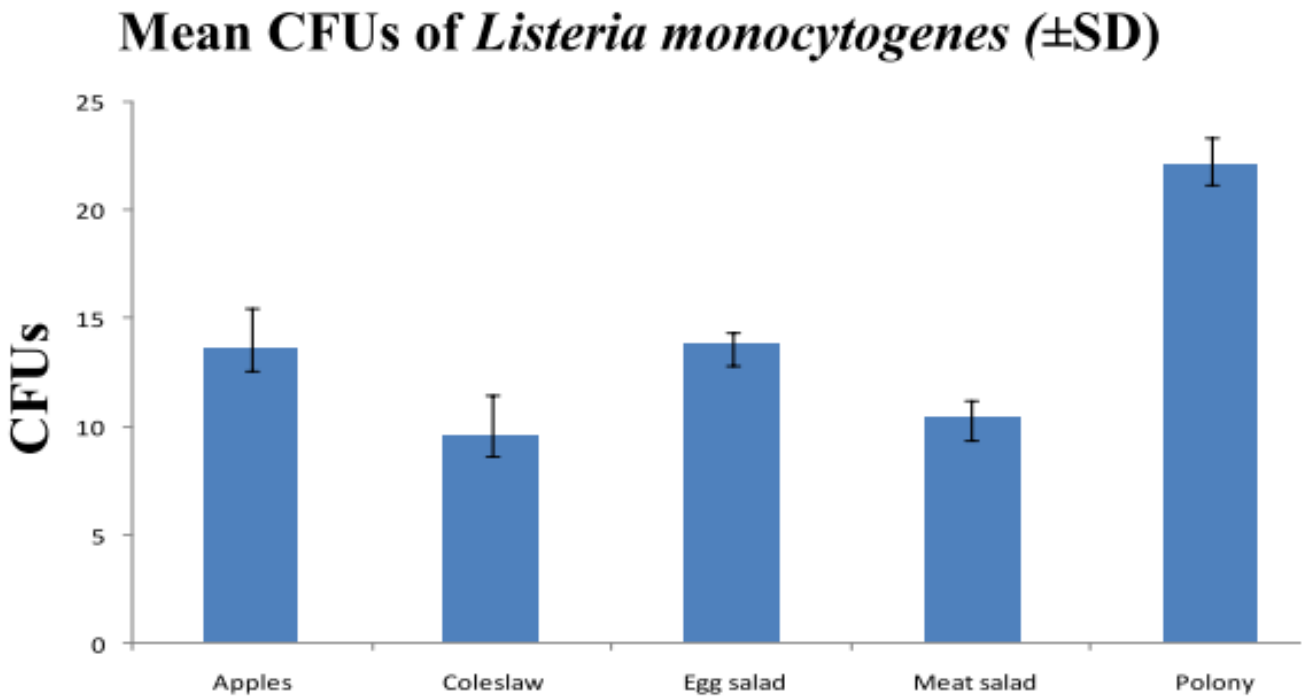

Figure 2.2 The prevalence of *Listeria monocytogenes* in RTE foods at retail Shop 1 (Windhoek) in South Africa (Mogomotsi and Chinsembu, 2012:68).

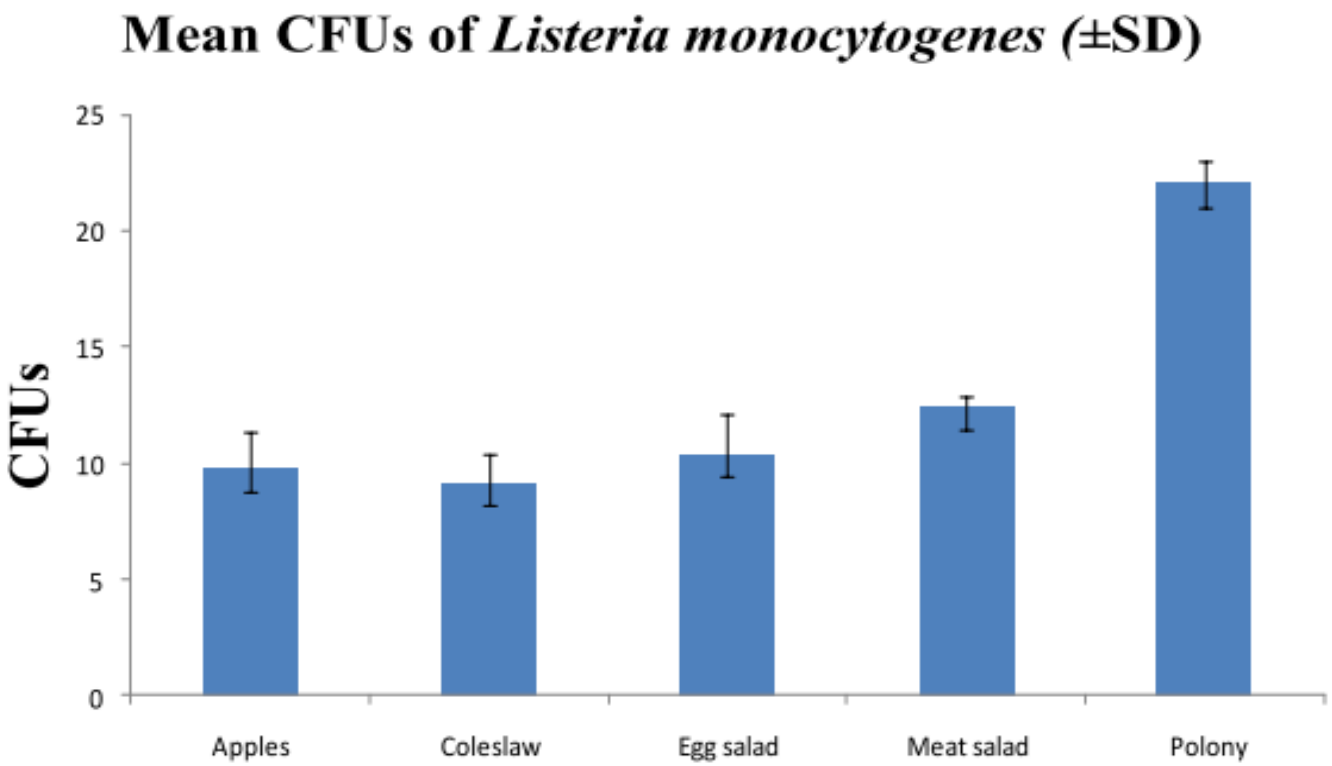

Figure 2.3 The prevalence of *Listeria monocytogenes* in RTE foods at retail Shop 2 (Eros) in South Africa (Mogomotsi and Chinsembu, 2012:69).

2.3 Food preservatives

Preservatives are substances that are used to prevent spoilage of food by microorganisms (Krishna, 2017:1). Food preservatives are important in the development of a new product. It is used to extend the shelf life of a product, making them last longer. In addition, this assists in maintaining their organoleptic properties such as colour, odour, texture and taste. The quality of food is important to retain, for the consumption of humans for health and safety measures.

Today there are different types of preservation methods. These methods are either used conventionally or used as a modern preservation methods (Sharif et al., 2017:145). Many of these preservation methods use added preservatives, which are categorized as either artificial preservatives, known as synthetic preservatives, or natural preservatives. Nowadays, consumers are more aware of how food products are processed, and the ingredients used during processing. This resulted in consumers being more health conscious and cautious against synthetic preservatives.

Synthetic or chemical preservatives are divided into two categories, i.e., anti-microbial agents and anti-oxidation agents. In this type of synthetic preservative only antimicrobial agents are used to inhibit the growth of microorganisms and are commonly used to prevent food spoilage, however, it is important to note that excessive consumption of foods with synthetic preservatives can be dangerous to consumers(Sharif et al., 2017:145-147). People who over-consume this preservative could reap the dangers of health complications such as asthma, kidney failure and cancer (Abdulmumeen et al., 2012:36-42). The use of chemical additives in food products has made consumers wary, leading to a higher interest in natural preservatives. According to Valley and Misso (2012:16-18), the use of sulphite as a preservative can activate different allergic reactions. Asthma, abdominal pains, nausea, urticaria (hives), diarrhea and seizures are common symptoms. These health concerns are dangerous. There is therefore an interest in the application of natural food preservatives to reduce the side-effects of synthetic compounds.

Natural preservatives are extracted from plants or animals. Which may also be used to prevent the development of microbes in food products and avoid the occurrence of food spoilage. The concern regarding synthetic chemical additives, as well as consumer awareness, has led food producers and food developers to develop foods preserved with natural additives.

2.4 Traditional preservation methods

2.4.1 *Sodium Chloride*

Traditionally, salting is a form of preservation. Salt in the form of sodium chloride (NaCl) added to polony preserves the food product, produce flavor, and solubilize proteins. NaCl has been regarded as a preservative that improves health by limiting and controlling the growth of foodborne pathogens and spoilage organisms (Doyle and Glass, 2010:44). However, increasing consumption of different processed foods that contain high levels of sodium is considered “a potential health threat” (CDCP, 2009:281). Excessive sodium intake is associated with adverse health effects including high blood pressure, development of hypertension and coronary heart diseases (Hollenberg, 2006:240). Sodium, is primarily consumed as salt (sodium chloride). In foods, NaCl contributes to many important attributes; this includes preservation, shelf life, and sensory qualities such as savoury flavour and texture (IFT, 2016:1).

Sodium is an important nutrient with the function of regulating extracellular fluid volume and the active transport of molecules across cell membranes. It improves the sensory properties of foods, by increasing saltiness, decreasing bitterness and increasing sweetness (Liem et al., 2011:694-706). Ultimately, sodium is essential for normal human functioning. The current sodium intake (1150mg per 100g) far exceeds the recommendation (2 grams sodium per day) for good health (Brown, 2009:791-795).

2.4.2 *Effects of sodium chloride* on health

Various studies show that high dietary sodium levels are associated with health problems including hypertension, cardiovascular disease (CVD), asthma and kidney stones. These are only a few potential threats to the human body caused by the intake of salt. Data shows that a high intake of sodium may affect the development and the severity of these conditions (He and MacGregor, 2009:363).

The majority of adults have either hypertension defined as untreated systolic blood pressure or prehypertension. Untreated hypertension is associated with increased incidences of diabetes, heart disease, strokes, and kidney diseases. Therefore, there is a universal agreement that interventions that reduce or prevent development of high blood pressure would significantly improve health (Pawelek, 1994:126-127). Hypertension is a recognised risk factor for cardiovascular disease and is often associated with other cardiovascular risk factors such as obesity, insulin

resistance, and elevated blood lipids, in a condition called metabolic syndrome. A comparison of urinary sodium excretion in more than 700 people with and without symptoms of the metabolic syndrome, found in higher levels of sodium excretion were significantly related to elevated blood pressure and to obesity (Doyle and Glass, 2010:44-47). High sodium intake increases blood pressure (Balamurugan et al., 2016:51-52). Roughly, 80% intake of various processed foods and meats are being consumed (Henney et al., 2010:90-95). Statistics states that the majority of the populations' sodium intake is more than the recommended levels. Figure 2.4 indicate that both male and females exceed the daily recommended sodium intake. The average sodium intake varies between 1500 and 2300 mg, of which both genders abused their limit, indicating that high sodium intake could potentially pose health risks such as heart failure and hypertension.

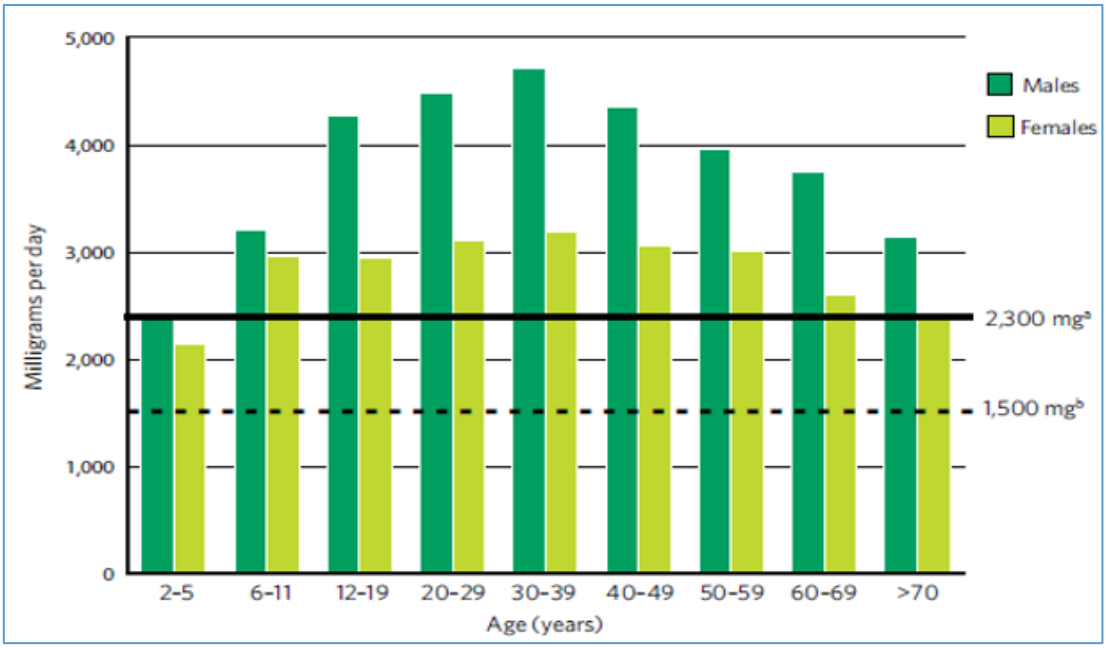

Figure 2.4 Illustration of an estimated mean of sodium intake, by age and gender in the years 2005-2006 (Palmer, 2011:5)

2.4.3 *Alternative approaches to determine the inhibition of L. monocytogenes*

The importance of the development of methods to reduce sodium in food products is clear. Following the publication by the WHO on sodium usage and intake, several countries have introduced legislation to reduce sodium intake in the past few years

(Jachnimowics-Rogowska and Winiarska-Mieczan, 2023:2). Therefore, there is an increase in the demand for shelf life extension and improved safety in processed meats. However, reduced sodium levels and the use of salt replacers do not support this need. According to Sansawat et al. (2013:1157) inhibition of *L. monocytogenes* in full (1.8 % salt) and low (1.0 % salt) sodium frankfurters (RTE processed meats) stored at 4 ºC, 7 ºC and 10 ºC showed growth of *Listeria* sp. This study also presented alternatives that minimizes the presence and controlled levels of *L. monocytogenes* in RTE processed meats. They prepared samples with organic acid salts as sprayed dried powders. Formulations containing salt replacers presented less colonies of *L. monocytogenes* in RTE processed meats. These results indicated that co-sprayed powders are viable alternatives to reduce or inhibit the growth *of L. monocytogenes* in RTE processed meats (Sansawat et al., 2013:1157).

Another study performed by Glass et al. (2013:2074), investigated alternatives to reduce the amount of sodium while maintaining protection against pathogenic growth in RTE processed meats. Six sliced cured, deli-style turkey models were tested, including a negative control (without antimicrobials), and a model preserved with a 3.2% lactate-diacetate blend. Four additional treatments with 0.3, 0.4 or 0.5 liquid sodium propionate (PC) or with a 0.4% proprietary ingredient, liquid sodium propionate and sodium benzoate (PBC) were tested at pH of 4.8 to 5.2 (Glass et al., 2013:2074). During the trials of this study, cured deli-style turkey slices were surface inoculated with *L. monocytogenes*. A total of 0.5 ml of liquid inoculum was spread over the top surface of the cured, deli-styled turkey models. Slices were then stacked and vacuum packed. Turkey models were divided and stored at 4 and 7ºC (Figure 2.5 and Figure 2.6). These models were observed and monitored for growth of Listeria populations at weeks 0, 4, 6, 8, 9, 10, 11 and 12 at 4ºC and 2, 4, 5, 6 and 7 weeks at 7ºC (Glass et al., 2013:2075-2077). In conclusion they have found that *L. monocytogenes* grew more rapidly on the treatments than the negative control without antimicrobials. As anticipated by the author, *L. monocytogenes* grew faster on cured turkey stored at 7 ºC. Cured turkey models showed more growth of *L. monocytogenes* stored at 7 ºC but slightly like cured turkey stored at 4 ºC (Figure 2.5 and Figure 2.6).

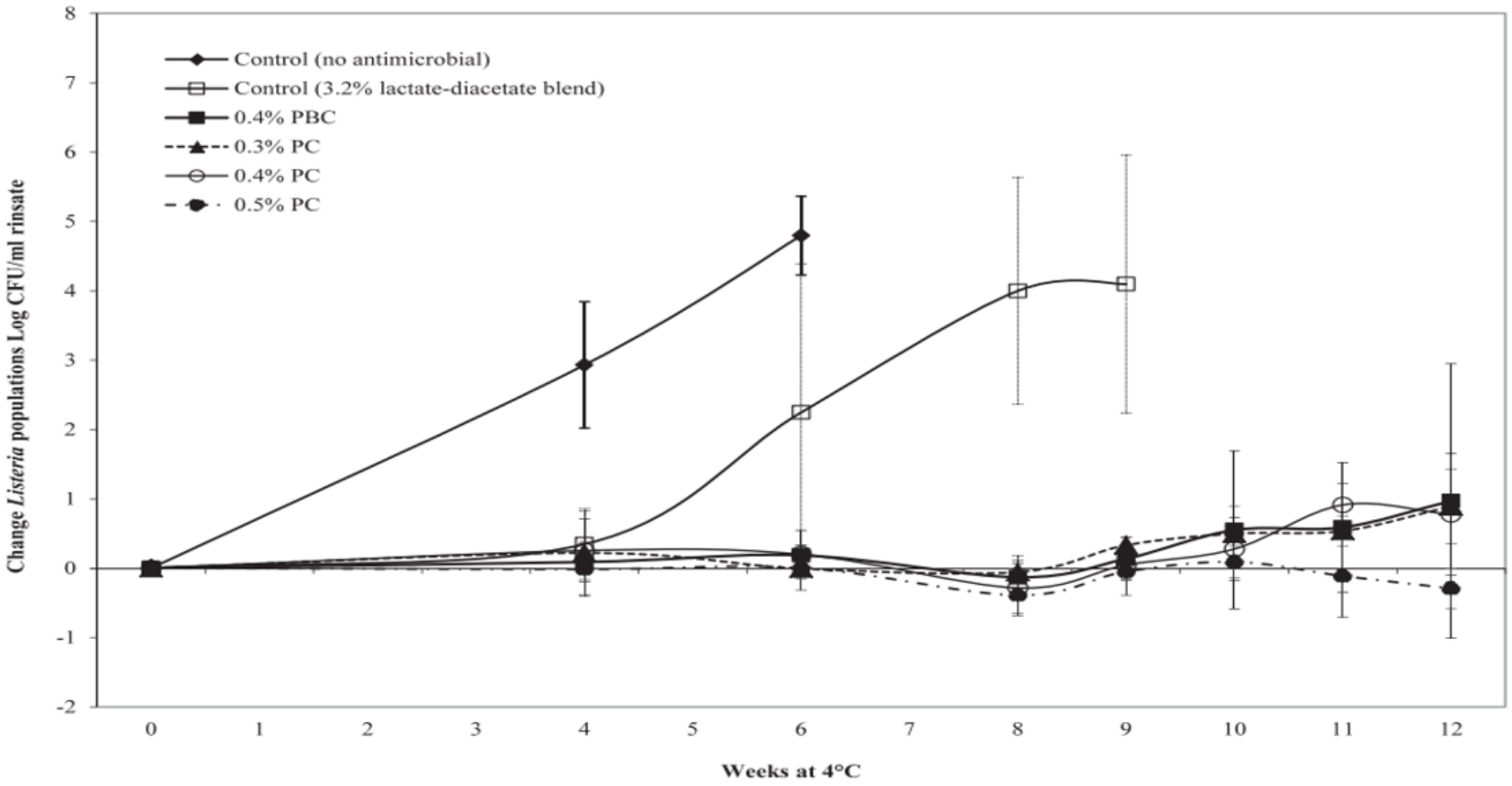

Figure 2.5 Changes in populations of *Listeria monocytogenes* in cured deli-styled turkey models stored at 4 ºC up to 12 weeks (Glass et al., 2013:2077).

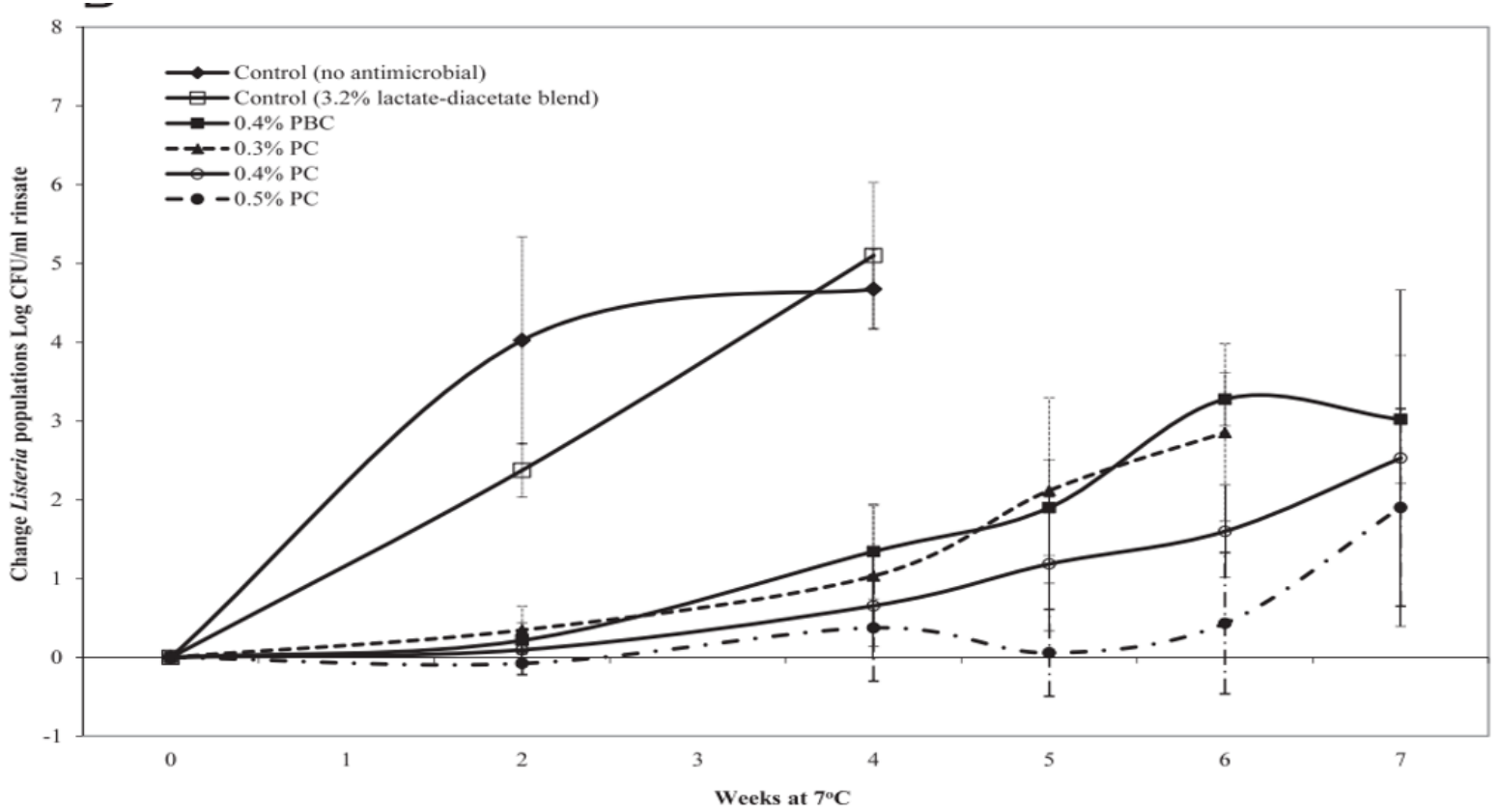

Figure 2.6 Changes in populations of *Listeria monocytogenes* in cured deli-styled turkey models stored at 7 ºC up to 7 weeks (Glass et al., 2013:2077)

2.5 Natural preservation methods

The shelf life of RTE processed meat products is considered to be an essential part of food safety for the SA consumer. Growth and survival of different pathogenic organisms such as *L. monocytogenes*, *E. coli*, *Staphylococcus* have the opportunity of growing in these meat products (Lucera et al., 2012:1-6). This can occur pre- or post-contamination of a meat product.

Over the past few years, SA consumers have become more informed about the use of chemical additives in food and the side effects it possesses. The known side effects in conventional preservatives in food contains nitrites, sulfates, sodium etc. Nitrates/ nitrites have been linked to leukemia, colon, bladder and stomach cancer (Lee and Paik, 2016:547). Previous research has also displayed certain pathogenic organisms that can withstand traditional preservation methods. This, however, is advantageous since it creates opportunities for the use of natural preservatives. Natural food additives have become popular due to consumer awareness. There are various natural preservation methods that effectively reduce microbial growth. Figure 2.7 shows the different individual natural preservation methods. The current study focuses on chitosan and rosemary (plant extract).

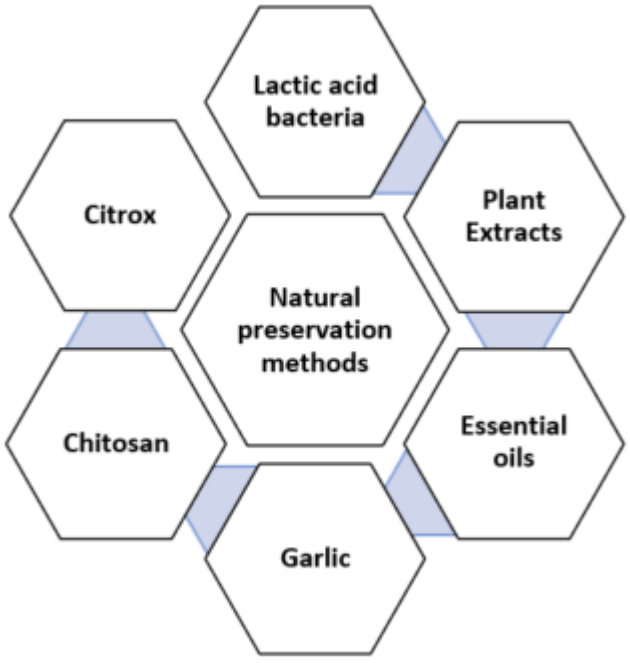

Figure 2.7 Different natural preservation methods used to employ microbial inhibitory activity (Hintz et al., 2015:1-12)

2.5.1 *Chitosan*

Chitosan is known as an all-natural, non-toxic dietary fibre attained by the deacetylation of chitin which originates from the exoskeleton of aquatic life (Ramawat and Mérillon, 2015:1-3). Chitosan has a multipurpose advantage in the production of RTE polony as it has both antimicrobial and antioxidant properties. These play an important role in the extension of shelf life, quality of a food product and the inhibition of organisms that cause spoilage. These properties support the use of chitosan in the food industry (Campos et al., 2011:1117). Other advantages include its contribution to weight loss and different health efficacies such as reducing blood loss, blood sugar levels, blood pressure and improvement of the digestive system. Chitosan is a natural biopolymer known for its exceptional polycationic nature, which contributes to its antifungal and antibacterial activity (Tiwari et al., 2009:5990). Previous researchers have demonstrated the antibacterial activity of chitosan against *E. coli* and *Staphylococcus aureus*. Research was done on chitosan (0.25, 0.5, 1.0) as an antimicrobial ingredient in pork sausage (Lee and Paik, 2016:547). Natural antimicrobial agents such as chitosan may offer unique advantages in controlling growth of *L. monocytogenes* (Table 2.2). It may increase the shelf life properties and the safety of food to consumers as indicated in Table 2.2. Thus, preventing outbreaks of the detrimental pathogenic organism *L. monocytogenes*.

2.5.2 *Plant extracts (rosemary)*

Plant extracts are used in a variety of food applications to preserve food quality. Rosemary (*Rosemarinus officinals L*.) is one of many plant extracts that shows strong antimicrobial and antioxidant effects (Inatani et al., 1983:521). Essential oils in herbs and spices such as rosemary, sage, basil, thyme and oregano are known for their strong capabilities in reducing the growth of microorganisms (Kročko et al., 2015). In addition to improved antioxidant properties, rosemary extract comes from natural sources and is beneficial in reducing food odours. Rosemary extract can in essence be applied for the prevention and reduction of *L. monocytogenes* growth. The concern for synthetic antioxidants and antimicrobials by consumers has led to many meat processors seeking alternative natural antioxidant and antimicrobial agents. Rosemary has been found to be effective in meat systems (Kročko et al., 2015:160-165). Although the study focuses on chitosan and rosemary as natural preservation method, Figure 2.8 displays other alternatives that could potentially inhibit microorganisms.

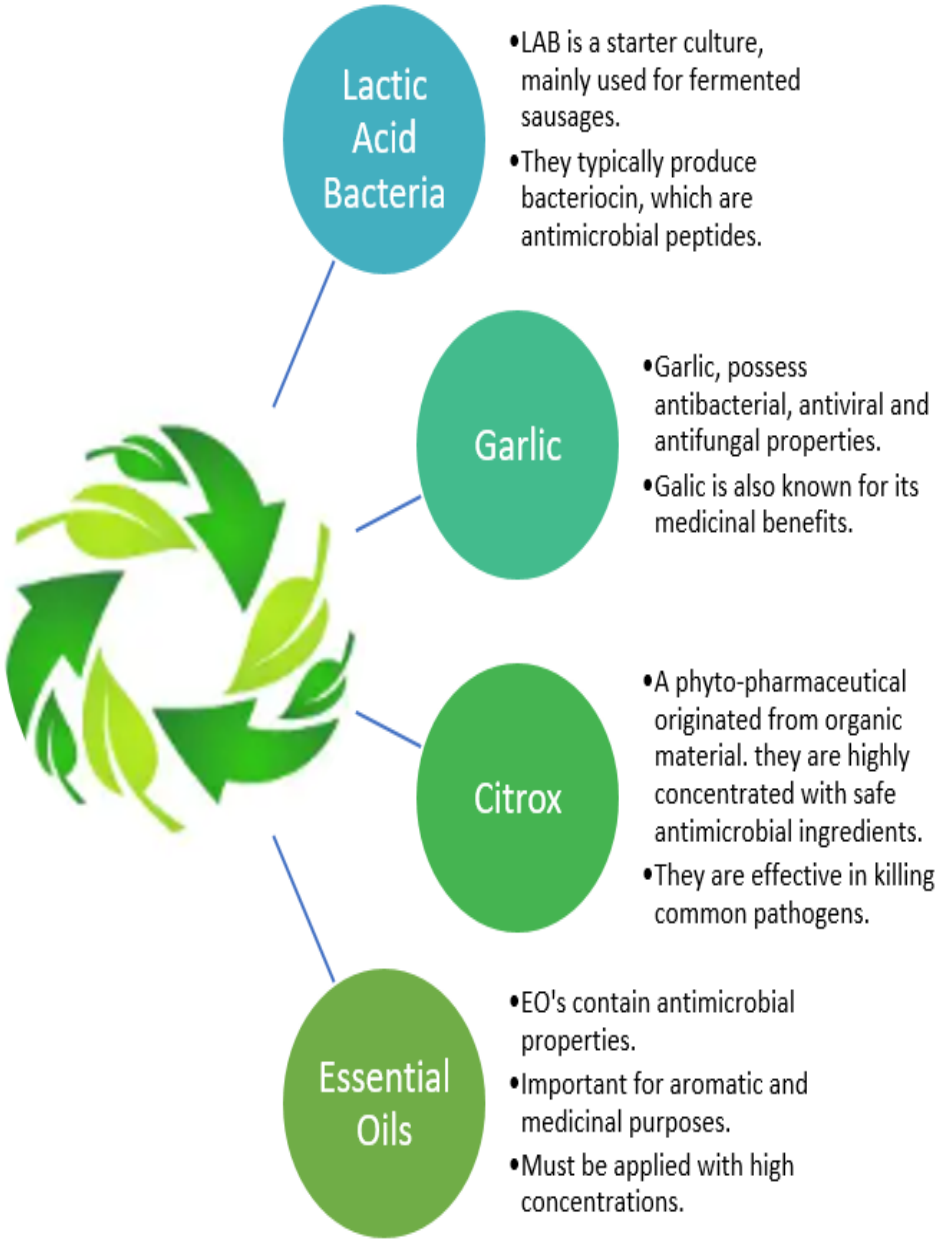

Figure 2.8 Other natural preservation methods and its benefits for inhibition of microorganisms (Hintz et al., 2015:2-10)

Table 2.2 The effect of natural antimicrobial agents applied to processed products based on food preservation and quality (Lucera et al., 2012:3).

Food product	Natural compound/ antimicrobial agent	Main results /microbial dynamic	Quality attributes	Reference
Red meat	5 ml/l clove oil 1 ml/l thyme oil 300 g/Kg tea catechins	*Listeria monocytoge*nes: 1.15 – 1.71 microbial log reduction	Increase in shelf life and a decrease in lipid oxidation	(Tiwari et al., 2009:5994)
Beef Hot dogs	5 ml/l clove oil 1 ml/l thyme oil 300 g/Kg tea catechins	*L. monocytoge*nes: 0.067-1.05 microbial log reduction	Increase in shelf life and a decrease in lipid oxidation	(Tiwari et al., 2009:5994)
Strawberries packaged under Modified Atmosphere Packaging (MAP)	Solution of 1% chitosan	Chitosan inhibited growth of microorganisms	Significantly affected microbiological stability of strawberries	(Lucera et al., 2012:3)
Fresh cut mushrooms	Coating containing 5, 10 and 20 g of chitosan	At 4 ºC for 15 days: chitosan coating inhibited growth of total bacteria, yeasts, and moulds	No change in quality attributes	(Lucera et al., 2012:3)
Meatballs Stored at 10 ºC	0.2 % rosemary, cranberry and lovage extracts	High risk product shelf life – 13.3 days	Rosemary extract was most effective on product shelf life	(Lucera et al., 2012:3)
Fresh chicken sausage	500 ppm of rosemary	Resulted in good meat quality	Rosemary improved meat quality	(Lucera et al., 2012:3)

2.6 Polony classification

Polony is a SA version of a finely ground pork and beef sausage, commonly known as bologna sausage, originally derived from the Italian city of Bologna. It is a cured and cooked meat product, stuffed into larger tubular nylon casings that can be removed before consumption (Cluff et al., 2017:143). The use of extensive meat trimmings such as beef, pork, pork rinds, fat and extension with soya proteins allow polony to be affordable. Polony is the highest volume processed meat across retail and food service sectors in SA (Cluff et al., 2017:143). The Agricultural product standards act (Act No. 119 of 1990) classifies polony as a comminute or finely chopped processed meat product, which is classified as a cured and heat treated product (Department of Agriculture, 2019:10).

The physical requirements of polony include a firm and fine texture, pink to light brown colour, cylindrical in shape, and no bone or grittiness. The outer surface should be clean and free of any foreign matter and no rancidness (Department of Agriculture Forestry and Fisheries, 2012:9). There are different types of polonies that are produced by the meat industry. Polony is a meat emulsion classified under dried and semi dried sausage, cooked smoked sausage and cooked meat specialities (Table 2.3). These polonies are classified, based on the characteristics of polony that fall under the different sausage categories. Bologna also known as “polony” have characteristics such as cured, comminute, and cooked meat products.

Today, the consumption of polony remains an important component of the global diet. Many South African consumers buy polony, as it is an important food component in many consumers’ lives. New product development have led to a whole range of different polonies in the market to-date. Retailer’s polony category ranges based on the flavour profiles of polony such as, chilli polony, French polony, mutton polony, chicken polony, beef pastrami, chicken pastrami and many more.

Table 2.3 Polonies are classified under sausage (R1283, 2019:21-23)

Classification	Characteristics	Examples
Fresh sausage	Fresh meat, uncured, comminuted, seasoned and usually stuffed in cases, must be cooked before serving	1. Fresh pork sausage 2. Bratwurst 3. SA Boerewors
Dried and semidry sausage	Cured meat, fermented air dried, may be smoked before drying, served cold	4. Gonoa salami 5. Pepperoni 6. Len Bologna 7. Summer sausage 8. Dry wors
Cooked sausage	Cured or uncured meats, comminuted, seasoned, stuffed into casings, cooked, and sometimes smoked, severed cold	9. Liver sausage 10. Liver cheese
Cooked, smoked sausage	Cured meats, comminuted, seasoned, stuffed into casings smoked and fully cooked, do not required further cooking, but some are heated before serving	11. Frankfurters 12. Bologna 13. Cotto salami
Uncooked, smoked sausage	Fresh meats, cured or uncured, stuffed, smoked but not cooked, must be cooked before serving	14. Smoked, country-style pork sausage 15. Mettwurst 16. Kielbasa
Cooked meat specialities	Prepared meat products, cured or uncured, cooked but rarely smoked, often made in loaves nut sold in slices, served cold	17. Loaves 18. Head cheese 19. Scrapple

2.7 Manufacturing of polony

Polony is a classic emulsified sausage product and is the simplest indication of luncheon meat. Polonies are good examples of value added processing that includes ordinary meat cuts and trimmings that are tenderised by processes such as grinding or chopping and may include salt, spice and other specific ingredients needed before cooking or other finishing processes (Advisory, 2016:1). Ingredients for polony include meat: a mixture of lean and fatty beef, pork or mutton. Fat: fat can be added when meat is too lean. Ice and water is included into the formula to control the temperature during the mincing process. Edible offal such as heart, liver, tongue or kidney must only be used where permitted and must be limited to 5% by mass of the product and must be declared on packaging and labels when used (Advisory, 2016:1-5). Other ingredients that are added to production process of polony include salt (0-5%) which improves the taste and acts as a preservative to prevent bacterial growth. Salt helps with the solubilization of meat proteins. Flavouring is added to improve taste and sodium ascorbates (500 ppm) reduces the curing time and acts as a colour stabiliser in minced and chopped products (Advisory, 2016:1-5). The casing used for polony is normally plastic casing holders. Figure 2.9 indicate and identifies the processing steps needed to produce a typical RTE polony.

The polony was produced by chopping the rind at a slow speed for 10 minutes using a bowl cutter until a 3 mm particle size was attained. Ice was added to form a slurry followed by the addition of soy and $NaHCO_3$. The mixture was bowl-chopped until a temperature of 15 ^oC was reached. The meat components, half ice and phosphate were added, and bowl chopped until the mixture was well blended. The remaining ingredients were added, and bowl chopped at a high speed to a final temperature of 14 ^oC. Filling into casings was done speedily to prevent emulsion breakdown due to protein denaturation. The polony was then cooked in a steam chamber or hot bath to a temperature of 68-72 ^oC. The temperature of the water should not increase above 75 ^oC. Once this process is completed, the product is cooled under running water to reach a temperature of 10 ^oC.

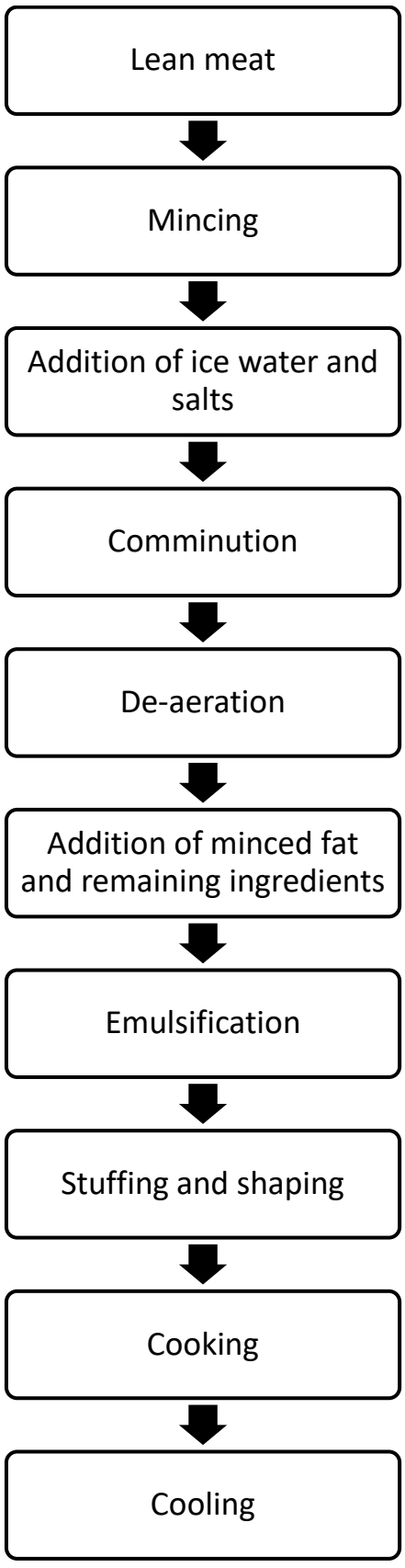

Figure 2.9 **An** overview of the production process to manufacture a RTE polony (Advisory, 2016:1) .

2.8 Endogenous factors affecting the shelf life of polony

2.8.1 *Temperature*

Temperature is a significant factor that influences the rate and level of microbial growth. According to Madigan et al. (2003), microorganisms can be divided into four groups namely: 1) Psychrophiles, with low temperature optima; 2) mesophiles with mid-range temperature optima; 3) thermophiles with a high temperature optima, and 4) hyperthermophiles, with very high temperature optima. Psychrophilic pathogens such as *L. monocytogenes*, mesophilic pathogens such as *S. aureus* and thermophilic pathogens such as *Clostridium botulinum* have been isolated from most food products (Hoffman et al., 2003:52).

In shelf life determinations of RTE foods, temperature has been shown to affect the lag phase and generation time of microbial growth. When temperature is reduced, the lag phase and generation time increase. Growth rate decreases, as the temperature approaches the minimum for growth where cell division ends (Herbert and Sutherland, 2000:101-103). An easier way to increase the shelf life of RTE polony would be to decrease temperature of processing areas. In the case of lower or reduced temperatures, more genera that are important could be eliminated from various meat products.

2.8.2 *pH*

Generally, spoilage microorganisms grow at a neutral pH of 7.0 (Kim, 2006:1). This is a result of proteins, which are more heat stable at their isoelectric point. Most spoilage bacteria function more effectively in neutral environments (Mossel et al., 1995:63). The pH of raw meat range from 5.2 – 5.7. Each organism has a specific pH requirements for optimum growth.

2.8.3 *Surface area*

The process of polony includes mincing/grinding of the meat and fat used. Minced meat creates a larger surface area. The larger surface area of minced meat poses more risks for contamination. Thus, creating increased surface area and mixing during the mincing operation and the transfer of product-to-product may lead or result in cross-contamination (Mathenjwa et al., 2012:23). Microorganisms associated with ground

beef processing include pathogenic microorganisms such as *L. monocytogenes* and *Salmonella* spp. (Mathenjwa, 2010:23).

2.8.4 *Water activity*

A parameter commonly known to measure the amount of water in foods for microbial growth is water activity (a_w). According to Mossel (1995), Gram-negative bacteria is normally more sensitive to reduced a_w than Gram-positive bacteria. RTE food products such as polony or boerewors have a water activity higher or equal to 0.97, favouring the most spoilage organisms including *E. coli*, which require a water activity of 0.96 – 0.97 for growth, as shown in Table 2.4. These specifics make RTE polony a highly perishable product.

Table 2.4 Minimal water activity levels required for growth of food-borne microorganisms at 25 °C (Dilbaghi and Sharma, 2007:6).

Groups of Microorganisms	Water activity
Most Bacteria	0.91 – 0.88
Most Yeasts	0.88
Regular Moulds	0.80
Halophilic Bacteria	0.75
Xerotolerant Moulds	0.71
Xerophilic Moulds and Yeasts	0.62 – 0.60

2.9 Exogenous factors affecting the shelf life of polony

2.9.1 *Packaging material*

The primary purpose of packaging is to protect foodstuffs during the different stages of processing such as distribution, storage and transport from contamination by dirt, microorganisms, toxic substances or loss of sensory quality (Organisation, 2020:1-2). The growth of different pathogenic and spoilage microorganisms will depend on the type of package material used (Siroli et al., 2017:1,9). Different packaging should assist with the prevention of spoilage, weight losses and enhance customer acceptability (Organisation, 2020:3).

2.9.2 *Gaseous environment*

The development of bacteria can be determined by the presence of molecular oxygen (Mathenjwa, 2010:21-24). If oxygen affects different meat products during their storage periods, it will affect the organoleptic analysis of the product. This may result in oxidation and rancidity of fats with undesirable off flavours (Organisation, 2020:1-4).

2.10 Microbial analysis

The most important concept of food microbiology is food safety. To ensure food safety, microbiological testing of food products such as testing for pathogens and spoilage organisms are necessary. Testing will confirm that non-beneficial microorganisms are present in food at acceptable levels (Hindman, 1997:838). There are several concerns regarding food safety, including food poisoning, food spoilage, and food preservation. Food legislation defines the acceptable levels of microorganisms in raw ingredients and the final product.

A review of sampling and microbiological testing undertaken by health service executives indicated that food samples tested for microbial contamination in 2007, where 79.5% were categorised as RTE products, while 20.5% were non- RTE (Committee and Authority, 2011:8). In 2008, RTE samples accounted for 73.9%, with non RTE samples making up the remaining percentage (Committee and Authority, 2011:14-16). This data actively highlights the predominance of ready-to eat products in microbial testing and underscores the higher risk of contamination in RTE products, as they are consumed without further cooking or processing.

Microbial analysis plays a vital role in assessing whether manufacturing control mechanisms prevents contamination effectively. Since microorganisms can survive and grow in RTE foods, routine testing ensures that the product is safe for consumption. In the case of RTE polony models, several microbial tests are performed, including those presented below to guarantee the safety and quality of the product.

2.10.1 *Total viable counts*

The plate count method is used to determine the total number of viable cells present in a sample. In a typical laboratory, the most used method for detecting the presence of a viable cell is to allow it to multiply and form visible colonies (Sanders, 2012:1-16).

2.10.2 *Yeasts and moulds*

Both yeasts and moulds cause various levels of deterioration and decomposition of food products, as they can invade and grow on virtually any type of food at any time. This is particularly problematic in plants that produce high sugar, low water activity or low pH products. Manufacturers that produce fruit products, baked goods, confectionary, and fermented dairy products can be at a high risk of yeast and mould contamination (Betts, 2016:10-11).

2.10.3 *Bacillus cereus*

Members of the genus *Bacillus* are generally Gram-positive, spore-forming rods, but some may display a Gram-negative reaction (Abriouel et al., 2011:201). *Bacillus cereus* is aerobic organism with large vegetative cells. It growth has a temperature range of 8-55°C, optimally around 28 to 35°C. *B. cereus* does not have a tolerance for low pH or a_w (Bottone, 2010:383-393).

2.10.4 *Staphylococcus aureus*

S. aureus are Gram-positive cocci that form spherical to ovoid cells. Staphylococci are catalase positive, oxidative-negative, facultative anaerobes. The bacterium is mesophilic with a growth temperature range between 7 and 48°C and an optimum at 37°C (Conley et al., 2006:1)

2.10.5 *Listeria monocytogenes*

L. monocytogenes is a foodborne pathogen that is responsible for the food related listeriosis. *L. monocytogenes* is difficult to detect with culturing methods and requires an enrichment step for confirmation tests. It is a Gram-positive non-spore-forming bacterium which is ubiquitous in nature and can grow under both aerobic and anaerobic conditions (Kurpas et al., 2018:49).

2.11 A developed preservation method used in the food industry (HACCP)

Hazard analysis and critical control point (HACCP) system (SANS 1330:2020, 2022:1-2), is a food safety management system that is used for the prevention of physical, chemical and microbiological hazards (Caswell and Hooker, 1996:775). HACCP operates by incorporating a systematic, logical, and scientific approach to control product and/or processing hazards. HACCP is a pervasive system, which covers the entirety of the food production process starting from the procurement of the raw materials all the way to the final consumer.

A HACCP plan is not a stand-alone program. It must be part of a large control program (Steps et al., 2018:27). It is a program that can be used to control conditions in a plant environment, which contribute to the overall safety of the product. The plan can help an auditor to identify the important steps during food processing and the safety measures. Some auditors may fail to distinguish between the important and relatively unimportant requirements of food safety. Factors that are critical to food safety maybe overlooked or underestimated due to the wording of the laws, frequently failing to distinguish between factors critical to food safety and those that are of an aesthetic or food quality nature.

The HACCP system is thereafter important in the development of food as it offers benefits to the regulator, processor and consumer safety (Mital, 2020:1-2). Prerequisite programs (SANS 10049:2019:1-4) which include procedures such as the current Good Manufacturing Practices (GMPs) and Standard Sanitation Operation Procedure (SSOP) form part of the HACCP system (Wallace and Williams, 2001:235-238). Prerequisite programs must be developed, implemented, and documented before attempting to put the HACCP system into place. These prerequisite programs provide fundamental environmental and operational conditions required to produce safe food products. HACCP can be implemented in various companies by making use of the following principles (Chavan, 2017:313):

Principle 1: Conduct a hazard analysis

Principle 2: Determine the critical control points (CCPs)

Principle 3: Establish critical limits

Principle 4: Establish monitoring procedures

Principle 5: Establish corrective actions

Principle 6: Establish verification procedures

Principle 7: Establish record-keeping and documentation procedures

The following principles facilitate the development and implementation of an effective HACCP system. It can be applied to each segment of the food industry. In the development of a HACCP plan, six preliminary tasks should be accomplished before the application of the HACCP principles to a specific product and process. The six preliminary tasks are:

1. Assemble the HACCP team
2. Describe the food and its distribution
3. Describe the intended use and consumers of the food
4. Construct a process flow diagram
5. Verify the flow diagram in an on-site verification
6. Conduct a hazard analysis

Previous research determined the impact of an implemented HACCP plan in a retail butcher's premises that produces different RTM meat products. Where a HACCP plan is implemented and followed, it resulted in a good hygienic conditions in the facility (Mathenjwa, 2010:31). The implementation of a HACCP plan could reduce the bacterial counts and specifically pathogenic organisms, thus creating a clean environment to produce different products. Figure 2.10 provides the necessary steps needed to determine the critical control points during the production of RTE polony models ensuring a safe to consume product used in Chapter 4 (Sensory analysis)

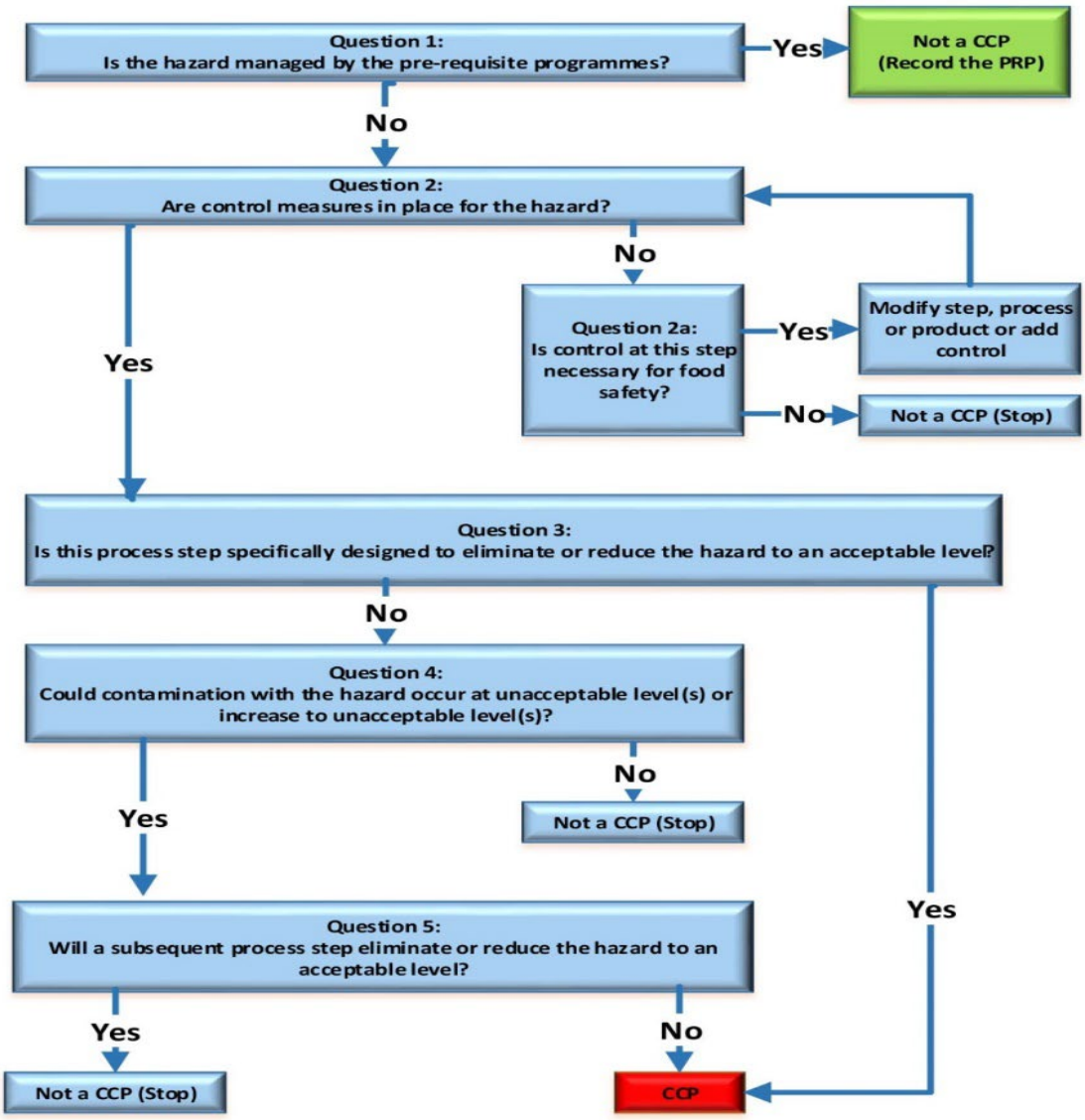

Figure 2.10 A decision diagram to allocate and determine the critical control points (CCPs) (Nenciu et al., 2022:128).

2.12 Conclusions

The role of chitosan and rosemary extract highlights the potential of using natural preservatives to increase food safety, create awareness of the health risks associated with high salt intake and inhibit the growth of *Listeria monocytogenes*. This study encapsulates the importance of exploring innovative natural preservation methods to ensure the safety, health and quality of food products. By utilizing chitosan and rosemary, the food industry will dominate by using natural alternatives to prevent pathogenic outbreaks and reduce health implications.

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CHAPTER 3

THE MICROBIAL QUALITY AND EFFECT OF CHITOSAN AND ROSEMARY EXTRACTS ON *LISTERIA MONOCYTOGENES* IN POLONY MODELS

ABSTRACT

The purpose of the study was to investigate the effect of chitosan and rosemary on the growth and survival of L. monocytogenes in four polony models at two different storage temperatures. The models were P1 (control sample), P2 (chitosan sample), P3 (rosemary sample) and P4 (chitosan and rosemary sample) and the storage temperatures were 4 and 10˚C. The models P2 to P4 were preserved using three different concentration levels of the same preservative; 0.5, 1.0, and 2.0% respectively. Polony model P1 contained NaCl compared to the other three models that were preserved with chitosan and rosemary and combinations thereof. The polony models were spiked with 10^3 log cfu/g of *L. monocytogenes* stored at 4 and 10˚C for 9 days. Dilution series were prepared from each sample and plated onto PALCAM agar, after which the plates were incubated at 37˚C for 48 hours under aerobic conditions. Chitosan and rosemary extracts in the formulations controlled and inhibited the microbial contamination against coliforms, TVC, and yeasts and moulds. The microbial counts indicated less growth on the models preserved with chitosan and rosemary (Models P2 – P3) compared to the control model P1. Both polony models preserved with natural preservatives at the respective concentration levels significantly reduced the growth of *L. monocytogenes* ($P < 0.05$). It was also observed that models stored at 4˚C exhibited slower growth than those stored at 10 ˚C ($P < 0.05$). The growth of *L. monocytogenes* over 9 days displayed similar growth patterns ($P > 0.05$) indicating that *L. monocytogenes* could have adapted to its environment over time. In conclusion, the use of chitosan and rosemary could potentially aid in the prevention and inhibition of *L. monocytogenes* in RTE polony compared to polony models produced with salt (NaCl) of which made the conditions conducive for contamination and growth of *L. monocytogenes*.

3.1 Introduction

Listeria monocytogenes gained recognition as a harmful pathogenic bacterium in South Africa over the last few years (Keet and Rip, 2021:40). An outbreak of listeriosis was identified in South Africa in 2017 (Smith et al., 2019:1). The National Institute of Communicable Disease (NICD) reported 1060 confirmed laboratory cases of listeriosis, including 216 deaths (Tchatchouang et al., 2020:1; National Listeria Incident Management Team, 2018:1). These cases were mostly associated with low to middle class buyers who consumed *L. monocytogenes* contaminated polony (Thomas, 2020:632). Ready to eat (RTE) foods such as polony are often consumed as uncooked foods resulting in a higher risk to public health (Gizaw, 2019:2). The safety and quality of these foods rely greatly on temperature control (Devlieghere et al., 2001:53-55). RTE foods such as polonies are produced with high quantities of sodium (Calliope and Samman, 2020:1). Although sodium serves as a great purpose in food in terms of flavour enhancers, leavening aids and provides nutritional content the detrimental health effects associated with sodium intake cannot be ignored (Henney et al., 2010:69-90).

L. monocytogenes has been isolated from a wide range of environments. The bacteria can spread rapidly from farms and processing facilities to retail stores and consumers, creating various conditions for processed foods to become contaminated (Doron, 2020:273; Zhu et al., 2017:6). The ability of the pathogen to grow at refrigeration temperatures (0 to 4 ˚C) and tolerance to sodium content (2 to 5 %), impacts the persistence and prevalence of this pathogenic bacterium (Magalhães et al., 2014:450-456). Food contaminated with salt tolerant and resistant *L. monocytogenes* can lead to listeriosis (Harper and Getty, 2012:1). RTE polonies with high sodium levels could potentially elevate and enable the growth and survival of the salt tolerant and salt resistant bacteria (Aalto-araneda, 2020:19).

Natural preservatives such as chitosan [(1, 4)-2-Amino-2-deoxy-β-D-glucan] and rosemary (*Rosmarinus officinalis* L.) extract could potentially reduce or inhibit the growth of *L. monocytogenes* (Tiwari et al., 2009:10; Al-Hijazeen and Al-Rawashdeh, 2019:27). According to Soultos et al. (2008:3) a concentration level of 1.0 % (w/w) chitosan was found to be most effective during experiments on boerewors, where chitosan was used in the formulation. Kim (2012:1-2) suggested that a concentration level of 0.026 % (w/w) rosemary, which is equivalent to 260 g/Kg, is an effective antimicrobial and antioxidant agent. Furthermore, the use of natural preservatives

could reduce health implications of sodium-based products such as hypertension, kidney damage, osteoporosis, headaches and chances of diabetes (Yu et al., 2021:2).

The control of the growth and survival of *L. monocytogenes* will help manage and minimize contamination in the food industry (Ireland, 2005:7-8). In addition, understanding the microbial carrying capacity is a crucial part of managing microbial populations and characterising the growth of *L. monocytogenes* (Marik et al., 2020:108). Chitosan and rosemary extract have the potential to act and serve as a natural preservative to reduce growth and survival of *L. monocytogenes* in RTE polonies (Saraiva et al., 2021:1-2; Culliney and Schmalenberger, 2020:1). Therefore, the present study aimed to evaluate and monitor the microbial quality, and the growth and survival of *L. monocytogenes* in polony models preserved with chitosan and rosemary extract stored at 4˚C and 10˚C.

3.2 Materials and methods

3.2.1 Source of materials

Chitosan powder (CAS-No: 9012-76-4) used in this study for the production of polony models was purchased from Sigma-Aldrich, Johannesburg, South Africa. All culture media were purchased from Science World, Cape Town, South Africa. The ingredients used for the formulation of polony models were sampled from retail stores based in Cape Town. The chemicals used in this study were of analytical grade. Frazer selective supplements and Palcam selective supplements were purchased from Anatech Instruments, Johannesburg, South Africa. The equipment used in this study was obtained from the Department of Food Science and Technology based in Bellville at Cape Peninsula University of Technology, South Africa.

3.2.2 Preparation of (*Rosmarinus officinalis L.)* Plant material

Rosemary (*Rosmarinus officinalis L.)* was sampled from a local supermarket in Cape Town, South Africa. Rosemary leaf samples were weighed, washed with distilled water and dried at 50 ˚C for 12 h (Cabinet drier, Model 1069616). The samples were trimmed, dried and ground into a fine powder using a laboratory blender (Waring Commercial, Model HGBTWTS3). The rosemary powder was filled into concealed plastic containers and stored at room temperature until use.

3.2.3 Production of polony models

The experimental design used in this study is shown in Figure 3.1. Four ready to eat (RTE) polony models were produced at different concentration levels of chitosan and rosemary, namely Model P1, a control which contained 1.15 mg per 100 g NaCl; Models P2, P3 and P4 were produced at (Concentration 1) 10 g/Kg chitosan and 0.26 g/Kg rosemary, (Concentration 2) at 20 g/Kg chitosan and 0.52 g/Kg rosemary and (Concentration 3) at 5 g/Kg chitosan and a 0.13 g/Kg rosemary (Table 3.1). Figure 3.2 indicates the polony models P1-P4 produced in this study. Each of the three concentration levels was prepared in triplicate. The methods used to produce the models are described in the following sections:

1. Production of polony without natural preservatives (Model P1)

Beef rind was bowl chopped at a low speed for 10 minutes until a 3 mm particle size was reached (Advisory, 2016:3). Ice was then added to form a slurry. Once the slurry was formed, soy and $NaHCO_3$ was added and bowl chopped (Freddy Hirsch, Model A-20) until a temperature of 15 ^oC was reached. The meat components, ice and phosphate were then added, and bowl chopped until the polony mixture was well blended. The remaining ingredients including 1150mg per 100g NaCl were then added and bowl chopped at a high speed to the final temperature of 14 ^oC. The emulsion was then filled speedily into polony casings (Freddy Hirsch, Cape Town, South Africa), preventing emulsion breakdown due to protein denaturation. The polony was then heat processed in a hot bath at a temperature of 68 – 72 ^oC for 30 minutes. The temperature of the water was monitored to stay below 75 ^oC. The product was cooled under running water to 10 ^oC. After production, Model P1 was stored at 0 ^oC in a refrigerator until further analysis.

2. Production of polony preserved with Chitosan (Model P2)

Liquid of fresh beetroot was extracted and kept aside at 20 ^oC until use. This was necessary to avoid using sodium nitrite/nitrate for the development of the pink colour since these salts also have antimicrobial effects. Ten grammes of granulated chitosan powder (Merck Life Science, CAS-No: 9012-76-4) were added to 300 ml of water, stirred well, and set aside until use. Rind was then bowl chopped (Freddy Hirsch, Model A-20) at low speed for 10 minutes until a 3 mm particle size was reached. A 300 ml aliquot of chitosan solution and 2 ml beetroot liquid extract were then added to the bowl chopper to form a slurry. Meat components and ice were added and bowl chopped until

a well-blended mixture was formed. The remaining ingredients were added and bowl chopped at a high speed until a final temperature of 14 ^{o}C was reached. The emulsion was filled into polony casings (Freddy Hirsch, Cape Town, South Africa) and heat processed as described above in section 3.2.3.1.

3. Production of polony preserved with rosemary (Model P3)

An amount of 0.26 g of rosemary powder (Pick n Pay supermarket) was added to 300 ml of water, stirred well and set aside until use. Rind was then bowl chopped (Freddy Hirsch, Model A-20) at low speed for 10 minutes until a 3 mm particle size was reached. Rosemary solution and beetroot liquid extract was then added to the bowl chopper to form a slurry. Meat components and ice were then added and bowl chopped until a well-blended mixture was formed. The remaining ingredients were added and bowl chopped at a high speed until a final temperature of 14 ^{o}C is reached. The emulsion was filled into polony casings (Freddy Hirsch, Cape Town, South Africa) and heat processed as described above in section 3.2.3.1.

4. Production of polony preserved with a combination of chitosan and rosemary (Model P4)

Ten grammes chitosan and 0.26 g/kg of rosemary powder were added to 300 ml of water, stirred well and set aside until use. Rind was bowl chopped (Freddy Hirsch, Model A-20) at a low speed for 10 minutes until a 3 mm particle size were reached. 300 ml chitosan and rosemary solution and beetroot liquid extract was added to the bowl chopper to form a slurry. Meat components and ice were added and bowl chopped until a well-blended mixture was formed. The remaining ingredients were added and bowl chopped at a high speed until a final temperature of 14 ^{o}C was reached. The emulsion was then filled into polony casings (Freddy Hirsch, Cape Town, South Africa) and heat processed as described above in a hot bath at a temperature of 68 – 72 ^{o}C for 30 minutes.

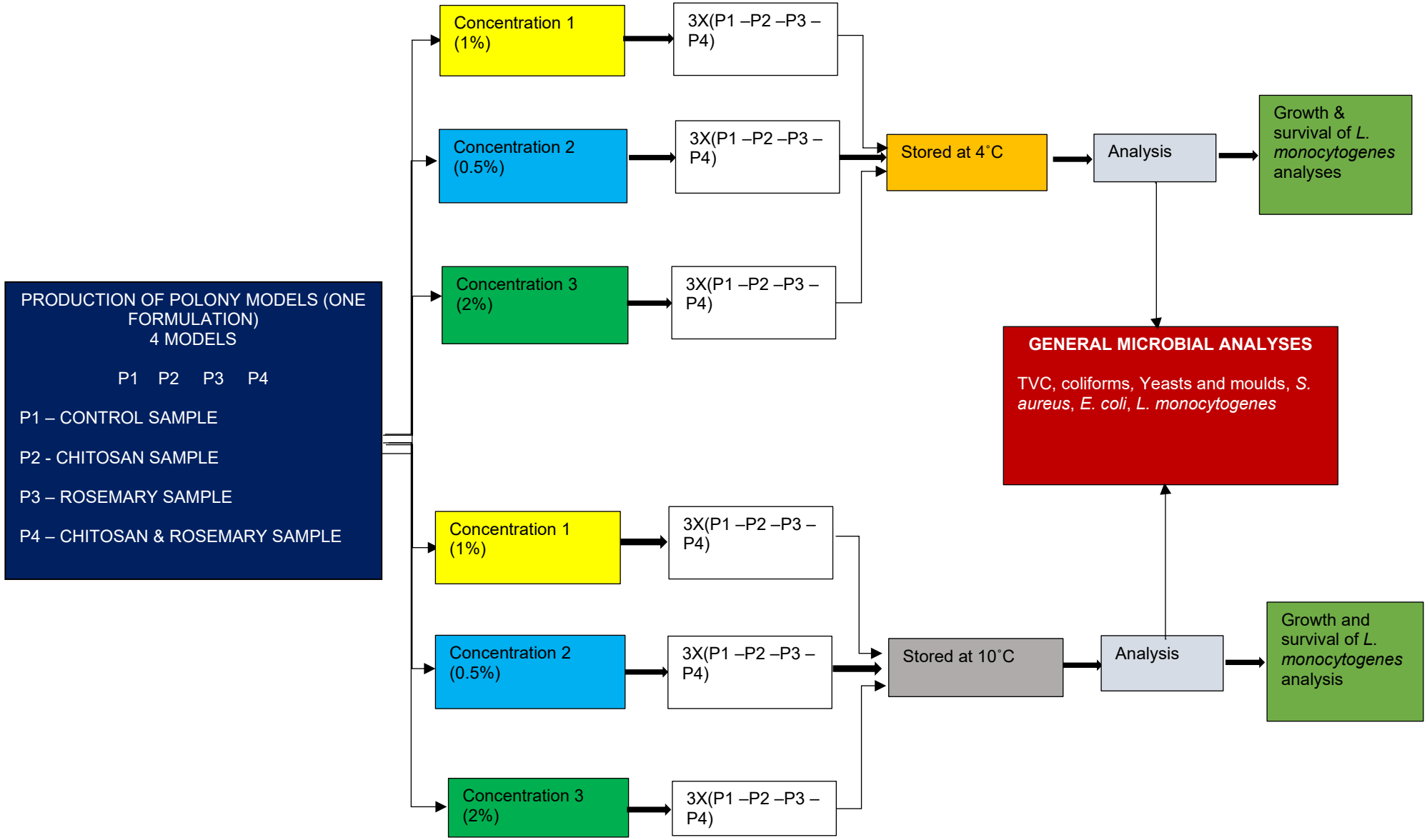
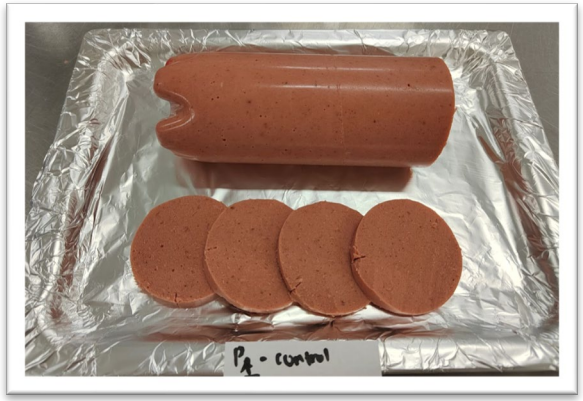


Figure 3.1 Experimental design for this study

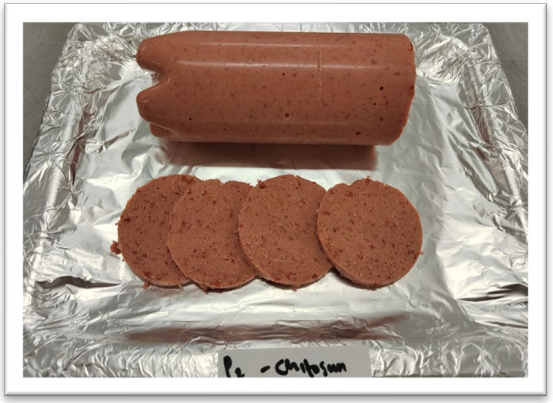
Table 3.1 Concentration levels of natural preservatives (Chitosan and Rosemary) used to preserve polony models

Concentration level	Model P1[1] Control	Model P2[2] Chitosan	Model P3[3] Rosemary	Model P4[4] Rosemary + Chitosan
Concentration 1	0.00 g/Kg	10 g/kg	0.26 g/kg	0.26 g/kg + 10 g/kg
Concentration 2	0.00 g/Kg	20 g/kg	0.52 g/kg	0.52 g/kg +20 g/kg
Concentration 3	0.00 g/Kg	5 g/kg	0.13 g/kg	0.13 g/kg + 5 g/kg

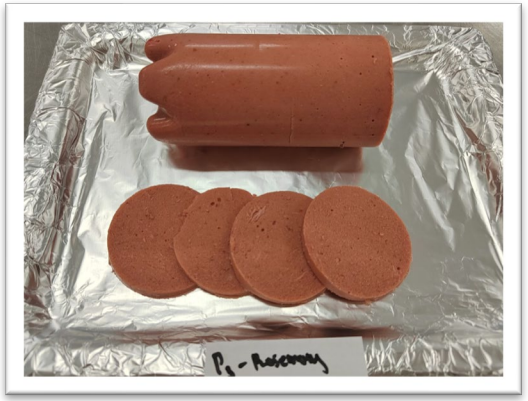
[1] P_1 – control sample (adding of salt)
[2] P_2 – Chitosan sample (Removal of salt, adding of natural preservative),
[3] P_3 – Rosemary sample (Removal of salt, adding of natural preservative)
[4] P_4 – Chitosan + Rosemary (Removal of salt, adding of both natural preservatives)



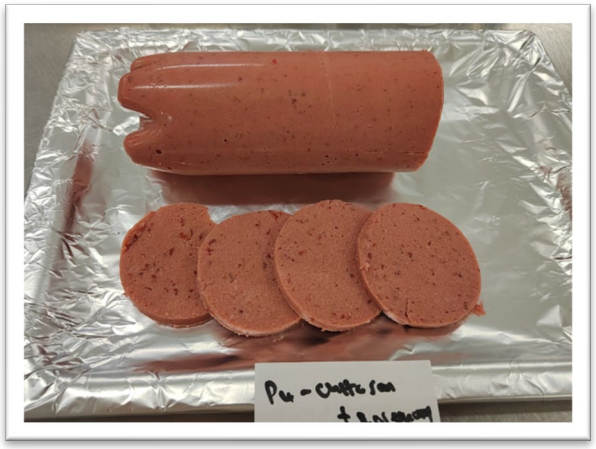
P 1



P 2



P3



P 4

Figure 3.2 Polony models P1-P4 produced in this study. P1 = control, P2 = chitosan, P3 = rosemary, P4 = chitosan and rosemary

3.3 Microbiology

Microbiological analysis was conducted to observe and monitor the bacterial growth in four polony models preserved with chitosan and rosemary extract over a period of five days at three different concentration levels. Microbial analysis included the presence and enumeration of colony forming units (cfu) of total coliforms, total viable counts (TVC), *S. aureus*, *E. coli*, *L. monocytogenes* and yeasts and moulds. Four separate polony samples, representing the four models P1-P4, were tested on days 0, 3, and 5 after production was completed.

3.4 Procedure for microbial analysis of uninoculated polony models

Uninoculated polony model samples were analysed for total coliforms (Division and Bag, 2005:1-5), TVC (Africa, 2007:1-8), yeasts and moulds (SANS 7954:1987, 2002:1-8), S*. aureus* (Standardization, 1999:2), *E. coli* (SANS, 2005:9) and *L. monocytogenes* (ISO11290-2, 1998:1). The presence or absence of these microorganisms was investigated by using 10 g of the sample mixed with 90 ml of sterile Ringer's solution placed in a sterile stomacher bag. The stomacher bag was then placed into a stomacher machine (Seward stomacher 1, model BA7020) for 2 minutes. This allowed for the homogenisation of the product.

The homogenised samples were serially diluted up to 10^{-4}. One ml of each dilution was pour-plated into respectively labelled Petri dishes. (Beyaz, 2014:194).

3.4.1 *Enumeration of total coliform*

One millilitre was pour-plated into respectively labelled Petri dishes with violet red bile agar (VRBA, Science World: C-MILB031-A) into triplicate sterile Petri dishes (Preparation et al., 2020:3). A thin layer of VRBA (Table 3.2) was added to the Petri dish. The plates were then swirled gently to mix with the dilution with the molten agar. The agar was allowed to set and the plates were incubated at 37 ˚C for 24 h (Leclercq et al., 2002:1631). The number of pink colonies, representing coliform growth, were counted and recorded.

3.4.2 *Detection of Escherichia coli*

One millilitre was pipetted into a correspondingly labelled petri dish and poured with violet red bile agar (VRBA, Science World: C-MILB031-A) in triplicate. (Arana et al.,

2013:2). A thin layer of VRBA (Table 3.2) was added to the Petri dishes. The agar was then allowed to solidify and incubated at 37 ˚C for 24 h (Leclercq et al., 2002:1631). The number of colonies were countered as per coliform count respectively. Thereafter the dishes were placed under an ultra violet lamp (UVL) to monitor florescent growth, which indicated the presence of *E. coli* (Wang et al., 2017:1).

3.4.3 *Detection of Total Viable Counts (TVC)*

From each dilution, 1 ml was pipetted into sterile Petri dishes in triplicate and covered with plate count agar (PCA, Science world: MISC001) (Table 3.2) (Arana et al., 2013:2). The agar was allowed to solidify at room temperature. The dishes were inverted and incubated at a temperature of 25 ˚C for 3 days (Bogomolny et al., 2013:4114). After 3 days, the number of colonies that were formed were counted and recorded.

3.4.4 *Detection of Staphylococcus aureus*

Detection of *S. aureus* was conducted by using the pour plate method. From each dilution, 1 ml was pipetted into sterile Petri dishes and poured with a thin layer of mannitol salt agar (MSA, Science world: MISC001) (Table 3.2) in triplicate (Moraes et al., 2021:9). The dishes were incubated at 37 ˚C for 24 h. After 24 h, the number of colonies that were formed was counted and recorded. *S. aureus* colonies were typically 1.0 -1.5 mm in diameter. They were black and shiny in colour, convex in shape with a white margin surrounded by clear zones (Harris et al., 2002:39).

3.4.5 *Detection of Yeasts and moulds*

From each dilution, 1 ml was pipetted into sterile Petri dishes and poured with a thin layer of potato dextrose agar (PDA, Science world: MISC001) (Table 3.2) in triplicate. The dishes were incubated at 25 ˚C for 3 days (Anonymous, 2022:40). After the incubation period, the number of colonies were counted and recorded (Fung, 2014:395).

3.4.6 *Detection of Listeria monocytogenes*

Four steps were involved in the detection of *L. monocytogenes*: pre-enrichment in selective liquid media, secondary enrichment, selective plating, and confirmation (Gasanov et al., 2005:853-855). For the pre-enrichment of *L. monocytogenes*, 25 g of

the food sample was weighed into a stomacher bag mixed with 225 mL half Fraser broth. The sample was incubated for 24 h at 30 ˚C. The second step involved a secondary enrichment step. 0.1 mL of the pre-enriched sample were transferred into a tube that contained 10 ml of Fraser broth. The inoculated Fraser broth was incubated at 37 ˚C for 24 h. After 24 h of incubation, the culture obtained was then used to streak onto PALCAM (PALCAM Listeria selective agar base. Anatech: FELAP20500) agar plates. These plates were incubated at 4˚C and 10˚C for 24 h. After incubation, a confirmatory test was conducted by performing Gram-staining to confirm the presence of *L. monocytogenes* (ISO, 1998:11290).

Table 3.2 Types of culture media used to isolate and identify microorganisms in the microbial analysis

Microorganism	Culture medium	Reference
Total viable count	Plate count agar	(Petersen and Mclaughlin, 2016:19-44)
Yeasts and moulds	Potato dextrose agar	(Petersen and Mclaughlin, 2016:19-44)
Escherichia coli	Violet red bile agar	(Petersen and Mclaughlin, 2016:19-44)
Staphylococcus aureus	Mannitol salt agar	(Petersen and Mclaughlin, 2016:19-44)
Total coliforms	Violet red bile agar	(Petersen and Mclaughlin, 2016:19-44)
Listeria monocytogenes	Palcam agar	(Petersen and Mclaughlin, 2016:19-44)

3.5 Growth and survival of *Listeria monocytogenes* in RTE polony models preserved with chitosan and rosemary extract

3.5.1 *Preparation of L. monocytogenes inoculum*

L. monocytogenes stock culture was activated by mixing with 1 ml Ringer's solution (Ringers, Science world: C-BR00052). The mixture was then transferred into 10 ml Frazer broth (Frazer broth base, Science world: MISC001) and incubated at 30 ˚C for 48 h. After 48 h, the broth changed colour from light brown to black indicating the presence of *L. monocytogenes*. From the broth, a loopful was streaked out onto Palcam agar plates (Thiel, 1999:1). The Palcam agar plates were incubated at 30 ˚C for 48 h (Figure 3.3). After incubation, colonies that displayed typical characteristics of *L. monocytogenes*, such as grey-green colonies with black halos, were counted to determine the colony forming units (CFUs). Serial dilutions of the culture were

performed prior plating to ensure that countable numbers of colonies were obtained, enabling accurate enumeration of the bacterial population in the original sample.

3.5.2 *Inoculation of RTE polony models P1-P4 with L. monocytogenes*

An aliquot of 2.5 ml of *L. monocytogenes* inoculum were artificially injected into 25 g polony samples, to a concentration of 1 000 CFU/g, in a bio-safety laminar flow cabinet. A total of 96 samples were injected on day 0. This included the four polony models (P1-P4) at three the different concentrations. 48 of the injected samples were stored at 4 ˚C and the other 48 samples at 10 ˚C. The samples were monitored and observed for the presence of *L. monocytogenes* on days 0, 3, 6, 9 of storage (Figure 3.3).

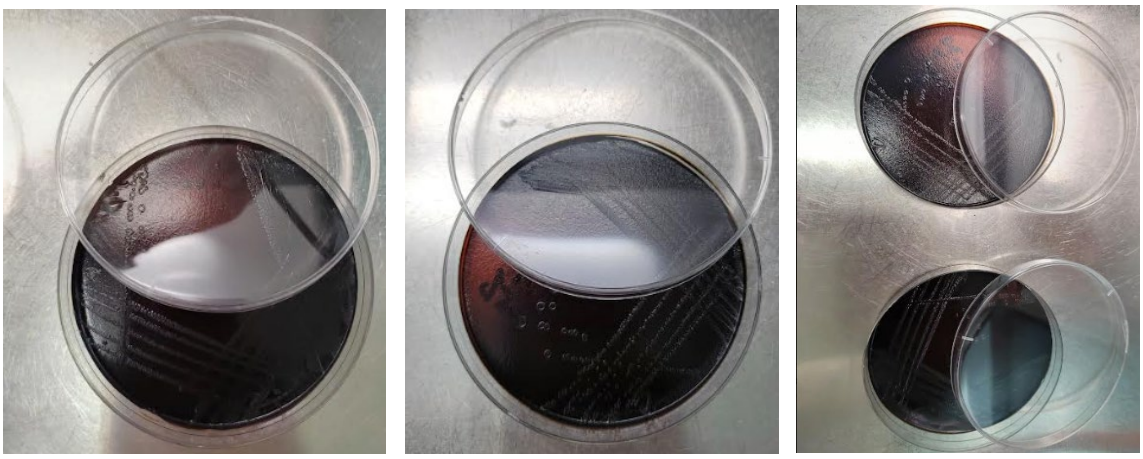


Figure 3.3 Presumptive presence of *L. monocytogenes* inoculum on Palcam agar used in this study

3.5.3 *Microbial analysis of inoculated L. monocytogenes polony models P1-P4*

To determine the growth of *L. monocytogenes* cells in polony models P1 to P4, an injected polony sample was removed from storage and transferred into a stomacher bag (Merck, South Africa) that contained 222.5 ml of Frazer broth. The sample was homogenised for 2 minutes using a stomacher machine (Seward stomacher1, model BA7020). From the homogenised sample, 1 ml was used to prepare a dilution series. 0.1% of each dilution was spread plated onto Palcam selective agar in duplicate. The Palcam plates were incubated at 37 ˚C for 48 h. The presumptive *L. monocytogenes* colonies were counted and recorded.

3.6 Statistical analysis

All microbial counts obtained for the different polony models were converted to log cfu/g and subjected to Analysis of Variance (ANOVA) using the statistical package SPSS, 2023 to determine significant differences between treatments (IBM – SPSS, 2023). The microbial quality of polony models in terms of different concentration levels, time (over nine days), polony models and different temperatures were compared by means of the Tukey HSD (honestly significant difference) test (Bland and Altman., 1995:304). The microbial quality of polony models stored at 4 ˚C and 10 ˚C were statistically compared using an Independent Sample Test (two-sample t-test) on microbial counts converted to log cfu/g. The mean difference was significant at the 0.05 level (IBM – SPSS, 2023).

3.7 Results and discussion

3.7.1 *Microbial quality of the polony models P1-P4*

As part of the production of the ready-to-eat meats, the four polony models were subjected to different microbial tests including total coliform count, total viable counts (TVC), *S. aureus*, *E. coli*, *L. monocytogenes* (not detected), and Yeasts and moulds counts. The polonies underwent microbiological tests to ensure that their quality was acceptable. The mean microbial counts for all four polony models in this study, are shown in Table 3.3. All polony models (P1-P4) were within their respective microbial quality limits and conformed to specifications.

Table 3.3 The mean microbial quality of polony models (P1-P4) preserved with Chitosan and Rosemary

	Average microbial counts (log cfu/g)[a]			
Microbial Tests	Control (P1)	Chitosan (P2)	Rosemary (P3)	Chit & Ros (P4)
Coliforms	1.14	-	-	1.45
TVC	1.53	1.46	1.50	1.46
S. aureus	-	-	-	-
E. coli	-	-	-	-

L. monocytogenes	ND	ND	ND	ND
Yeasts and Moulds	1.04	-	1.34	1.23

TVC= Total viable counts; Zero colonies noted as (-); a = Values represent the means of triplicate determinations.

1. Coliforms count

Coliforms are categorised as indicator organisms which are used as hygiene indicator organisms. The mean coliform count for P1 was 1.14 log cfu/g (Table 3.3), whereas P2 and P3 indicated no growth. The chitosan and rosemary model (P4) showed a higher mean count (1.45 log cfu/g) compared to P1 however, it was still within the specified limits. As per the International Commission of Microbiology Specification of Foods (ICMSF) guidelines the polony Models P1 and P4's coliform count were within the acceptable levels (<100 log cfu/g) (Kumari et al., 2019:204).

2. Total Viable counts

The mean TVC for all four models ranged between 1.46 to 1.53 log cfu/g where the maximum concentration of TVC was 1.53 log cfu/g for P1 and 1.46 log cfu/g for P4. The accepted level for TVC for ready to eat meat products is 10^3 - < 10^5 indicating that TVC was well within the acceptable limit for RTE meat products (Sin-bin et al., 2014:7).

3. Staphylococcus aureus

No growth for *S. aureus* was observed in any of the polony models (Table 3.3). The absence of *S. aureus* indicated safe food handling and processing practices and precise preventative measures were put in place to inhibit growth of microbes, while production of RTE polony models and microbial testing occurred (Kadariya et al., 2014). According to Thwala, Madoroba, Basson and Butaye (2021), *S. aureus* could potentially cause a wide range of infections (Thwala et al., 2021:1). Therefore, it is crucial to handle and store food correctly and follow food safety guidelines to reduce the risk of foodborne illnesses (Thobaben, 2010:534).

4. E. coli

The mean count per dilution cfu/g for *E. coli* was 0 cfu/g. As a result of the clean VRBA plates shown by the microbial count for P1 to P4, no *E. coli* colonies were present as reported in Table 3.3. Since there are no structured standards for meat products from South Africa, the SANS guidelines, Australian recommendations, NICD and ICMSF were all followed to ensure that all microbial counts were within specification. According to the food standards mentioned, the satisfactory criteria for *E. coli* in RTE foods is a level of <3 cfu/g (Fasnz, 2001:3-6). *E. coli* counts exceeding 100 cfu/g are considered unacceptable (Yohans et al., 2022:301). *E. coli* causes a number of symptoms, such as diarrhoea, abdominal pain and vomiting (Yohans et al., 2022:300). In extreme circumstances, renal failure and death may result (Williams et al., 2014:257). To avoid contamination and a lower chance of contracting a foodborne disease, it is crucial to keep *E. coli* levels in RTE foods within the established limits (Bintsis, 2017:238). In this study, polony models P1, P2, P3 and P4 were well within acceptable limits (Tables 3.3 to 3.6).

5. Yeasts and moulds

Table 3.3 indicate the average count of yeasts and moulds ranged from 1.04 log cfu/g to 1.34 log cfu/g. The acceptable level of yeast and moulds in RTE meat products ranged between 4.0 x 10 – 1.71 x 10^4 log cfu/g (Mousa et al., 2014:83-85). The levels of yeast and moulds in polony Model P1 to P4 indicated that there was little to no chance for spoilage. The low-level microbial count on the polony models indicated a safe to consume product preserving good quality polony.

3.7.2 Growth and survival of L. monocytogenes in polony models preserved with chitosan and rosemary extract

The effects of chitosan and rosemary preservation on growth and survival (G&S) of *L. monocytogenes* in polony models were investigated during this study. Time, temperature, concentration, and the produced polony models (P1, P2, P3 and P4) are recognised to be important parameters that can affect the G&S of the pathogen. During this study the four polony models were produced at three preservative concentration levels, and stored at 4 ˚C and 10 ˚C for nine days, a period used to stimulate shelf life conditions for RTE products

1. Polony models

The average count of RTE polony models (P1, P2, P3 and P4) contaminated with the pathogen *L. monocytogenes* ranged from 2.02 log cfu/g to 2.23 log cfu/g (Figure 3.4). There was growth in all four polony models, as expected. Following incubation (30 ˚C for 48 h) a significant difference ($p < 0.05$) was noticeable between P1 and the models preserved with chitosan and rosemary. The growth of *L. monocytogenes* in the control sample was significantly higher ($p < 0.05$) compared to the models produced with natural preservatives. The control sample (P1), which was produced using salt (NaCl) and its current batch pack used in normal RTE polonies, exhibited 2.23 log cfu/g of *L. monocytogenes*. Matle et al. (2020) reported that *Listeria* can endure NaCl up to 20% (w/v). Additionally, high salt levels could potentially increase the risk of a listeriosis outbreak due to its halotolerant behaviour and unique adaptability (Osek et al., 2022:5-13). Producing RTE food products with natural preservatives could have a positive effect in preventing growth of *L. monocytogenes*. The *L. monocytogenes* colony counts in model P2 was significantly lower than P1 ($p < 0.05$), while P2 and P4 did not differ significantly ($p > 0.05$) (Figure 3.4).

The difference ($p < 0.05$) between Models P1 and P2 indicated that the natural preservatives used, do in fact, contribute to less growth of *L. monocytogenes,* as hypothesized in this study. According to a study done by Kim and Kim (2007:273), a natural preservative such as chitosan could potentially be used to inhibit the growth of pathogenic bacteria (Kim and Kim, 2007:273). No significant difference ($p > 0.05$) in growth inhibition was observed between polony models produced with chitosan and rosemary extract. The observations made indicate that added salt could potentially increase the growth of *L*. *monocytogenes*, whereas producing RTE polony with natural preservatives such as chitosan and rosemary decrease the growth of *L. monocytogenes.* El-Zehery et al. (2022:2582) suggested that chitosan and nano-chitosan have potential antimicrobial properties against the pathogenic bacteria. El-Zehery et al. (2022:2582) also found chitosan to be effective with other strains of *Listeria*. This increases the potential to use chitosan as a natural preservative against *L. monocytogenes* and other foodborne pathogens (El-Zehery et al., 2022:2582). Figure 3.4 also indicates a slight increase in the growth of *L. monocytogenes* from P2 to P3, although there was no significant difference between the natural preservatives, the decrease in growth from P1 to P3 indicated a significant difference ($p < 0.05$). A study done by Youssef et al. (2022:73), suggested that the use of rosemary led to a

lesser microbial count compared to untreated samples and showed signs of inhibition of microbial growth of the pathogen by 10^3 log cfu/g (Youssef et al., 2022:72-74).

In conclusion, Figure 3.4 summarised that all polony models automatically showed growth since a 10^3 log cfu/g was injected into each model, with the highest counts in P1, averaging 2.23 log cfu/g. In contrast, the models preserved with chitosan and rosemary showed significantly lower growth rates ($p < 0.05$). Specifically, the *L. monocytogenes* counts in P2 were significantly lower than in P1, though P2 and P4 were not significantly different ($p > 0.05$). The higher growth level indicated that P1 may be less effective in inhibiting *L. monocytogenes* compared natural preservatives used in this study.

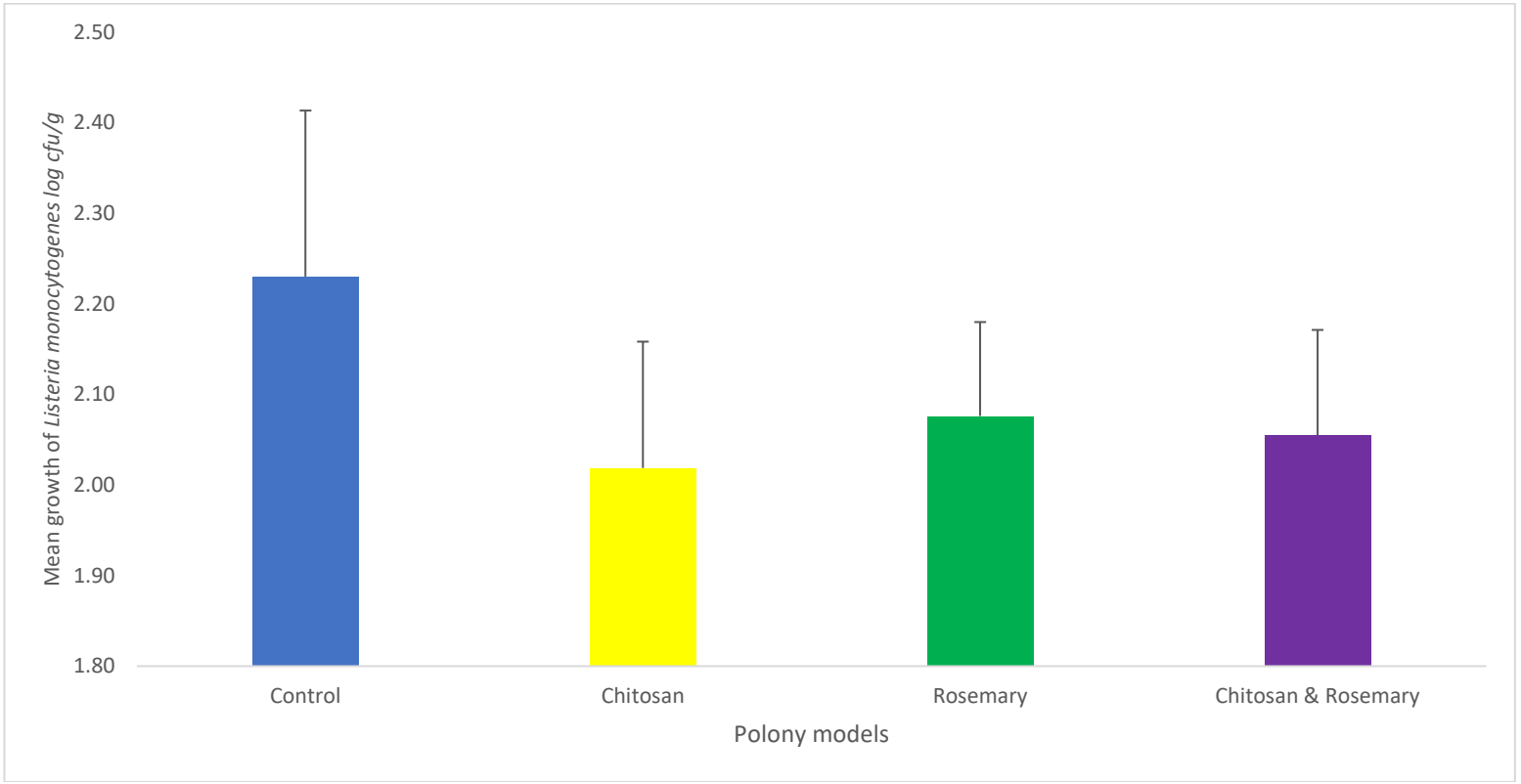


Figure 3.4 Growth of *L. monocytogenes* after 9 days incubation at 4 ˚C in RTE polony models preserved with natural preservatives

2. Concentration of natural preservatives

Concentration in this study refers to the level of natural preservatives used in the polony models. Natural preservatives such as chitosan and rosemary extract have the potential to inhibit the growth of *L. monocytogenes*, and higher concentrations of these preservatives may ultimately have a higher inhibitory effect (Mei et al., 2019:1-12). However, it is important to note that their maybe a limit of effectiveness at high concentrations and the over use of preservatives may negatively impact food quality, safety and organoleptic qualities (Musyoka et al., 2018:1-2). Therefore, it is important to find a balance between the growth of the pathogen, maintaining the quality of the product and finding a concentration that is most effective.

Concentration levels of the four polony Models artificially injected with *L. monocytogenes* are summarised in Figure 3.5. Colony counts obtained with the models ranged between 1.97 log cfu/g to 2.24 log cfu/g. P1 with 0.5% g/Kg concentration level was not significantly different ($p > 0.05$) to that of P1 with a 1.0% g/Kg concentration level and P1 with a 2.0% g/Kg concentration level. Figure 3.5 clearly indicated a higher growth level of *L. monocytogenes* with P1 at 0.5% g/Kg concentration and 2.0% g/Kg concentration. P2 with a 0.5 g/Kg concentration level shared the same trend. A significant difference ($p < 0.05$) occurred compared to P2 with a 1.0% g/Kg concentration level and that of P2 with a 1.0% g/Kg concentration level. According to studies done by Soultas et al. (2008) and Kim (2012) a concentration level of 1.0% w/w chitosan and a 0.26 g/kg rosemary was found to be most effective and obtained effective antimicrobial and antioxidant properties (Soultos et al., 2008:1150; Kim, 2012:12330). It is evident that polony models (P1) produced with salt (NaCl) increases the risk of growth of *L. monocytogenes* compared to those models produced with natural preservatives.

Concentration level P3 (0.5 g/Kg) and P3 (2.0% g/Kg) was significantly different ($p < 0.05$) with added rosemary than P3 (1.0% g/Kg). There was no significant difference ($p > 0.05$). With P3 at 1.0% g/Kg concentration level. Similarly, to the findings of Kim (2012:273), rosemary shows less growth of *L. monocytogenes* between concentration levels 0.5% and 1.0% g/Kg with growth of 2.06 and 2.06 log cfu/g compared to that of a 2.0% g/Kg concentration level with a growth rate of 2.14 log cfu/g. Chitosan and rosemary extract both displayed its potential for their antimicrobial properties, particularly in inhibiting the growth of *L. monocytogenes*. During this study the synergistic effect was adopted, ultimately to observe how effective

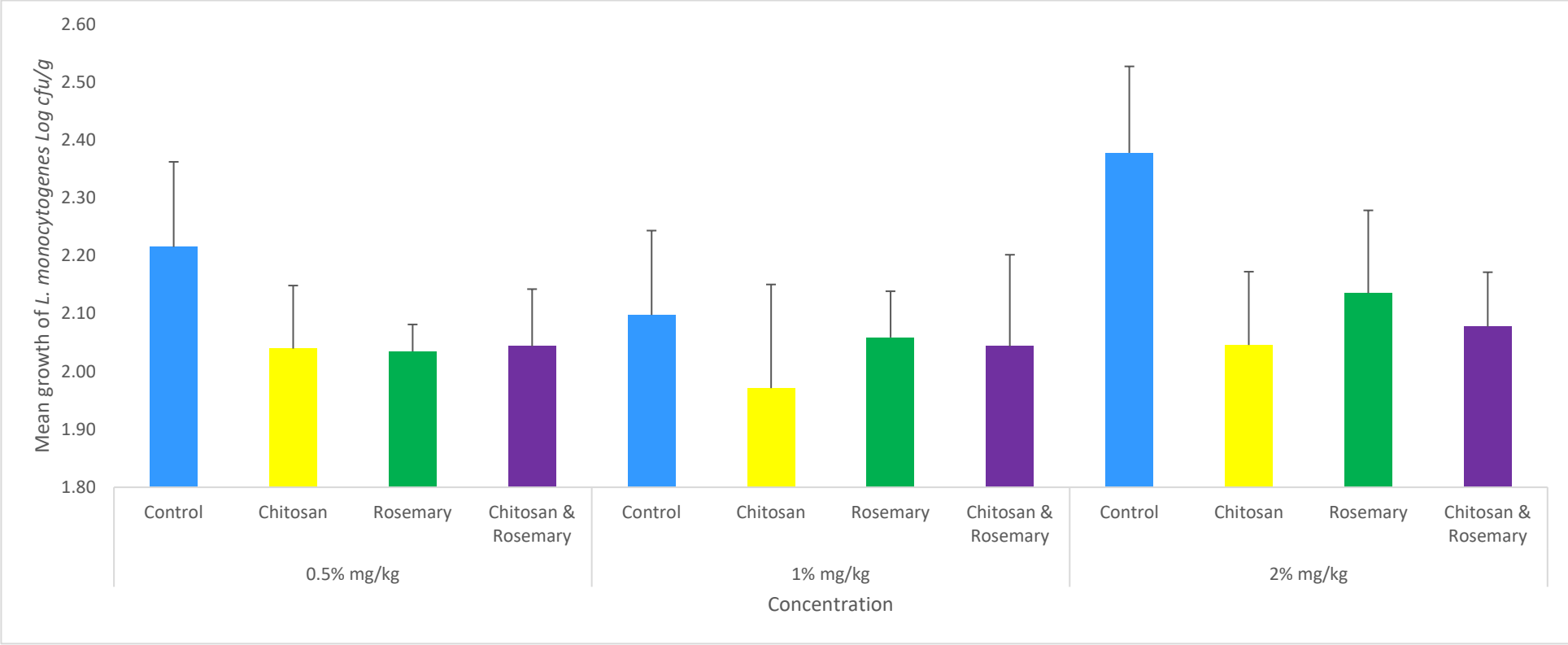


Figure 3.5 Growth of *Listeria monocytogenes* following inoculation into RTE polony models containing natural preservatives at three concentration levels

(antimicrobial activity) chitosan and rosemary will react when combined (El-Zehery et al., 2022:2583-2584).

In Figure 3.5, P4 (Chitosan and rosemary) at all concentration levels indicated no significant difference ($p > 0.05$) with the other polony models and its concentration levels. In fact P4 (0.5 g/Kg) and P4 (1.0 g/Kg) indicated the same growth of 2.04 log cfu/g and a slight increase in growth of 2.08 at P_4 (2.0 g/Kg). Although there is no significant ($p > 0.05$) difference in colony counts regarding Model P4 at three different concentration levels, it is evident that *L. monocytogenes* grows slower at 1.0 g/Kg concentrations level in polony models P1, P2 and P4.

3. Storage temperatures

The four polony models artificially injected with *L. monocytogenes* were analysed for growth of the pathogen stored at 4 and 10 ˚C for nine days (Figure 3.6). The mean colony count of *L. monocytogenes* in polony models stored at different temperatures ranged from 1.99 log cfu/g to 2.30 log cfu/g. Storage temperatures 4 and 10 ˚C significantly ($p < 0.05$) influenced the growth of *L. monocytogenes* in polony models produced with chitosan and rosemary extract. The growth of *L. monocytogenes* can be explained by the findings of Szczawinski et al. (2017) who described the colony counts of *L. monocytogenes* in RTE ham stored at 15 ˚C to be higher than storage at 3-6 ˚C. The results found in the study showed that storage temperature had a statistical significant influence on the growth of *L. monocytogenes* (Szczawiński et al., 2017:45). P1 stored at 4 ˚C was significantly ($p < 0.05$) lower than P1 stored at 10 ˚C. For P2 stored 4 ˚C, there was a significant ($p < 0.05$) difference compared to that of P2 stored at 4 ˚C. P3 samples ranged between 2.04 and 2.11 log cfu/g. For the rosemary sample, there was a significant ($p < 0.05$) difference amongst P3 stored at both 4 and 10 ˚C. The one way analysis of variance between the 4 and 10 ˚C had a statistical significant ($p < 0.05$) influence in growth of *L. monocytogenes* between the groups and within the groups. The Tukey HSD test presented a significant difference ($p < 0.05$) during the multiple comparison test within the samples stored at 4 and 10 ˚C.

During this study, the overall conclusion drawn after storage at 4 ˚C exhibited slower growth than that of polony models stored at 10 ˚C, as hypothesised. The difference between these temperatures (4 and 10 ˚C) lies in the use of natural preservatives and rate of bacterial growth. However, natural preservatives and storage temperatures are not the only key factors needed to inhibit the growth of *L.*

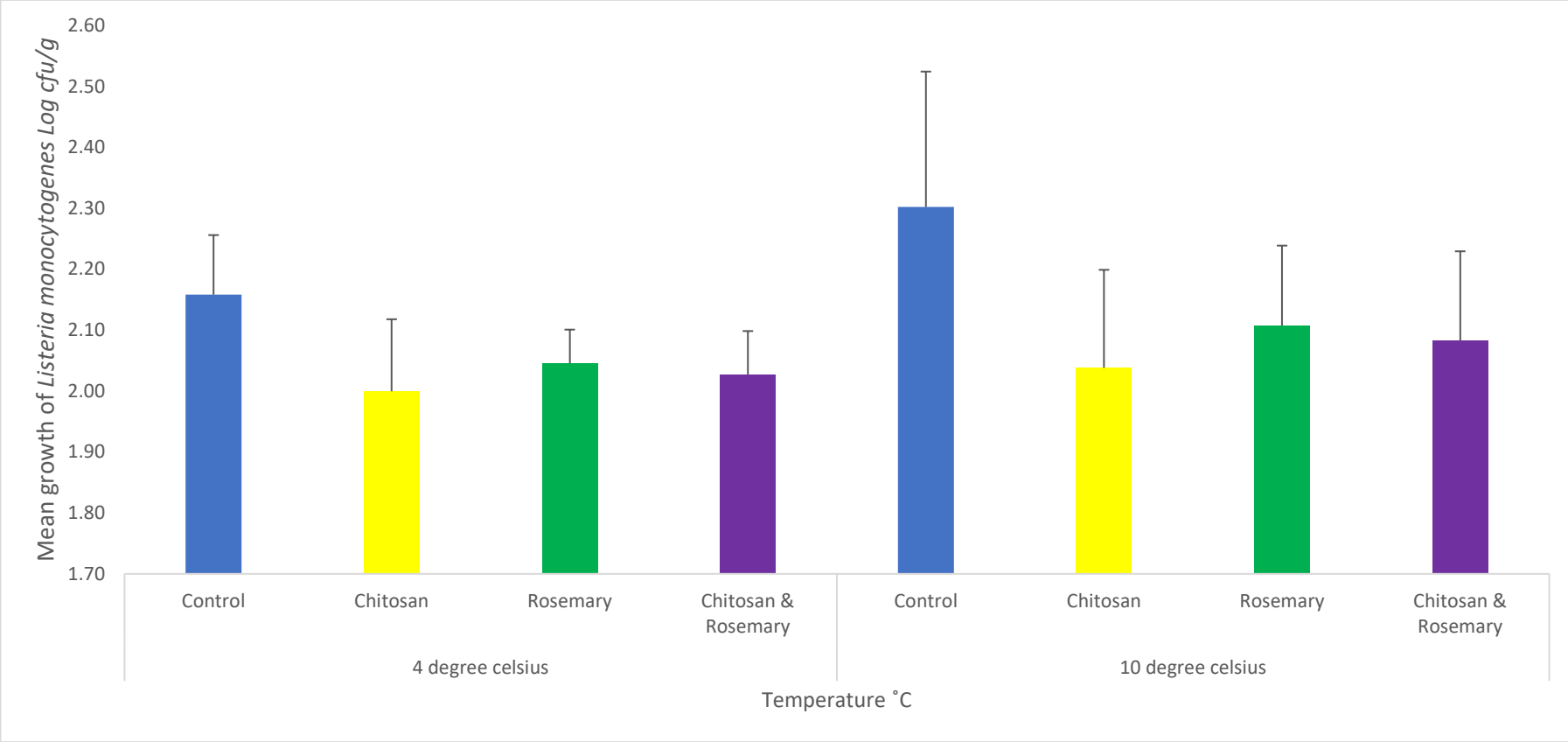


Figure 3.6 Growth of *L. monocytogenes* following inoculation into RTE polony models preserved with natural preservatives stored at different temperatures (4 and 10 ˚C)

monocytogenes (Osek et al., 2022:13). Essential factors such as proper food handling, adherence to food safety guidelines and storage conditions are crucial to prevent the growth of the pathogen in polony models (Mkhungo et al., 2018:126).

4. Time

The growth of *L. monocytogenes* in polony models were observed over days 0, 3, 6 and 9 at 4 and 10 ˚C. The growth of *L. monocytogenes* in polony (P1-P4) over 9 days were all similar ($p > 0.05$). The National Institution of Health (2008), suggested that if the growth pattern for *L. monocytogenes* were the same for a 9 day period, it indicates that bacterium is able to adapt to the environment it is in (National Institutes of Health, 2008:6-7). In this study, the growth of *L. monocytogenes* can be influenced by many factors, including temperature, time, water activity and the presence of preservatives or competing microorganisms (Igo et al., 2022:987-988). Therefore, monitoring and observing the interaction of storage temperature and time against inoculated polony models are important (Figure 3.7). The mean colony count of *L. monocytogenes* in polony stored at 4 and 10 ˚C for days 0, 3, 6 and 9 ranged from 2.03 log cfu/g to 2.20 log cfu/g (Figure 3.8). Although there is no significance ($P > 0.05$) a slight increase was observed over time when polony models were stored at 4 and 10 ˚C. Polony stored at 4 ˚C on day 1 yielded less growth of *L. monocytogenes* to that of day one at 10 ˚C. On day 3 (4 ˚C) similar growth patterns were observed from 2.09 log cfu/g to a growth count of 2.10 log cfu/g on day 3 (10 ˚C). Polony on day 6 yielded less growth of the pathogen when stored at 4 ˚C to that on day 6 at 10 ˚C. Day 9 carried the same trend, when polony models were stored at 4 ˚C with less growth compared to that of day nine at 10 ˚C. Overall it appeared that time (days) acted as an important parameter when influenced by other factors, including temperature and the presence of *L. monocytogenes* (Poimenidou et al., 2009:7184-7187). Therefore, it is important to adhere to food safety procedures when handling and storing of RTE polony, and to discard any product that exhibit signs of spoilage or contamination (De Oliveira Mota et al., 2021:1).

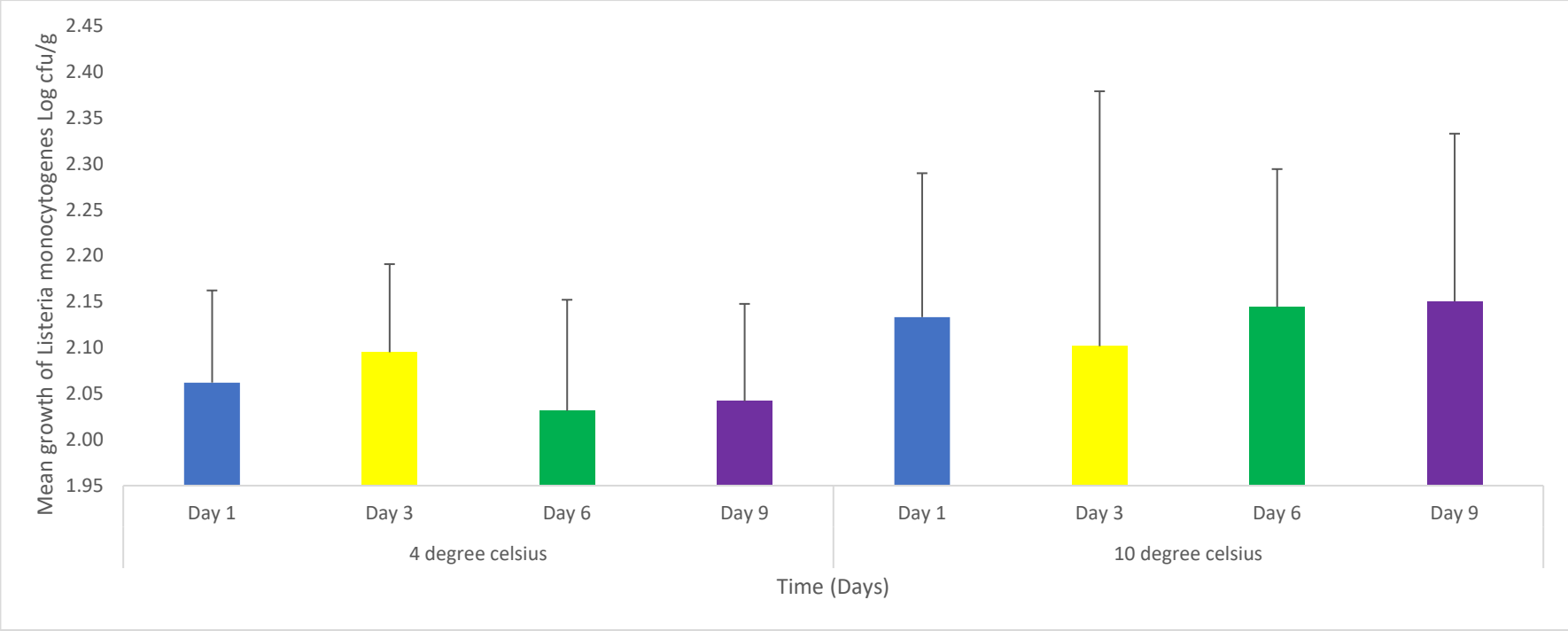


Figure 3.7 Growth of *L. monocytogenes* following inoculation into RTE polony models preserved with natural preservatives stored at different temperatures (4 and 10 ˚C) over nine days (0, 3, 6 and 9)

3.8 Conclusions

This study has demonstrated that the microbial quality of RTE polony can be significantly improved through the use of natural preservatives, such as chitosan and rosemary extract, either individually or in combination. The findings of this research study suggest that these natural preservatives are effective at inhibiting the growth and survival of *L. monocytogenes* compared to synthetic preservatives used in the polony batch pack for P1. The observation around the reduction in microbial contamination against coliforms, TVC and yeasts and moulds, highlights the potential of chitosan and rosemary as viable natural alternatives to synthetic preservatives. The results observed in this chapter underscore the importance of optimising concentration levels, storage conditions and time to enhance the efficiency of the antimicrobial properties used in this research study. Therefore, the growth and survival of *L. monocytogenes* in polony models preserved with chitosan and rosemary is recommended to develop safer and more effective preservation strategies in the food industry and to prevent the risk of microbial contamination.

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CHAPTER 4

INFLUENCE OF CHITOSAN AND ROSEMARY EXTRACT AS NATURAL PRESERVATIVES ON POLONY MODELS, MICROBIAL QUALITY AND CONSUMER PREFERENCE FOR THE VARIOUS TREATMENTS

ABSTRACT

High sodium contents are linked with health implications and the adaptation of bacteria. Chitosan and rosemary have been proclaimed to have good antimicrobial and antioxidant properties that stem from the natural world. Chapter 4 builds on the findings from Chapter 3 by evaluating the consumer's acceptability, sensory characteristics and preferences, while continuing to ensure product safety. New polony samples were prepared as outlined in Chapter 3, Section 3.2. This study therefore aimed to explore the impact of chitosan and rosemary as preservatives in polony models through microbial and sensory analyses to ensure both product safety and consumer appeal. This study compared polony models preserved with chitosan and rosemary (P2-P4) to a control model using salt (P1). Microbial analysis was conducted for coliforms (SANS 4832), yeasts and moulds (SANS 7954)*, E. coli* (SANS 7251), *S. aureus* (SANS 6888) and *Listeria monocytogenes* (SANS 11290), with all models showing no growth except for total viable counts (TVC), where P1 had the highest growth at 1.83 log cfu/g compared to P2 (1.60 log cfu/g), P3 (1.50 log cfu/g) and P4 (1.60 log cfu/g). All polony models produced in the current study were within their legislative safety limits and used for sensory evaluation. Consumers rated the acceptability of the polony models, with 42% showing a preference for P1, while others expressed their acceptance/preference for the different polony models as well. The overall preferred model chosen by the consumers in this study was P2 with a score of 38% compared to the other models. The outcome of this research study indicated that RTE polonies preserved with chitosan are preferred by consumers, thus assisting with the dual approach for microbiological and consumer-driven aspects, providing insight into the potential of natural preservatives that may be used in the food industry in the context of enhancing food safety and meeting consumer's expectations.

4.1 Introduction

For the past few years, the meat industry together with the South African Department of Health (DoH) have been fundamentally involved in reducing, replacing and removing sodium chloride (NaCl) in foods, to achieve three main goals: reducing consumer salt intake, reducing the outbreak of pathogens and improving the health of consumers that exist in the world (Nurmilah et al., 2022:1-3, Department of Health, 2017:1). Thus far, South African meat manufacturing companies have implemented sodium replacing formulations and techniques to reduce and conform to restructured health regulatory limits (Koen et al., 2021:14-15). Although salt replacers are generally effective concerning the taste qualities of food, concerns exist concerning their effectiveness as preservatives as well as the safety of these compounds to consumers (Barcenilla et al., 2022:1-3). The use of natural preservatives in the development of ready to eat (RTE) processed meats provides the opportunity to remove or reduce NaCl in food. The aim was to produce more healthy foods and while still reducing pathogen growth (Yu et al., 2021:1). Natural preservatives have the potential to improve and enhance the microbiological safety, shelf-life and quality of food products (Baptista et al., 2020:1). The role of these preservatives include: reduction of microbial contamination, maintenance of the quality of the product during storage and thereby providing safe alternatives (Bag et al., 2022). This study focused on producing RTE polony models preserved with chitosan [(1, 4)-2-Amino-2-deoxy-β-D-glucan] and rosemary (*Rosmarinus officinalis* L.) extract by evaluating the influence on consumers through preferential sensory analysis based on a 7-point hedonic scale (Tiwari et al., 2009*:*5991; Al-Hijazeen and Al-Rawashdeh, 2019:29). In addition, this study also analysed the sensory characteristics of polony models after microbiological safety tests of the polony models.

The effectiveness of natural preservatives was recently demonstrated in a study in Iran which determined the effect of chitosan and rosemary essential oils on the microbiological quality and organoleptic characteristics of chicken burgers during storage (Farokhzad et al., 2023:1). It was demonstrated that natural preservatives (chitosan + rosemary essential oil nanoliposomes) were effective in controlling the growth of microorganisms, slowing down sensory changes, and increasing shelf-life. In another study, El Bayomi et al. (2023) experimented and evaluated the sensory characteristics of chitosan and rosemary essential oils (REO) in treated and untreated rabbit meat. They concluded that the chitosan and REO positively impacted and

improved the sensory properties of the rabbit meat samples compared to untreated control samples. Limited research and only a few trials have been conducted to evaluate the effect of natural preservatives on the sensory qualities of products (Baptista et al., 2020:1-3). Most research studies on chitosan and rosemary focused on their antimicrobial and antioxidant properties, as well as their influence on shelf-life (Nieto et al., 2018:1; Muñoz-Tebar et al., 2023:1-2). Generally, these studies do not consider the implications of consumer's preferences in product development and the extensive game changing research in the food production and retail realm (Bogue and Sorenson, 2007:277).

The purpose of this study was to produce polony models (P1-P4) preserved with chitosan and rosemary extract, assess their safety through microbial analysis and evaluate consumer preferences and sensory characteristics to determine the effectiveness of these preservatives. Therefore, the research study emphasized the potential of natural preservatives by using chitosan and rosemary to improve microbiological safety and overall product quality. Specifically, the study addressed and focused on the production of RTE polony models preserved with chitosan and rosemary extract, by assessing their impact on consumers through preferential sensory evaluation. The primary goal of this study was to create healthier food options by using natural preservatives in the near future, while still suppressing the growth of the pathogen, *Listeria monocytogenes*.

4.2 Materials and methods

4.2.1 Source of materials

Chitosan powder (CAS-No: 9012-76-4) used in this study for the production of polony models was purchased from Sigma-Aldrich, Johannesburg, South Africa (Africa, 2023:1). All media used in microbial analyses were purchased from Science World, Cape Town, South Africa. Ingredients used for the production of the polony models were sampled from retail stores based in Cape Town. The chemicals used in this study were of analytical grade. Palcam Selective Supplements were purchased from Anatech Instruments, Johannesburg, South Africa. All equipment utilized was from the Department of Food Science and Technology based in Bellville at the Cape Peninsula University of Technology, South Africa.

4.2.2 Processing of rosemary plant material

Rosemary plants were washed with water and dried at 50˚C for 12 h (cabinet drier, Model 1069616). The leaves were then finely cut using sterile kitchen knife and ground using a laboratory blender (Waring Commercial, Model HGBTWTS3). The powdered rosemary was filled into plastic containers and stored at room temperature until use.

4.2.3 Extraction of colourant for polony models

The method used was adapted from Jin et al., (2014:472) . Red beet as a natural colourant was used in the emulsion of polony models P1, P2, P3 and P4.

4.2.4 Production of polony models with natural preservatives

Upon receiving the ingredients sourced at local supermarkets, powdered rosemary and fresh liquid beetroot extract was prepared and kept aside until use. The chitosan (P2)[5], Rosemary (P3)[6] and Chitosan & Rosemary (P4)[7] solutions were prepared separately in 500 ml beakers and sealed until use.

The rind was then bowl chopped (Freddy Hirsch, Model A-20) at a low speed for 10 minutes until a 3 mm particle size was reached. The respective preservative solutions and beetroot liquid extract were added to the bowl chopper to form a slurry. Meat components and ice were added and bowl-chopped until a well-blended mixture was formed. The remaining ingredients were added and bowl-chopped at a high speed until a final temperature of 14 ºC was reached. The emulsion was then filled into polony casings (Freddy Hirsch, Cape Town, South Africa). The polony was heat treated in a water bath at 68-72 ºC for 30 minutes. The temperature of the water kept below 75 ºC. The product was then cooled under running water to reach a temperature of 10 ºC (Figure 4.2).

This process was then repeated using the other preservatives until all three polony models were developed and stored in a refrigerator at 4-6 ºC until further analysis.

[5] 10 g/Kg of granulated chitosan powder was added to 300 ml of water.
[6] 0.26 g/Kg of rosemary powder was added to 300 ml of water.
[7] 10 g/Kg chitosan and 0.260 g/Kg of rosemary powder was added to 300 ml of water.

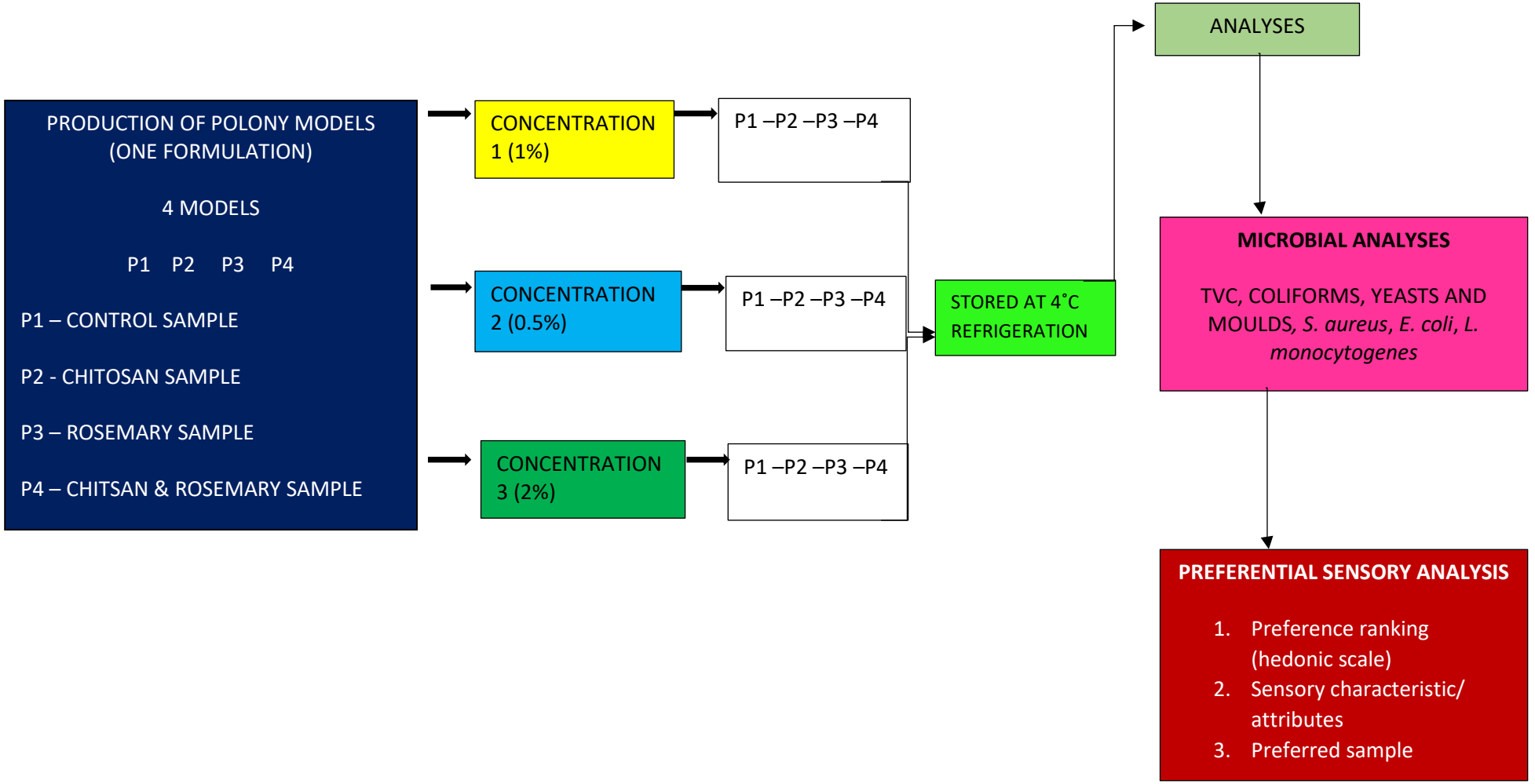


Figure 4.1 Experimental design for Chapter 4

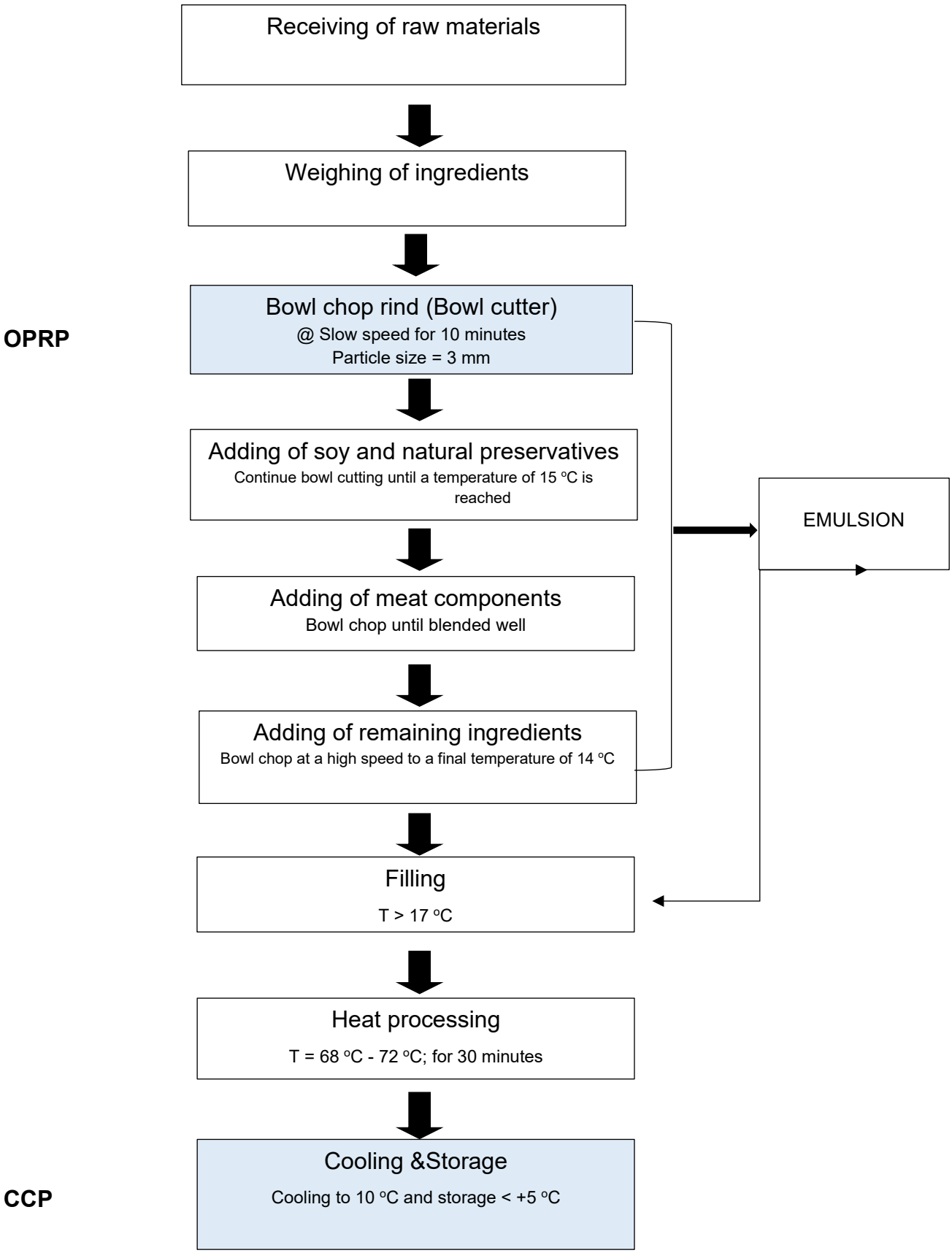


Figure 4.2 Process flow for polony models preserved with chitosan and rosemary extract. OPRP, operational prerequisite program; CCP, critical control point

4.2.5 Production of polony models without natural preservatives

Upon receiving the ingredients sourced by local supermarkets, rind was bowl chopped at a slow speed for 10 minutes until a 3 mm particle size was reached. Ice was then added to form a slurry. Once the slurry was formed, soy and $NaHCO_3$ was then added to the bowl chopper (Freddy Hirsch, Model A-20) until a temperature of 15 ^oC was reached. The meat components, half ice and phosphate were added and bowl chopped until the mixture was well blended. The remaining ingredients were added and bowl chopped at a high speed to the final temperature of 14 ^oC. The emulsion was then filled into polony casings. The polony was heat processed in a water bath at 68 – 72 ^oC for 30 minutes. The temperature of the water was kept below 75 ^oC. The product was then cooled under running water to reach a temperature of 10 ^oC.

4.3 Implementation of innovation preservation techniques in the production of polony models (P1-P4)

A growing component of the world's food production includes meat and meat products (Aratchilage et al., 2020:1-2). However, meat is commonly associated and implicated in food outbreaks (Lianou et al., 2022:521). The most recent pathogenic food pathogen outbreak in South Africa was caused by *Listeria monocytogenes* (Tchatchouang et al., 2020:1). Therefore, good manufacturing and hygienic practices are put in place to ensure the control and prevention of microbial contamination (Tropea, 2022:1). For example, in 2023, a study published by Siluma et al. (2023) suggested commercial butcheries in South Africa require training to improve hygiene practices of meat handling. Similarly, in 2014, conclusions were made by Kim et al. (2014:1-2) to better educate the industry using hazard analysis critical control point (HACCP) management systems. According to the Engeljohn et al. (2003) the Food Safety Inspection Service (FSIS) amended their regulation and further requires a HACCP plan whilst producing RTE processed meats or poultry. Therefore, part of this study included HACCP plans and procedures that were implemented during the production of polony models P1, P2, P3 and P4.

4.3.1 Hazzard Analysis Critical Control Point (HACCP) as a practical control method

During the production of polony models P1, P2, P3 and P_4, HACCP, as specified by the United States Food and Drug Administration (US FDA, 2006:1), was adopted as

an additional systematic method to increase the quality and shelf-life of the RTE polony models (LaBorde, 2020:1-2). One of the main objectives for this study was to adopt HACCP principles and plans to prevent microbial growth, comply with food safety regulations and enhance customer confidence.

4.3.2 HACCP ANALYSIS

During the processing of RTE polony models, potential hazards (B, C, P, A)[8] were identified through a HACCP analysis, taking into consideration their acceptable levels, associated risks and corresponding control measures (FAO, 2023:1-14). The shaded steps in Figure 4.2, represented two possible significant hazards that could occur during the production of the RTE polony models. The shaded steps were then used during the hazard analysis (Table 4.1).

Table 4.1 explains the hazards in detail. The essential highlighted steps in Figure 4.2 guide the progression of the polony model process, aiding in the identification and management of the potential hazard. Although the BCPA (biological, chemical, physical and allergen hazards) approach was used in this study to identify potential hazards (Schmidt and Newslow, 2007:3), only biological, physical and chemical hazards were identified. Other contributing factors that played a key role in this study was to assess the risks and prioritize the control measures. By identifying the acceptable levels of which consumer health, contamination and regulatory compliance must be considered to ensure the safety of the consumer as well as maintaining the quality of the product. During the hazard analysis, the severity and likelihood of each hazard was determined and evaluated. Preventative measures were then created to mitigate the risks that could potentially occur.

[8] B- biological, C- Chemical, P- physical, A- allergen

Table 4.1 Hazard analysis for the production of polony models (Schmidt and Newslow, 2007:7). B, biological hazard; C, chemical hazard; P, physical hazard; A, allergen

PROCESS STEP	IDENTIFY B / C / P / A AT EACH STEP	ACCEPTABLE LEVEL	RISK ANALYSIS	CONTROL MEASURE(S)
Step3: bowl chopping/cutting	**B** – Bacteria build-up in the bowl cutter/ between blades etc. Common pathogens found to look out for: *L. monocytogenes, S. aureus, E. coli, Salmonella*	Recommended acceptable level of these pathogens must be less than 100 CFU/g. *L. monocytogenes* and *Salmonella* spp will not be present in a 25g processed meat product ding to SANS Generally not present due to GMP and PRPs put in place this ensures good CIP's procedures preventing contamination	Likelihood = L Severity = H Significance = L x H = H If procedures and implementation plans are not followed, this could be High Risk. Since the bowl cutter is used daily, it should be cleaned properly. Temperature should follow as per process flow chart. This also ensures growth of bacteria	Ensure GMPs are followed; ensure that equipment and blades of the bowl cutter are cleaned properly (before production and end of production). This prevents cross contamination Do weekly microbial analysis on product and weekly swabs Keep temperature record as per flow diagram,
	C- Cleaning Reagents	Not present Make use of the proper cleaning reagents. Possibly food grade chemicals with acceptable levels followed by MSDSs	Likelihood = L Severity = M Significance = L x M = M If the recommended dosage on cleaning detergents label are followed, it increases the adverse effects	Cleaning chemicals must be listed in the MSDSs Implement PRPs for cleaning schedules and procedures
	P- Nails, glass, hair and any other foreign objects	Not Present – PRPs and GMPs are followed. There should be zero tolerance for physical hazards	Likelihood = L Severity = H Significance = L x H = H If any physical hazard is found in food product it could cause illness or harm to the customer, hence this will be considered	Implement suitable PRPs for personal hygiene and employee facility, as well as PRP for cleaning and contamination control of hazards

			significantly High	
Step 9: Storage	**B**- Potential Pathogenic bacteria - *L. monocytogenes* - *S. aureus* - *E.coli* - *Salmonella spp* - *Clostridium spp* - *Campylobacter jejuni* If storage temperature is not controlled. Growth of these bacteria can start to grow	In most processed meat products, the recommended acceptable level is <100 CFU/g *L. monocytogenes* and Salmonella spp will not be present in a 25g processed meat product According to SANS	Likelihood = L Severity = H Significance = L x H = H High risk, if the final product is not stored at temperatures < 5 °C	Make sure that storage temperature is controlled and maintained. This will inhibit the growth of organisms. Use PRPs to ensure storage unit is maintained.
	C- Food Additives	Product has an acceptable level of sodium as regulated in R214 of 20 March 2013)	Likelihood= L Severity = H Significant= L x H =H High risk. High levels of sodium in foodstuffs can compromise human health.	Use the recommended amount of sodium as regulated by government laws.
	P- Nails, glass, hair and any other foreign objects	Not Present – PRPs and GMPs are followed. There should be zero tolerance for physical hazards	Likelihood = L Severity = H Significance = L x H = H If any physical hazard is found in food product it could cause illness or harm to the customer	Implement suitable PRPs for personal hygiene and employee facility, as well as PRP for cleaning and contamination control of hazards

4.3.3 HACCP PLAN

During the production of polony models, a HACCP plan was constructed to help minimize and eliminate the risk of contamination (Awuchi, 2023:1). Both, operational prerequisite programs (OPRP's) and critical control points (CCPs) were taken into consideration to lower the risk of contamination (Figure 4.2). The HACCP plan included two hazards that were identified, i.e. the bowl chopping (OPRP) and storage process (CCP) steps (Table 4.2). The OPRP for bowl chopping addresses the temperature during this process as well as bacterial build up that could potentially cause harm. The temperature was consequently regularly monitored, every 5 minutes. To address potential buildup of bacteria, cleaning in place (CIP) procedures were practiced. This OPRP aims to maintain food safety and prevent possible hazards throughout the bowl chopping and storage process (Haines, M., Fishback, P., and Rhode, 2010:4-5). The HACCP plan (Table 4.2) provides measures to control deviations during bowl chopping and storage. Corrective actions involved further adjustments such as relocating the polony models to a colder storage area (storage process) and cleaning of blades (bowl cutting process) to avoid the build-up of bacteria. The implementation of a HACCP plan in this study was of utmost importance when producing RTE polonies. The systematic approach of HACCP lead to the control of potential hazards in this production process, ensuring the maximum level of food safety.

Table 4.2 Hazzard analysis Critical Control Point (HACCP) plan for processing of RTE polony models (Krishnakumar, 2019:9)

SIGNIFICANT HAZARD AND PROCESS STEP	POSSIBLE HAZARDS	A CCP or OPRP (JUSTIFICATION)	CRITICAL LIMIT	STUDENT MONITORING	CORRECTION & CORRECTIVE ACTION	STUDENT RECORD
Step 3: Bowl chopping /cutter	**B** – Bacteria build-up in the bowl cutter/ between blades etc. Common pathogens that could be present: 1. *L. monocytogenes* 2. *S. aureus* 3. *E. coli* 4. *Salmonella*	OPRP Justification: This is not a CCP, As good CIPs procedures are practiced as part of the PRPs implemented at the university. Microbial analysis and swabs are also done regularly. This Inhibits and reduces the likelihood of contamination.	Maintain at appropriate temperatures given in the process flow, for this specific step Temperature @ 15 ˚C	What: Rind emulsion and meat temperature was monitored	Correction: 1. Clean blades according to procedures put in place 2. Add adequate ice to reduce the temperature if it's too high	PRP200 checklist
	C – none			When: checking temperature of bowl cutting process and when meat emulsion is completed		
	P – none			Who: Researcher/ student	Corrective Action: Keep track or record of temperature checks	
	A – none			With what: calibrated thermometer was used		
Step 9: Storage	**B** – Micro-organisms/bacteria	CCP Justification: This processing step is significantly crucial. This step is used to prevent or reduce microorganisms to an acceptable level if product is stored correctly	Stored at temperatures <+5˚C	What: storage temperature of polony @ 0 - 4 ˚C	Correction: do maintenance on the storage equipment. If broken replace it	PRP151 Checklist
	C – none			When: during this stage of the process		
	P – none			Who: Researcher/ student	Corrective Action: Student do checks to maintain the storage unit.	
	A – none			With what: A calibrated thermometer		

4.4 Microbial analysis

For the microbiological analysis, coliform count (Leclercq et al., 2002:1631), *Escherichia coli* (SANS 7951, 2005:1-13), total viable count (TVC) (SANS 4833:2007, 2007:1-8), *Staphylococcus aureus* (SANS 6888-2, 1999:1-28), Yeast and mould count (SANS 7954:1987, 2002:1-7), and *Listeria monocytogenes* (ISO, 1998:1-35) were enumerated and detected following the methods that were described by Regulations Governing Microbiological Standards for Foodstuffs and Related Matters (Notice:, 2001:1-7). The samples P1, P2, P3 and P4 on respective refrigerated storage conditions were opened under the microbial inoculation laminar flow (Lasec Group, Model BBS-V1300) following aseptic conditions. 10 g of the respective polony model was blended with a 90 ml sterile ringer's solution (Science World, South Africa) in a stomacher bag and serial dilutions were organized as per requirement. The pour plate technique was used to assess and analyse samples in duplicate to determine the presence of these organisms.

4.4.1 *Enumeration of coliforms*

One 1 ml was pipetted from each dilution and poured with violet red bile agar (VRBA, Science World: C-MILB031-A) into Triplicate sterile plates (Preparation et al., 2020:1-2). A thin layer of VRBA was added to the sterile plate. The agar was then allowed to set and incubated for 24 h at 37 ˚C (Leclercq et al., 2002:1631). The number of pink colonies that developed on the agar plate, a sign of coliform growth, were counted and recorded.

4.4.2 *Detection of Escherichia coli*

One ml was pipetted from each dilution and poured with violet red bile agar (VRBA, Science World: C-MILB031-A) into duplicate sterile plates (Arana et al., 2013:2-3). A thin layer of VRBA was added to the sterile plate. The agar was then allowed to set and placed in an incubator for 24 hours stored at a temperature of 37 ˚C (Leclercq et al., 2002:1631). The number of pink colonies that developed on the agar plate, a sign of coliform growth were observed, counted and recorded.

Thereafter the plates were placed under an ultra violet lamp (UVL) to monitor florescent growth which indicated the presence of *E. coli* (Wang et al., 2017:1).

4.4.3 *Detection of Total Viable Counts*

From each dilution, 1 ml of the dilution sample was pipetted into sterile petri dishes and poured with plate count agar (PCA, Science World: MISC001) in triplicates (Arana et al., 2013). The plates were then allowed to solidify at room temperature. Sterile plates were then inverted and incubated at a temperature of 25 ˚C for 3 days (Bogomolny et al., 2013:4113).

After 3 days, the number of colonies that were formed were then observed, counted and recorded.

4.4.4 *Detection of Staphylococcus aureus*

Detection of *S. aureus* was conducted by using the pour plate method (Moraes et al., 2021:8-14). From each dilution, 1 ml of the dilution sample was pipetted into sterile petri dishes and poured with a thin layer of mannitol salt agar (MSA, Science World:MISC001) in triplicates (Moraes et al., 2021:8-14). The plates were then allowed to solidify at room temperature. Shortly after that the plates were incubated at 37 ˚C for 24h. After 24h, the number of colonies that were formed were observed, counted and recorded.

S. aureus are 1.0 -1.5 mm in diameter. They are colonies that are black and shiny in colour, convex in shape with a white margin surrounded by clear zones (Harris et al., 2002:39-44).

4.4.5 *Detection of Yeasts and moulds*

From each dilution, 1 ml of the dilution sample was pipetted into sterile petri dishes and poured with a thin layer of potato dextrose agar (PDA, Science World: MISC001) in triplicates. The plates were incubated at 25 ˚C for 3 days (Anonymous, 2022:39-40).

After the incubation period, the number of colonies were observed, counted and recorded (Fung, 2014:395-396).

4.4.6 *Detection of Listeria monocytogenes*

Four steps were involved in the detection of *L. monocytogenes*: Pre-enrichment in selective liquid media, secondary enrichment, selective plating and confirmation. For the pre-enrichment of *L. monocytogenes*, 25 g of the food sample was weighed into a stomacher bag mixed with 225 mL half Fraser broth. The sample was incubated for

24h at 30˚C. The second step was conducted by a secondary enrichment, 0.1 mL of the pre-enriched sample was transferred into a tube that contained 10 ml of Fraser broth. The inoculated Fraser broth was then incubated at 37˚C for 24h.
After 24 hours of incubation, the culture obtained was then used to streak onto PALCAM agar plates. These sterile plates were then incubated at 4˚C and 10˚C for 24 hours. After incubation a confirmatory test was conducted by performing a gram stain test to confirm the presence of *L. monocytogenes* (ISO, 1998:1-35).

4.5 Sensory analysis

Fifty people from the Department of Food Science and Technology, Faculty of Applied Sciences, Cape Peninsula University of Technology were selected as panelists to conduct sensory tests in a sensory laboratory, equipped with single booths to fit the criteria designed for sensory analysis (Boork et al., 2022:71). The sensory analysis laboratory was located at the Department of Food Science and Technology, Cape Peninsula University of Technology (Bellville). Sensory tests were conducted twice (two groups of twenty-five) on the same day and were carried out between 11 a.m. and 4 p.m. Samples of approximately 25 g were prepared (stored @ 4 ºC) in the research and development (R&D) kitchen and were served in small white foam paper bowls. The samples were coded with numbers that end with 0, 2, 4 and 5 representing the different RTE polony models P1, P2, P3 and P4. At each booth, panelists were presented with water used to cleanse the mouth between sample testing (Song et al., 2018:8). Each panelist was asked to sign a consent form and read the instructions thoroughly. Panelists were also informed about the study's aim and research objectives. After sensory tests were completed, panelists were rewarded with a lollipop.

Sensory panelists evaluated acceptance and preference of four treatment samples that were prepared with natural preservative Chitosan and Rosemary, its combination and a controlled sample. A total of four samples P1, P2, P3 and P4 were served to the panelists. Panelists were asked to rank the samples using a 7-point scale 7- I like it extremely, 6- I like it very much, 5- I like it, 4- neither like it or dislike it, 3- I dislike it, 2- I dislike it very much and 1- I dislike it extremely (García-Gómez et al., 2022:2). Participants were instructed to comment on attributes such as taste, colour, odour and appearance of which would influence the score of the polony model. Lastly, panelists evaluated the most preferred sample and second most preferred sample. It

is important to note that sensory analysis of the RTE polony models were approved by the ethics committee of the Faculty of Applied Sciences of the Cape Peninsula University of Technology. Panelists were also made aware of the natural preservatives used in these samples.

4.6 Statistical analysis

Data obtained from microbial counts of coliforms, total viable count, yeasts and moulds, *S. aureus*, *E. coli* and *L. monocytogenes* were converted to log cfu/g. Data for microbial analysis ensured that the RTE polony models produced were safe to consume. The sensory analysis data collected from panelists was recorded using Microsoft Office Professional Plus 2016 in Excel. Statistical analysis for sensory evaluation on consumer preference and acceptability by means of ranking, were done using statistical package SPSS 2023 to determine significant differences between treatments (IBM-SPSS, 2023). The overall consumer preference and acceptability (ranking) measured for the RTE polony models P1, P2, P3 and P4 were analysed by means of cross tabulation and Pearson's chi-square test to determine significant differences at the 0.05 level (IBM-SPSS, 2023).

4.7 Results and discussion

4.7.1 Microbial quality of polony models

The microbial analysis for the control model (P1), chitosan model (P2), rosemary model (P3) and chitosan and rosemary model (P4) are detailed in Table 4.3. For microbial quality and human consumption, six different microbiology tests were performed to ensure the safety of the different polony models for the consumption before conducting sensory evaluation.

The yeasts and moulds count were recorded at less than 10 cfu/g, as indicated in Table 4.3. This is a positive indication of the hygienic quality of the polony models, as the growth of yeasts and moulds could potentially lead to spoilage, off flavours and harmful toxins. No Coliforms or E. *coli* were detected in any of the four polony models plated on Violet Red Bile Agar (VRBA), further confirming the absence of these microorganisms. The Total Viable Counts (TVC) were 1.83 log cfu/g for P1, 1.60 log cfu/g for P2, 1.50 log cfu/g for P3 and 1.60 log cfu/g. The South African National Standard states the acceptable limit for TVC in processed meat products (<1000 cfu/g)

(SANS 885:201X, 2009:3-33), indicating that TVC in P1-P4 were well below the maximum allowable limit. Moreover, this suggests that the microbial load in the RTE polony models produced were minimal, which is an important aspect of ensuring consumer safety and shelf life stability. The low counts recorded for TVC further indicates that polonies produced under strict food safety conditions limited the microbial contamination.

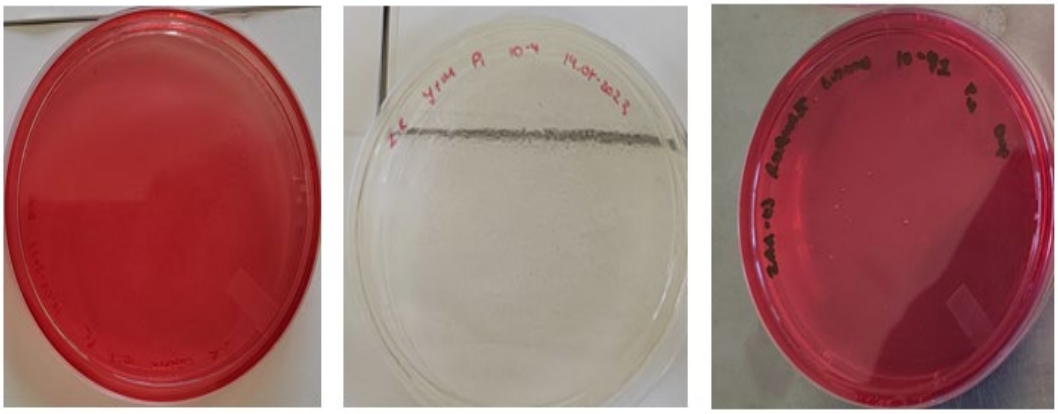
All four polony models plated on mannitol salt agar resulted in no growth, indicating a presumptive negative for the presence of *S. aureus*. The absence of *Staphylococcus aureus* was deemed necessary, as this pathogen is a major concern in the food industry due to its ability to cause severe gastrointestinal illness. The negative result indicated good hygiene through the production process. *Listeria monocytogenes* was not detected on the Palcam Agar plates, thus indicating that the polonies passed the safety microbial tests and were deemed safe for consumption.

Before undergoing sensory evaluation and the consumption of food, it is important to ensure that food safety and the quality of food are not compromised by microbial contamination (Mkhungo et al., 2018:126). De Oliveira Mota et al. (2021:1) suggested the implementation of different environmental monitoring programmes to increase food safety and quality of processed foods. Evidently, microbial analysis of the polony models ensured that the product is safe for consumption (Figure 4.3). Evidence in Table 4.3 during microbial tests, also proved that less growth occurred on those models preserved with chitosan and rosemary extract compared to the control model P1. Thus, suggesting that the antimicrobial activity of chitosan and rosemary extract assisted with the quality and prevention of microbial growth in polony models preserved with natural preservatives.

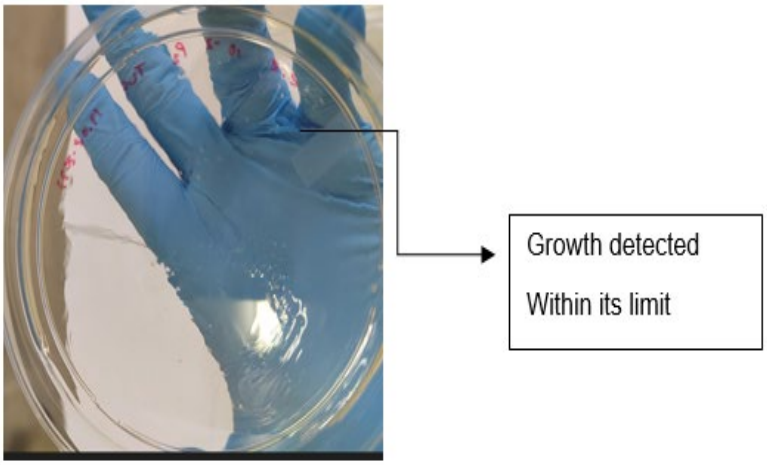
Table 4.3 Microbial analysis for polony models preserved with chitosan and rosemary extract

Microorganisms (Culture medium)	**Average colony count per dilution (log cfu/g)**			
	Control model (P1)	**Chitosan model (P2)**	**Rosemary Model (P3)**	**Chitosan + Rosemary Model (P4)**
Coliforms (VRBA)	-	-	-	-
Escherichia coli (VRBA)	-	-	-	-
TVC (PCA)	1.83	1.60	1.50	1.60
Staphylococcus aureus (MSA)	-	-	-	-
Yeasts and moulds (PDA)	-	-	-	-
Listeria monocytogenes (PALCAM)	ND	ND	ND	ND

ND= Not Detected, - = no growth, TVC= total viable count



L. monocytogenes Yeasts and Moulds *S. aureus*



TVC

Figure 4.3 Indication of the presence of microorganisms plated on different agars for microbial analysis

4.7.2 Sensory evaluation

Cross Tabulation and Pearsons Chi-square Tests (White, 2004:179-193) were performed to interpret the sensory evaluation data. The results summarised from panelist's acceptability hedonic rating (1-7) tests and attributes offered valuable insight to the preferential sensory result of the panelists for the different polony models used in this study (Figure 4.4).

1. Consumer acceptability of polony models preserved with natural preservatives

The sensory analysis results are summarised in Figure 4.4. Hedonic scores are presented for the overall consumer's response for the four polony models produced in this study. P1 received the highest rating (42%), indicating that panelists preferred this model, while 8% of the panelists "disliked" it and 14% of the panelists "neither liked nor disliked it. 18% of the panelists "liked it very much" and 18% of panelists "liked it extremely much". With regards to Model 3 (rosemary), 8% of consumers "disliked it", but 22% "liked it". 22% of the consumers "like it very much" and 26% of the consumers "liked it extremely much." The chitosan and rosemary polony model (Model 4), received different responses from consumers, with a significant ($p < 0.05$) portion "neither liking nor disliking" (26%) the sample and lower percentages expressing extreme dislike of the sample. The P2, similar to that of P4, displayed mixed ratings with 8% of the consumers that "dislike it extremely much", 20% of the consumers "dislike it very much", 12% of the consumers "disliked it", 18% of the consumers "neither like nor disliked it", 18% of the consumers "liked it", 16% of the consumers "liked it very much" and 8% of the consumers "liked it extremely much". Surprisingly, all the sensory panelists presented mixed reviews for Model 2 preserved with chitosan, making it extremely difficult to differentiate whether it tasted good or bad.

Based on the sensory scores and the Pearson Chi-Square tests, p-values were extremely low (0.000), indicating a significant difference ($p < 0.05$) between variables (Govender et al., 2019:9). Figure 4.4 also displays the differences ($p < 0.05$) between samples and its consumer ratings. In summary, the results shown in Figure 4.4 provided strong evidence of the associations between the different variables.

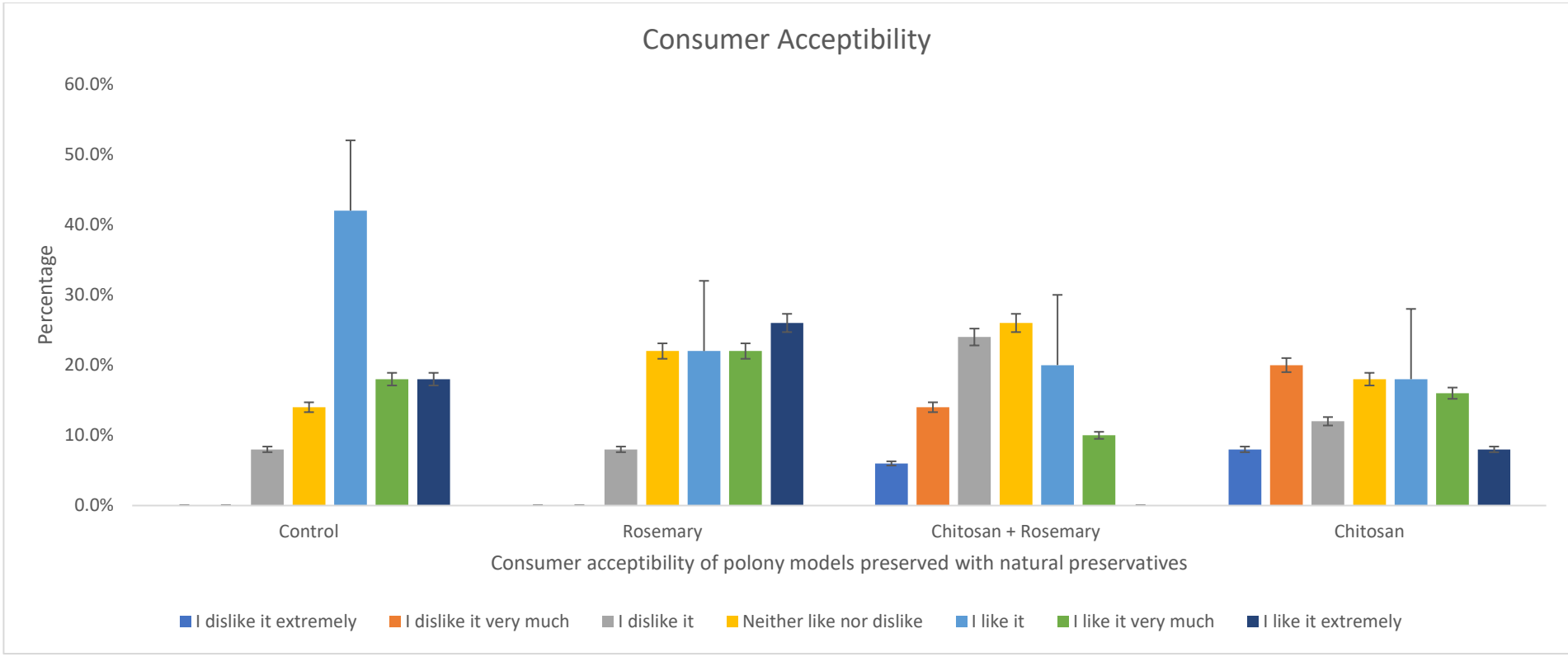


Figure 4.4 Consumer acceptability rating (1-7 hedonic scale) of Polony models P1-P4 preserved with chitosan and rosemary extract, and a combination of chitosan and rosemary extract

2. Consumer response by evaluating the organoleptic characteristics of polony models P1-P4

The panelist's response for the sensory characteristics of polony models P1-P4 are shown in Table 4.4. The participants rated organoleptic sensory characteristics (taste, odour, texture and appearance) of the polony models as "good", "satisfactory" and "bad". Table 4.4 also indicates the percentage of the participants who gave their ratings for the respective sensory organoleptic characteristics. The overall results indicated that most of the participants rated taste, odour, texture and appearance as "good" for the control (Model 1) and rosemary (Model 3) models compared to chitosan and rosemary in combination (Model 4). Similarly, in P2, the attribute scores were generally rated as "good". However, specific attributes like taste, odour and texture received mixed reviews, with some being rated as "satisfactory" while others were marked as "bad". The sensory evaluation elicited varied responses from the consumers which could have impacted the consumers overall acceptability shown below in Table 4.4.

Table 4.4 Number and percentages of participants who rated the polony model for the sensory characteristics evaluated (n = 50)

Polony models	Rating	Taste	Odour	Texture	Appearance
	Good	26[a] (52)[b]	28[a] (56)[b]	34[a] (68)[b]	50[a] (100)[b]
Control	Satisfactory	18 (26)	17 (34)	11 (22)	0 (0.0)
(Model 1)	Bad	6 (12)	5 (10)	5 (10)	0 (0.0)
	Good	31 (62)	27 (54)	27 (54)	50 (100)
Rosemary	Satisfactory	14 (28)	20 (40)	16 (32)	0 (0.0)
(Model 3)	Bad	5 (10)	3 (6)	7 (14)	0 (0.0)
	Good	8 (16)	7 (14)	7 (14)	50 (100)
Chitosan &	Satisfactory	24 (48)	27 (54)	26 (52)	0 (0.0)
Rosemary	Bad	18 (36)	16 (32)	17 (34)	0 (0.0)
(Model 4)					
	Good	20 (40)	22 (44)	17 (34)	50 (100)
Chitosan	Satisfactory	16 (32)	13 (26)	16 (32)	0 (0.0)
(Model 2)	Bad	14 (28)	15 (30)	17 (34)	0 (0.0)

[a] Number of participants; [b] Percentage of total number of participants

Individually, the participant's preference for taste, odour and texture are shown in Figures 4.5 to 4.7. The data represented in Figure 4.5 demonstrate the organoleptic property "taste" and panelist's preference and acceptability of the RTE polony models produced in this study. The obtained data in Figure 4.5 showed that polony preserved with rosemary had the highest taste scores which rated as "good', while the combination of chitosan and rosemary showed the lowest scores. The data in the same figure also showed that data obtained between the control model and combination of chitosan and rosemary were significantly higher ($p < 0.05$). Similarly, the combination of P2 and P4 were significantly different ($p < 0.05$). The participants in the study preferred P3 and P1 ($p > 0.05$). The findings illustrated in Figure 4.5 corresponded to the finding reported by Kholy and Aamer, (2017:23-24). Kholy and Aamer (2017) reported that adding rosemary essential oils (REO) to its product increased the sensory characteristics. Similarly, Bachir and Benattouche (2013) found that adding REO to yoghurt samples resulted in the best flavour profile. Therefore, producing polony models preserved with rosemary would could enhance the taste and flavour profile of the RTE polony.

The organoleptic sensory characteristics for odour in RTE polony models were assessed by participants (Figure 4.6). The results revealed that polony models 1, 2 and 3 generated the highest odour scores, and were rated as "good", while the Model 4 received the lowest scores. The panelist's preference for odour differed between the different polony models ($P < 0.05$). Participants seemingly favoured Models 1, 2 and 3 ($p > 0.05$). A study conducted by Farokhzad et al. (2023:1) indicated that chicken burgers preserved with chitosan may alter the sensory characteristics of products. However, a study performed by Abo-Samaha et al. (2022:1) proved that different nugget treatments containing rosemary had "good" acceptability for taste and odour. Thus, supporting the increased consumer's odour preference for the rosemary model shown in Figure 4.6.

The data represented in Figure 4.7 demonstrated the organoleptic property "texture" for the participants/ consumer's preference and acceptability of the RTE polony models produced in this study. The preference for the texture of the control model was significantly higher ($p < 0.05$) compared to the rest of the polony models. The least favoured texture was the chitosan and rosemary model, however, there was a 52% uncertainty.

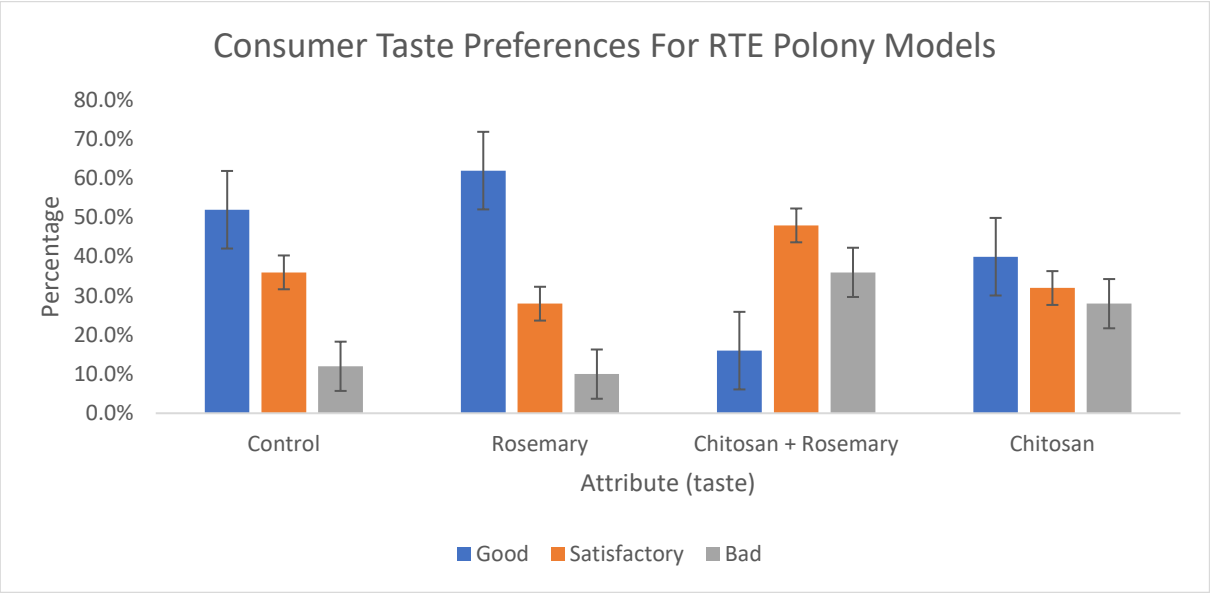


Figure 4.5 Consumer taste preference for RTE polony models produced in this study

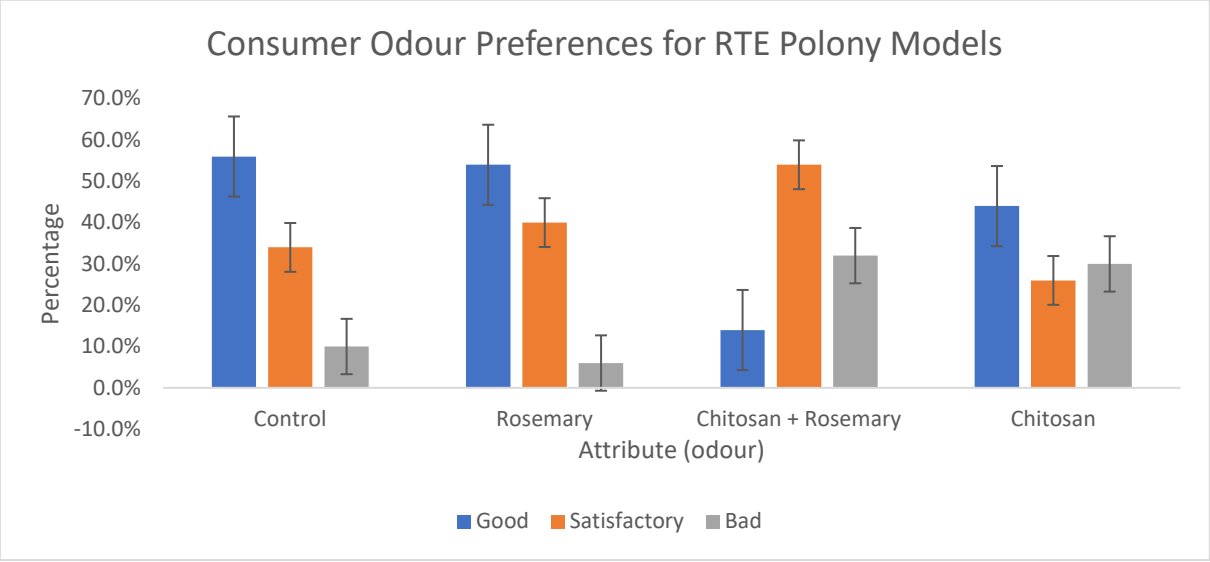


Figure 4.6 Consumer odour preference for RTE polony models produced in this study

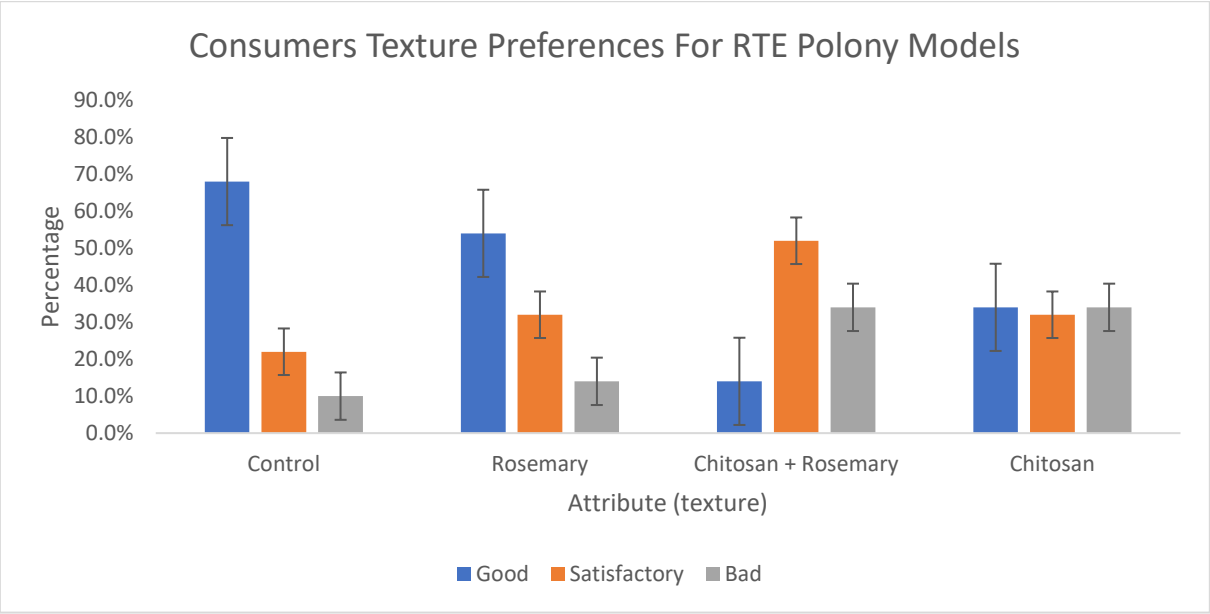


Figure 4.7 Consumer texture preference for RTE polony models produced in this study

Although the control sample was favoured the most as displayed in figure 4.7., it can also be assumed that participants/ customers used in the sensory evaluation was not familiar with the different polony models compared to the regular polony eaten by majority of the Western Capes people.

3. Consumer overall preference for the polony models used in this study

The overall effect of the consumer preferences for polony models preserved with and without natural preservatives were assessed. The results in Figure 4.8 demonstrated the results for the consumer's preferred first choice and preferred second choice. As displayed in Figure 4.8, the control model for both choices were quite similar ($p > 0.05$) with 32% and 34%. This suggests a relatively balanced distribution in the control models produced in this study. The rosemary model indicated the second choice was significantly higher ($p < 0.05$) compared to the first choice, indicating that the consumer chose the second choice as the most preferred sample (24%) when preserving the polony with rosemary. For the polony preserved with both chitosan and rosemary extract, consumers indicated that the first choice (26%) was significantly higher compared to the second choice (16 %).

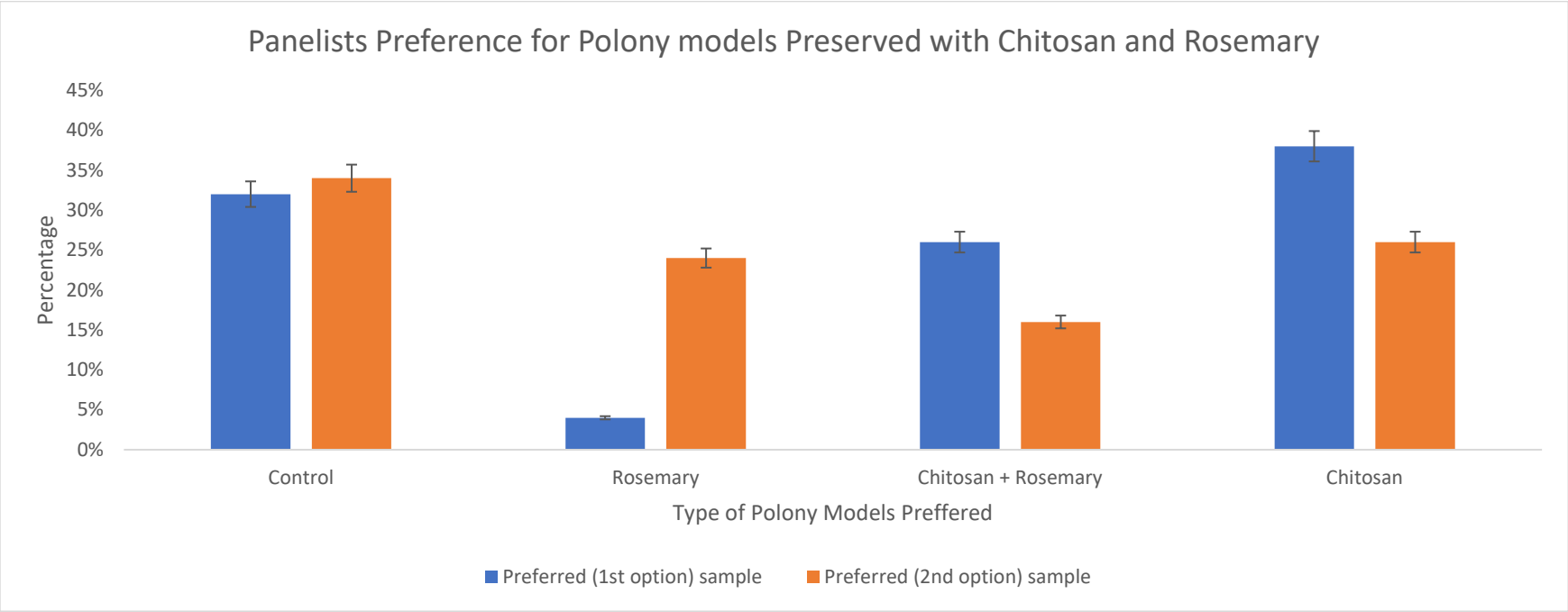


Figure 4.8 Consumers/ participants preferences for polony models preserved with chitosan and rosemary extract

The overall preferred model chosen by the participants in this study was the chitosan (Model 2) with 38%. Figure 4.8 indicated that the second most preferred polony model was observed as P1. The findings collated in this study showed the panelists clear preference choices for the chitosan model. A recent study done by El Bayomi et al. (2023) concluded that rabbit samples treated with a chitosan coating together with rosemary assisted with the quality and sensory attributes. Thus, indicating that the most preferred models were not significant ($p < 0.05$) between the control, Chitosan and Chitosan and Rosemary models.

4.8 Conclusion

The results of this study showed that the inclusion of the natural preservatives chitosan and rosemary improved the antimicrobial properties of the polony models. Total Viable Count (TVC) of the polony models decreased in the presence of chitosan and rosemary compared to the control model which may be attributed to the antimicrobial effect in chitosan and rosemary. In addition, the chitosan model used in the sensory evaluation was most preferred in this study followed by the control sample, indicating that natural preservatives used in this study appeared to the sensory attributes of the polony models produced. Therefore, this study concluded that chitosan should be use as a potential resource as a natural antimicrobial inhibitor in the meat industry.

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CHAPTER 5
GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 General summary and conclusion

The aim of this study was to investigate and evaluate the growth and survival of *Listeria monocytogenes* in polony models preserved with chitosan and rosemary extracts. This was achieved by developing four polony models; a control sample (P1) containing NaCl and three other models (P2 – P4) preserved naturally with chitosan and rosemary extract. Each of the polony models was inoculated with 3 log cfu/g of *L. monocytogenes*, These natural preservatives were produced at three different concentration levels and stored at 4 and 10˚C to determine whether the natural preservatives chitosan and rosemary present in the polony models, reduced or inhibited (antimicrobial properties) the growth and survival of *L. monocytogenes.*

For the first experimental chapter (Chapter 3), the study was designed with the aim to develop and investigate the four polony models (P1 – P4) for preservation against coliforms, yeasts and moulds, TVC, *E. coli*, *S. aureus* and *L. monocytogenes*. The polony models produced were screened for microbial contamination to ensure that they were safe for consumption. The results obtained during the screening tests indicated no growth for *E. coli, S. aureus* and *L. monocytogenes*. Also, no growth was observed with the control. Therefore, no claim could be made indicating that chitosan and rosemary, included in the models, inhibited the growth of these organisms. However, the growth recorded for coliforms, TVC and yeasts and moulds showed less growth on models P2 – P4 compared to model P1 indicating the possibility that polonies preserved with salt could increase the growth of organisms compared to those polonies preserved with chitosan and rosemary. Chitosan and rosemary preserved in the polony models (P2 –P3) displayed the potential to act as antimicrobial agents. All four polony models exhibited no growth or growth within recommended limits, indicating that their production process was hygienic.

The third objective was to determine the growth and survival (G&S) of *L. monocytogenes* in polonies preserved with chitosan and rosemary extract. These G&S studies were important in this research chapter as they would determine the safety, shelf-life and quality of the polony models. The G&S study is considered to prevent *L. monocytogenes* from multiplying, using chitosan and rosemary as antimicrobial agents. If *L. monocytogenes* was able to grow and survive in the polony models under

different storage conditions, it could possibly pose serious health risks to consumers. Therefore, it was important to evaluate the growth and survival of *L. monocytogenes* in RTE polony models preserved with chitosan and rosemary extract under different conditions to ensure the safety and quality of the product. The results obtained during the research chapter indicated that less growth of *L. monocytogenes* was detected in polony models preserved with chitosan and rosemary (P2 – P4) compared to the model preserved with NaCl (P1). Thus, indicating that the chitosan and rosemary assisted in the inhibition of *L. monocytogenes*.

The first hypothesis predicted that polony models preserved with chitosan and rosemary would inhibit the growth and survival of *L. monocytogenes*. This was observed as the polony model P1 had the highest growth of *L. monocytogenes* whereas polony models preserved with chitosan and rosemary (models P2 – P4) had less growth. With regards to the storage temperature, polony models too shared the similar result when stored at different temperatures. Hence, this hypothesis was accepted according to its objectives.

The fourth and final objective was to conduct the preferential sensory evaluation using a consumer panel for the most preferred polony model (P1 –P4). The results indicated that the polony models were safe for consumption. This indicated that the hygienic production of the polony was done successfully. As for preferential evaluation of the polony models by the panel, the control model P1 was preferred and the panels second preference choice was polony model P2. However, the panelists had mixed emotions when tasting the polony models when assessing the organoleptic properties.

The final hypothesis predicted that polony model P4 would be the preferred option during the sensory evaluation. However, the panellists used during the evaluation opted for a polony that was similarly produced to polonies bought in the supermarket. This, however, did not stop the panellist from sharing their second preferred choice, polony model P2, a model preserved with chitosan. Therefore, the hypotheses that a polony model preserved with a natural preservative could assist with the prevention of a listeriosis outbreak, as well as a polony model that contains a natural preservative, assuring higher health benefits, could change the way food manufacturing companies operate and support the inhibition of *L. monocytogenes*. Hence, this hypothesis was accepted.

To conclude, all objectives and hypotheses of the study were met and partially met, as the evidence in the research chapters indicated that natural preservatives such

as chitosan and rosemary in the different polony models displayed antimicrobial activity, thus also assisting with the prevention of *L. monocytogenes* in polony models P2 – P4 as compared to polony model P1 preserved with NaCl. The following conclusions can be made from this study:

1. Chitosan and rosemary preserved in polony models have good antimicrobial properties.
2. Chitosan and rosemary work well at 1% concentration level.
3. From the polony models produced, model P1 preserved with salt (NaCl) displayed higher growth of *L. monocytogenes* as compared to the models preserved with natural preservatives.
4. Natural preservatives have the potential to prevent listeriosis outbreaks. Replacing salts with more natural options would help to prevent consumers falling ill of high blood pressure and other health issues.
5. Chitosan and rosemary have also been accepted with consumer's pallets, increasing the chances of developing more products with these natural preservatives.
6. Natural preservatives assist with developing healthier food options while addressing the risks of pathogen growth concerns.

5.2 Recommendations

It is recommended that further research be done on the susceptibility of *L. monocytogenes* and exploration of concentration levels, while this research study found that polony models preserved with chitosan and rosemary at a 1% concentration level were effective in inhibiting the growth and survival of the bacteria. It would be beneficial to find the optimal concentration for maximum antimicrobial efficiency. It is also recommended that future studies investigate a longer shelf-life of polonies preserved with chitosan and rosemary extract. This study also recommends that further development of the polony models be worked on to ensure a more consumer friendly taste to meet the consumer's expectations and improve overall acceptability of future polony models preserved with chitosan and rosemary.