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Heat inactivation and survival of *Listeria monocytogenes* during polony production and shelf life

By

Onalenna Onneile Molaletsi

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DECLARATION

I Onalenna Onneile Molaletsi declare that this dissertation submitted for the degree MSc Food Science at the University of Pretoria, has not been previously submitted by me for a degree at this university or at any other institution of higher learning.

Onalenna O. Molaletsi

DEDICATION

I dedicated this work to my son, Anaya-Letlotlo Tshimo, I love you. Also to God the provider, who gave me life and brought me thus far.

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ABSTRACT

Heat inactivation and survival of *Listeria monocytogenes* during polony production and shelf life

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Onalenna Onneile Molaletsi

Supervisor: Prof. Elna. M Buys

Degree: MSc Food Science

Food safety continues to be a challenge due to re-occurring foodborne illness outbreaks such as listeriosis. The recent listeriosis outbreak in South Africa, in which polony was implicated, prompted for the need to strengthen the food safety systems and the application of more stringent measures to facilitate the production of safe polony products. The study was aimed at determining the effect of the conventional polony processing method against *Listeria monocytogenes* strains, as well as assessing the applicability of predictive tertiary models in estimating the growth of the strains in polony during the product shelf life.

The first phase of the study involved the bacteriological analysis (total plate counts-TPC, lactic acid bacteria-LAB, and *Listeria* spp.) of polony emulsion and processed (heat-treated to a core temperature of 72℃ and cooled to 10℃) polony, as well as physiochemical characterisation of polony. To determine the processing effect on *L. monocytogenes*, polony emulsion was inoculated (5 log CFUg^{-1}) with strains of *L. monocytogenes* (159/10, 69, 732), processed and analysed for *L. monocytogenes*.

Processing affected LAB, TPC, and all strains of *L. monocytogenes* investigated; a bacterial reduction of more than 5 log CFUg^{-1} was achieved. Strain heterogeneity influenced the extent to which processing affected *L. monocytogenes* strains as shown by significant differences ($p<0.05$) between percentage log MPNg^{-1} reductions of the strains. *L. monocytogenes* strain

69 had the lowest percentage log MPNg^{-1} reduction (61%), followed by strain 732 (71%), and strain 159/10 had the highest percentage log MPNg^{-1} reduction (91%). Polony had pH of 6.21±0.2, a_w of 0.95±0.08, 20±2% fat content and 68±1.8% moisture content.

The second phase of the study involved the investigation of the growth of *L. monocytogenes* strains and LAB in polony during the product shelf life (storage at 4℃ for 12 weeks). Specific maximum growth (μ_{max}) and lag phase duration (λ) of *L. monocytogenes* strains were determined. Predictive tertiary models selected based on their availability and the microorganisms of interest were used to estimate LAB and *L. monocytogenes* growth in polony.

The models used for the estimation of *L. monocytogenes* strains were the "growth of *L. monocytogenes* in RTE cured meats" model available in MicroHibro software, the "Broth growth" model in ComBase software, and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model in FSSP software. Growth of LAB in polony was estimated using two models; the "growth of LAB in ground beef" model in MicroHibro and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model in FSSP. Model performance was evaluated by comparing predicted growth data and observed growth data. The coefficient of determination (R^2), bias factor (B_f), accuracy factor (A_f) and root mean square error (RMSE) were used as indices for the evaluation of the model performances.

L. monocytogenes strains and LAB were able to survive and grow during refrigerated storage. Strain heterogeneity influenced growth ($p<0.05$) of *L. monocytogenes* as shown by varying μ_{max}, and λ of the strains. The growth of *L. monocytogenes* strains in polony, predicted by the "growth of *L. monocytogenes* in RTE cured meats" and the "growth of *L. monocytogenes* and LAB in chilled seafood and meats" models in MicroHibro and FSSP software packages gave acceptable and fail-safe prediction (B_f between 0.87 and 1.43). The ComBase Broth growth model gave unacceptable predictions (B_f >1.43) of *L. monocytogenes* strains in polony. The "growth of LAB in ground beef" and the "growth of *L. monocytogenes* and LAB in chilled seafood and meats" models gave acceptable predictions of LAB growth in polony.

Correct implementation of the polony processing method, coupled with good hygiene procedures, can lead to the production of safe polony. The South African polony industry can also explore the field of predictive food microbiology to strengthen and supplement their surveillance and food safety management systems hence protect human life while saving time and money.

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CHAPTER 1: PROBLEM STATEMENT

Food safety is a critical component of food nutrition and security, particularly in developing countries where the socio-economic status of a majority of the population is poor. The incidence of foodborne illnesses in developing countries is a major concern. In 2009 the World Health Organisation (WHO) estimated a total of 800 000 deaths due to diarrheal diseases in children worldwide, of which 80% of the cases were in developing countries (Ortega David, 2017). South Africa has experienced its food safety challenges, particularly those due to food pathogens and pesticides and antibiotic residues in food (Boatemaa et al., 2019).

The most recent food safety issue in South Africa is the listeriosis outbreak, which the WHO dubbed the world's largest outbreak (Allam et al., 2018, Boatemaa et al., 2019). A total of 1060 confirmed cases were recorded, with a 30% mortality rate and economic losses of more than US$ 285.4 million (Boatemaa et al., 2019, Olanya et al., 2019). The outbreak was traced to a ready-to-eat manufacturing plant in Polokwane, RSA, and polony was found to be the culprit (Boatemaa et al., 2019, Olanya et al., 2019, Smith et al., 2019). Polony is a South African version of the commonly known bologna sausage. The product is cheap and easily accessible to most people hence the most consumed RTE meat product in the country (Boatemaa et al., 2019), with significant contribution to the country’s economy in both the domestic and export markets (Olanya et al., 2019).

Production and preservation of polony rely primarily on heat processing and refrigerated storage to slow down the growth of *L. monocytogenes*. The pathogen, however, is reported to have the ability to survive harsh processing and preservation conditions, due to its ability to sense and respond to the various stresses it come across (Boor, 2006, NicAogáin and O’Byrne, 2016). The general response mechanism of the pathogen is through the activation of the stress-responsive *sigB* (σ^B), hence inducing the activity of stress response proteins of the pathogen (Ferreira et al., 2014).

Isolation of *L. monocytogenes* in polony and polony processing environment and its implication on the outbreak, called for a prompt response to strengthen the food safety measures in South Africa (Olanya et al., 2019, Smith et al., 2019). The measures include pre-emptive *L. monocytogenes* inactivation and detection to enhance control of the pathogen (Allam et al., 2018). Pathogen inactivation studies on polony are paramount, as no work has been conducted on the product, and the industry depends on data from experiments done on *L. monocytogenes* under laboratory conditions in laboratory media. According to Abee et al. (2016), a process that kills *L. monocytogenes* in one product may not be sufficient to do so in another product due to the dynamic response of *L. monocytogenes* strains to changing environmental conditions, which affects the efficiency of conventional methods.

The application of predictive microbiology models can also assist the South African polony industry in timely pathogen detection. The models can accurately predict the growth of *L. monocytogenes* in response to relevant stresses applicable to a specific food product, taking into account the intrinsic and extrinsic characteristics of food (Mejlholm and Dalgaard, 2015). Developed countries have explored the use of predictive microbiology, while in South Africa information on the application of the models is lacking.

Application of predictive models can provide cost-effective substitutes for traditional microbiological testing, especially in South Africa, where end-product testing is the main food safety assurance practice. When appropriately constructed and applied, they can be used as the ultimate rapid method (McMeekin et al., 2002). Assuring the effectiveness of the conventional polony processing method in inactivating *L. monocytogenes* strains, together with the application of predictive microbiology models in estimating the growth of the pathogen in polony, will provide information that can aid in strengthening food safety management procedures in South Africa, and consequently help to prevent the incidence of listeriosis outbreaks.

Therefore, the objective of this study is to determine the effect of the conventional polony processing method on *L. monocytogenes* strains and to determine the applicability of predictive models in estimating the growth of *L. monocytogenes* strains during polony shelf life.

CHAPTER 2: LITERATURE REVIEW

2.1 OVERVIEW OF THE MICROBIOLOGICAL SAFETY ISSUES IN THE WORLD

The past decades have seen an alarming rate of the increase of foodborne infections such as salmonellosis and listeriosis worldwide (Meng and Doyle, 2002, Lund, 2015). The patterns of foodborne disease outbreaks are impacted by, among others, the globalisation of the food supply chain (De Oliveira et al., 2011). Because of food trade globalisation, there is a more extensive production that involves a complex supply chain with numerous sites hence an increased risk of microbiological contamination and possibly the increase in incidences of foodborne outbreaks (Ronholm et al., 2016).

The occurrences of foodborne illnesses remain a notable cause of sickness and avoidable death globally despite the advancement of Food Science and Technology and a growing concern raised by international food safety groups (Akhtar et al., 2014). The major reason behind this is the ever-changing epidemiology of microbiological foodborne illnesses principally because of consumers' more adventurous eating habits and demand for more convenience foods (Hedberg et al., 1994, Meng and Doyle, 2002, Newell et al., 2010, Dewey-Mattia et al., 2018). The emergence of newly recognised pathogens, some of which are developing resistance, and the evolving food processing technologies, also affect the epidemiology of foodborne pathogens (Meng and Doyle, 2002, Akhtar et al., 2012).

Developed economies are no exception to foodborne illness outbreaks as the estimations of the occurrence of the illnesses are at least one-third of the population annually (EFSA, 2017). It has, however, been reported that there is a contrast between food safety issues in developed countries and in developing economies (Weinstein, 2018). The reason for this is due to developed countries' ability to manage and implement proactive strategies that reduce the risk of producing and delivering unsafe food products (Elkhishin et al., 2017). Their advancement in scientific implementation strategies on food hazards has brought more stringent food safety standards, improvements, and implementation in food processing hence an increased risk reduction (Ross and McMeekin, 2003, EFSA, 2017, Weinstein, 2018).

Developing economies experience foodborne outbreaks at a considerably higher rate than developed countries, with 80% of the world cases reported in developing countries (Weinstein, 2018). The socioeconomic status in developing societies, which is characterised by low income, lack of education, and poor water quality, is a major factor in the accessibility of safe food to the vulnerable population (Akhtar et al., 2014, Weinstein, 2018).

Documentation of the prevalence of foodborne incidences and surveillance is recognised as a potential determining factor for the fast deteriorating economic growth in underdeveloped countries (Akhtar et al., 2014); however, this is the most neglected area in the control of the foodborne diseases in the developing world (Akhtar et al., 2014). The assessment of the prevalence of outbreaks is not well documented because of poor surveillance and monitoring systems in developing countries (Akhtar et al., 2014). The developing world lack surveillance programs to capture information on foodborne cases based on the assumption that cases are hardly reported, and traditional food storage and preparation conditions prevent opportunities for growth (Todd and Notermans, 2011).

In the year 1998, The World Health Organisation collaborated with African countries to initiate the “Integrated Disease Surveillance & Response” (IDSR) in the region concentrating on illnesses of main concern such as cholera and diarrheal diseases in children under five (WHO, 2013). After the year 2005, the International Health Regulations (IHR) was founded, and IDSR was obliged to take account of the outbreaks of contaminated food and foodborne illnesses in the reporting system. Unfortunately, there is still the scarcity of statistics on foodborne epidemics because of the disintegration of food safety programs in Africa, hence insufficient exploitation of resources, replication of activities, and lack of partnership amid the countries and stakeholders of the continent (Akhtar et al., 2012). Out of 33 African countries expected to report to the Global Food Network databank; only 11 states reported their data as of 2012, and with only one country being a regular reporter (Paudyal et al., 2017).

Sufficient information regarding the pathogen contamination, its evolution, and persistence in food products, and its infection dosage is paramount in the assessment of the effect of the pathogens on public well-being (Doyle and Erickson, 2006). A science-based approach is essential in assembling and analysing information to expand the quality of public health decisions (Doyle and Erickson, 2006, De Oliveira et al., 2011). It is, therefore unavoidable that developed approaches be designed and instigated concentrating on managing and controlling hazards (Nychas et al., 2016).

2.2 INCIDENCES OF FOODBORNE OUTBREAKS AROUND THE WORLD

Throughout the world, the leading cause of morbidity and mortality is foodborne pathogens, hence epidemiological investigations and interventions on foodborne illnesses are critical in the public health infrastructure development (Ronholm et al., 2016). WHO reported that millions of people globally suffer from foodborne diseases yearly (Meng and Doyle, 2002), including 800 000 death of children under ten years of age, of which over 80% of the cases are in developing countries (Weinstein, 2018). According to Akhtar et al. (2014), 30% of the population in the USA, Australia, and India suffer from foodborne illnesses annually, indicating the severity of the problem.

Diarrheal related death among children in developing countries estimated at 1.8 million yearly, signifying a major economic liability due to foodborne illnesses (Ross and McMeekin, 2003, Akhtar et al., 2014). It has been reported that in the USA, 76 million cases and 5 000 deaths are recorded annually due to microbiologically contaminated food (Meng and Doyle, 2002, Lund, 2015). According to Lund (2015), *Salmonella* infections are the most commonly occurring infection reported (17.6 illnesses in 100 000 people) followed by *Escherichia coli* 0157 (0.9 illnesses in 100 000 people) then *Campylobacter*, *L. monocytogenes*, *Shigella*, and *Yersinia* infections, respectively.

L. monocytogenes outbreaks are rare compared with those caused by pathogens like *Salmonella*. However, they get substantial attention when they occur since they usually have some seriously affected cases and even deaths as shown in Table 2.1 (Todd and Notermans, 2011), making it the third leading foodborne illness cause of death, with case fatality rates of 20-30% (Churchill et al., 2006).

Foodborne outbreak incidences disrupt food supply and create food insecurity, cause economic losses, social disruptions, as well as environmental implications (Elkhishin et al., 2017, Whitworth et al., 2017). The possible effect of food safety outbreaks on a food business can be overwhelming, with a single episode capable of bringing unbelievable economic losses (Ronholm et al., 2016). It is estimated that the cost of recalls due to *L. monocytogenes* in the food industry in the US is estimated to be $1.2-2.4 billion per annum (Orsi et al., 2011). In contrast, the medical cost and productivity/premature death loss due to the pathogen may be about $2.3 billion annually (Orsi et al., 2011). Ronholm et al. (2016), stated that the economic study of food safety associated costs revealed that it is much economical for a producer to capitalize on preventing events of the foodborne outbreak than the cost after an event.

Table 2.1: Listeriosis outbreaks in different countries (Doyle and Erickson, 2006, Elkhishin et al., 2017, WHO, 2018)

Country/Region & year	**Cases**	**Deaths**	**Product**	**Estimated Economic loss**
USA-2011	19 336	378	n.r	n.r
USA-2013	6	1	Cheese	Recall and destroying the affected product
South Africa 2017-2018	1 068 cases	218	Polony (RTE meat sausage)	Over R300 million
USA-2019	17	2	Deli sliced meats	n.r n.r
Spain-2019	222	9	Chilled roasted pork meat	n.r

*n.r; not reported

2.2.1 Listeriosis in South Africa

Sporadic cases of listeriosis have been reported in South Africa from as early as 1977, with the first outbreak reported in Gauteng province in 1977/78 with an average mortality of 43%

(Dramowski et al., 2018, Manganye et al., 2018, Smith et al., 2019). From 2017 to 2018, the country experienced what the World Health Organisation dubbed the world's largest listeriosis outbreak (Allam et al., 2018, Boatemaa et al., 2019, Olanya et al., 2019, Kaptchouang Tchatchouang et al., 2020).

Before then, listeriosis was not a notifiable disease in the country because incidences of infection were low (Dramowski et al., 2018). The 2017-2018 a listeriosis outbreak was recorded, with 1068 confirmed cases and 218 deaths translating to a mortality rate of 30% (Allam et al., 2018, Boatemaa et al., 2019, Smith et al., 2019, Kaptchouang Tchatchouang et al., 2020). This prompted the National Department of Health to add listeriosis to their list of notifiable diseases (Manganye et al., 2018). The cases were widespread throughout the country, with a majority (57%) recorded in Gauteng province, 13% in the Western Cape, and 7% in Kwa-Zulu Natal provinces (Kaptchouang Tchatchouang et al., 2020).

L. monocytogenes sequence type 6 (ST6) was identified by laboratory and epidemiological studies as the causative agent of the outbreak (Smith et al., 2019). The strain was isolated from clinical samples of the victims, the processing environment, and processed meat commonly known as polony in the Tiger Brands' Enterprise Foods factory in Polokwane, South Africa (Dramowski et al., 2018, Boatemaa et al., 2019, Olanya et al., 2019, Smith et al., 2019).

Economic losses due to the outbreak were quite devastating, amounting to case-fatality costs of about US$ 260 million, hospitalisation costs of US$ 10.4 million, and productivity losses of US$ 15 million (Olanya et al., 2019, Kaptchouang Tchatchouang et al., 2020). These costs exclude product recall and disposal costs.

2.3 *Listeria*: HISTORY AND TAXONOMY

The genus *Listeria* was historically considered to consist of only six species, namely, *L. monocytogenes, L. grayi, L. innocua, L. ivanovii, L. welshimeri* and *L. seeligeri* (Rocourt and Buchrieser, 2007, Sibanda and Buys, 2017). According to Radoshevich and Cossart (2018), it was later on, found that only two species are pathogenic, *L. monocytogenes* and *L. ivanovii*.

L. monocytogenes was first named *Bacterium monocytogenes*; because of its characteristic monocytosis. The pathogen was isolated from guinea pigs (Radoshevich and Cossart, 2018). The microorganism was later renamed *Listerella hepatolytica* in 1927 by Prie who later in 1940 renamed the organism *L. monocytogenes* (Farber and Peterkin, 1991). Following its initial description, Gill and Nyfeldt in 1929 confirmed its isolations from sheep and humans (Farber and Peterkin, 1991). The bacterium was used as a model for the investigation of cell-mediated immunity (Farber and Peterkin, 1991).

The interest in *L. monocytogenes* increased hastily around the 1980s because of the growing trend of incidences of foodborne outbreaks, prompting the food manufacturers, government, and researchers to conduct more research on the pathogen hence an increase in published literature (Farber and Peterkin, 1991).

2.3.1 Characteristics, ecology of growth and survival

Listeria spp. are small rod-shaped facultative anaerobes (diameter of 0.5 μm and length 1-2 μm) (Rocourt and Buchrieser, 2007, Radoshevich and Cossart, 2018). The cells are usually found as single units; still, they can be expressed as short chains arranged in 'V' or 'Y' forms or palisades (Rocourt and Buchrieser, 2007). Most of the species are resistant to environmental stresses which make *L. monocytogenes* a concern in the food industry (Radoshevich and Cossart, 2018). *L. monocytogenes* is an intracellular (+) non-spore former pathogen which can grow between temperatures of -0.5℃ to 50℃ (Farber and Peterkin, 1991, McLaughlin et al., 2011, Radoshevich and Cossart, 2018). It has been described as a multifaceted pathogen with an ability to switch between the environment, food, and host (McLaughlin et al., 2011).

L. monocytogenes occurs in agricultural, aquaculture, and food processing environments (Buchanan et al., 2017). The pathogen occurs naturally in the plant, soil, and surface water samples, in silage, sewage, and faeces (Farber and Peterkin, 1991, Radoshevich and Cossart, 2018). According to Buchanan et al. (2017), the pathogen occurs in the abdominal tract in humans, and most people are carriers of the bacteria. The microorganism's ability to acclimatize to a wide range of environments may justify its wide distribution in nature, as well as synchronising the shift from soil-dwelling saprophyte to a lethal pathogen (McLaughlin et al., 2011).

Growth and survival of *L. monocytogenes* are highly dependent on intrinsic and extrinsic factors (Chen et al., 2018). The organism grows in temperatures between -1.5℃ and 50℃ (Farber and Peterkin, 1991), and exhibit tumbling and motility as survival mechanisms. The organism shows its tumbling and motility in narrow temperatures, as shown in Table 2.2. below, due to its peritrichous flagella (Farber and Peterkin, 1991)

Table 2.2: Growth requirements of *Listeria monocytogenes* (temperature, pH, water activity) (Farber and Peterkin, 1991)

Parameters	**Minimum**	**Optimal**	**Maximum**
Temperature, ℃	-1.5	30-37	50
pH	4.0	6.0-8.0	9.6
Water Activity	0.90	0.97	-

In addition to the above growth requirements, the food composition, such as oil or fat and water between particulates of the food, may support growth. Also, food micro-structure may affect the survival and growth of *L. monocytogenes* (Buchanan et al., 2017).

2.3.2 Evolutional lineages of *L. monocytogenes*

An in-depth comprehension of the evolution and ecology of *L. monocytogenes* is paramount in source tracking and attribution in cases of foodborne disease outbreaks (Orsi et al., 2011). *L. monocytogenes* strains are characterised into lineages and subgroups according to their inherent conserved gene-specific markers (Rocourt and Buchrieser, 2007). According to Rocourt and Buchrieser (2007), each species of the organism is defined by the presence or absence of specific genes. DNA/DNA hybridization studies have revealed the genetic content and evolution of species of the pathogen being at the forefront of serogrouping (Roberts et al., 2006, Rocourt and Buchrieser, 2007, Orsi et al., 2011).

Genotypic and phenotypic approaches such as ribotyping and pulse-field electrophoresis, have made it possible for the classification of *L. monocytogenes* isolates into lineages

(Rocourt and Buchrieser, 2007, Orsi et al., 2011). Previously, *L. monocytogenes* were grouped into only three lineages, termed lineage I, II, and III (Roberts et al., 2006, Rocourt and Buchrieser, 2007), and later lineage IV was discovered (Roberts et al., 2006, Orsi et al., 2011). The ecological niches of the different lineages are somewhat different but overlapping (Orsi et al., 2011).The lineages and sources of *L. momocytogenes* isolates are shown in Figure 2.1.

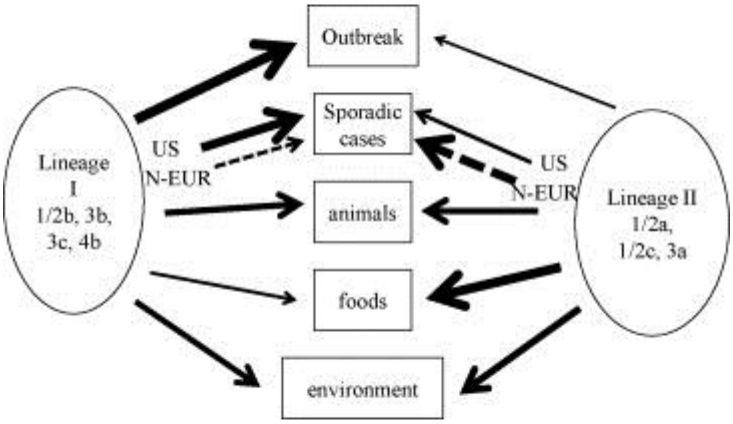


Figure 2.1: Lineages, serotypes and sources of *Listeria monocytogenes* isolates (Orsi et al., 2011)

Lineages I and II serotypes are frequently isolated from several sources, while lineage III and IV serotypes are reported to be rarely isolated and predominantly from animal sources (Orsi et al., 2011). Lineage I serotypes have been connected with most human listeriosis cases, and they were found to be overrepresented among human isolates (Chen et al., 2009, Orsi et al., 2011). Lineage II serotypes are frequently isolated from food and food environments, from the natural and farm environments as well as from animal listeriosis cases (Graves et al., 2005, Hong et al., 2007, Chen et al., 2009). Orsi et al. (2011) stated that these lineage serotypes are abundant in the food and food production environments, with several studies attaching this characteristic to their ability to form persistence traits.

The characterisation of *L. monocytogenes* isolates of different lineages and serotypes have revealed the phenotypic differences and unique features that help clarify the differences in the ecological and host niches of the strains (Orsi et al., 2011). The differences in the phenotypic characteristics affect the isolates potential to withstand unfavourable growth conditions, their distribution in the environment as well as their ability to cause an infection (Buncic et al., 2001, Orsi et al., 2011).

Lineage I isolates have a listeriolysin S hemolysin, a characteristic absent in other lineages, its isolates are clonal and have a low prevalence of plasmids because of its mechanisms that limit the attainment of foreign DNA by horizontal gene transfer (Rasmussen et al., 1991, Chen et al., 2006, Orsi et al., 2011). Lineage II isolates possess more plasmid; a feature that confers resistance to toxic environmental substances, hence making the strains resistant to even the bacteriocins (Orsi et al., 2011). The isolates have high recombination rates than any of the lineages, which helps to enable the strains adaptation to the various environments (Orsi et al., 2011). The strains were also found to be virulence-attenuated because of the premature stop codon mutations in *inlA* and *prfA* (Chen et al., 2006). Lineage III strains are very sensitive to environmental stress; this explains the rare isolation of the strains (Orsi et al., 2011).

Table 2.3: *Listeria monocytogenes* lineages: A summary (Orsi et al., 2011)

Lineage	**Serotypes**	**Genetic features**	**Sources**
I	1/2b, 3b, 3c, 4b	Lowest diversity and recombination among the lineages	Commonly isolated from various sources
II	1/2a, 1/2c, 3a	The most diverse lineage with high recombination levels	Food, food environments and natural environments
III	4a, 4b, 4c	Very diverse, recombination levels between those for lineage I and II	Isolates mostly from ruminants
IV	4a, 4b, 4c	Very few isolates analyses	Most isolated from ruminants

2.4 *L. monocytogenes* AND RTE FOOD PRODUCTS

Listeriosis is estimated to be 99% foodborne in the USA (Buchanan et al., 2017). Of particular importance with regards to the transmission of foodborne listeriosis is ready to eat foods (RTE), especially those that undergoes handling such as slicing and packaging after the application of a listericidal process (Buchanan et al., 2017). RTE foods are food intended for direct human consumption without the need for further cooking (EC2073, 2005). RTE foods include food that is eaten raw or handled and processed before consumption, in such a way that there is no further listericidal step (Buchanan et al., 2017).

RTE meat products have gained relevance driven by consumer demands on convenience foods (Horita et al., 2018), the demand, however, comes with increasing concern for safety and quality (Nikmaram et al., 2018). These meat products are susceptible to contamination with *L. monocytogenes* and being implicated in listeriosis outbreaks. In most cases, the pathogen contaminated food after the cooking step, usually during slicing and packaging, and the bacteria continues to multiply during the product shelf life (Nikmaram et al., 2018). Processed RTE meat products have caused many listeriosis infections and outbreaks in the USA, EU, Nigeria (Horita et al., 2018, Kaptchouang Tchatchouang et al., 2020), and in South Africa (WHO, 2018).

According to Buchanan et al. (2017), occasional listeriosis outbreaks have commonly been connected to RTE food products that are kept for prolonged periods at chilled temperatures. Chill storage allows the growth of *L. monocytogenes*, allowing it to reach high numbers before the time of consumption (De Oliveira et al., 2011, Lund, 2015). United States Department of Agriculture- Food Safety and Inspection Services have reported that chances of finding RTE food products, such as deli meats, positive for *L. monocytogenes* significantly increase when the food contact surface is positive for *L. monocytogenes*. Several vehicles may transmit *L. monocytogenes* into processing environments, and the organism may persist due to poor cleaning and sanitation, poor design, and poor food safety management (Buchanan et al., 2017). According to Ronholm et al. (2016), the pathogen can exist in food production areas for a prolonged period.

Food can be contaminated throughout different stages of production; from farm to processing, to storage, transport, and at the consumers' disposal (Elkhishin et al., 2017). To give assurance of food safety, food producers should implement effective food safety management systems, and they should never rely solely on end-product testing (Nychas et al., 2016).

Good combination of food safety interventions should be applied to mitigate the potential contamination of RTE foods with *L. monocytogenes* (De Oliveira et al., 2011, Lund, 2015). These include frequent food contact sanitation and testing, thorough inspection and monitoring as well as controlling critical parameters throughout the food life cycle (De Oliveira et al., 2011, Lund, 2015, Nychas et al., 2016). The life cycle of the food product should be monitored and extended beyond just the processing stage but even to the retailer, the consumers' storage, and preparation facilities (Nychas et al., 2016, Uyttendaele et al., 2016).

2.4.1 *L. monocytogenes* **in polony**

A South African bologna sausage, commonly known as polony, was the cause of listeriosis outbreak in the country (Dramowski et al., 2018). Polony is the most popular, highly consumed, and cheapest RTE meat product in South Africa (Cluff et al., 2017). The product is affordable because it is produced from a combination of cheap meat cut, mostly meat trimmings, and edible offal. In 2015, the sales of polony were 107 000 tonnes, contributing significantly to the country's economy (Cluff et al., 2017).

Polony is a commuted, cured, and heat-treated RTE meat product (SABS885, 2011). Basic polony ingredients include beef, pork fat, iced water, soy protein, NaCl, spices, sodium tri-polyphosphate, and sodium nitrite (Cluff et al., 2017). According to SABS885 (2011), the minimum total meat protein, actual meat content, and actual lean meat of the product should be 60%, 25%, and 15% respectively. The maximum added rinds and chicken skin should be 15% and 40% respectively (SABS885, 2011). The Department of Agriculture, Forestry, and Fisheries, RSA regulation for compulsory specifications for processed meat products states that the composition of polony shall be as specified in the SANS 885 standard.

According SABS885 (2011); the standard for processed meats, total viable count of polony should be less than 10^6 CFUg^{-1} , *E. coli* < 10 CFU, *Salmonella* should be absent in 25 g of the sample, and *S. aureus* should be <20 CFUg^{-1} while *L. monocytogenes* should be <100 CFUg^{-1}

2.4.2 Polony production

Mincing; the meat (beef offcuts, kidneys, hearts, chicken skin, or pork) is chopped in a mincer, mincer plate diameter of 5mm. Meat used should be boneless and free from bone chips, gristles, and cartilages.

Emulsification; is the extensive comminution of the meat, pork fat, iced water, and soya protein in a bowl cutter to achieve a homogenous batter. During the process, large particles of the protein sarcolemma are transformed into a homogeneous paste, the salts, iced water, nitrites, and phosphates solubilise myosin and actin, hence turn into liquefied material (Mapanda, 2011, Cluff et al., 2017). Salts and spices aid in microbial growth prevention and also add flavour to the product, nitrites are curing agents, and sodium triphosphate raises product pH (Mapanda, 2011, Cluff et al., 2017).

Mixing; the minced meat and emulsified ingredients are combined in a mixer together with more spices, preservatives, salt, ice water, and sugar in the mixer to produce a batter.

Stuffing/filling; as soon as the meat batter is ready, it is filled into artificial polony casings, usually nylon casings. Leaving the batter for prolonged periods before stuffing can cause souring, leading to a reduction in pH, hence unacceptable taste and a reduction in the water holding capacity (Mapanda, 2011).

Cooking; stuffed polony emulsion is cooked in a steam cabinet or hot water to an internal temperature of 72°C. Cooking time is determined by the product's diameter. Heating firms the product as proteins gelatinise, hence giving the final product its characteristic firm, meatloaf texture.

Cooling; immediately, the product reaches the desired internal temperature; it is cooled using iced water in a cooling chamber. Cooled products are stored refrigerated.

2.5 *L. monocytogenes* RESPONSES TO ENVIRONMENTAL STRESSES ENCOUNTERED IN THE FOOD CHAIN

L. monocytogenes experience many stresses in food and food-related niches, most of which are intended at food preservation and the production of safe food to the consumers (Boor, 2006). In this context, the word 'stress' is used to refer to any environmental perturbation that negatively impacts cell growth rate and survival (NicAogáin and O'Byrne, 2016). The stresses that *L. monocytogenes* encounters include low water activity, osmotic shock, mainly due to the addition of preservatives such as salt, cold stresses during refrigeration storage, heat stresses during thermal treatments and low pH (Boor, 2006, NicAogáin and O'Byrne, 2016).

However, *L. monocytogenes* is versatile and highly adaptable to a variety of environmental stresses along the food chain, hence improved control measures are required, particularly in ready to eat meat products (NicAogáin and O'Byrne, 2016, Buchanan et al., 2017). The pathogen has been reported to survive at 3M NaCl concentrations, which is too high for most bacteria, and can grow at temperatures of -0.4℃ (NicAogáin and O'Byrne, 2016), as well as at low pH and low water activity (NicAogáin and O'Byrne, 2016).

2.5.1 *L. monocytogenes* general stress response

The capability of *L. monocytogenes* to live and persist in the food and food production environments, is supported by its stress response mechanism (Boor, 2006, NicAogáin and O'Byrne, 2016). The general bacterial stress response explains how bacteria that have been exposed to mild stress adapt and become resistant to harsher stress conditions and at times even develop cross-protection to other stresses (Boor, 2006). Tolerance and adaptation to stress can be explained partially by the general stress response of the microorganism (Moorhead and Dykes, 2003). The mechanism of this kind of adaptation is that the gene and protein expression profiles of the cell are changed as the stress-responsive $sigB$ (σ^B) is activated and consequently induces the activity of stress response proteins in the cell (Ferreira et al., 2014).

Generally, bacterial gene expression alterations are controlled at the transcriptional level through association changes between the catalytic core of the RNA polymerase and the sigma factors in the cells and consequently reconfigure gene transcription to provide homeostatic and protective functions to cope with stress, as shown in Figure 2.2 (Moorhead and Dykes, 2003, Boor, 2006). RNA polymerase is an enzyme that is in control for identifying suitable genes under specific environmental conditions, and for generating the mRNA transcripts that can be translated into new proteins (Boor, 2006). When a sigma factor links with a core RNA polymerase to form an RNA polymerase holoenzyme, it leads the holoenzyme to identify preserved DNA motifs called promoter sites that head gene sequences (Boor, 2006).

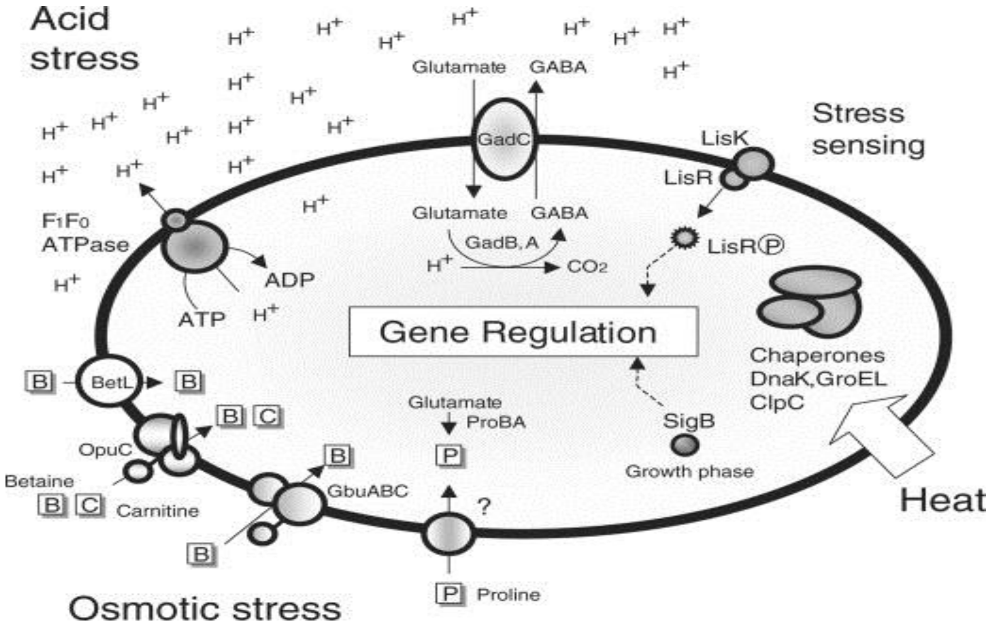


Figure 2.2: The general stress response of microorganisms against various stresses (Boor, 2006)

In *L. monocytogenes*, sigma factor, known and denoted *SigB* (σ^B), has been identified as a major contributor and regulon to the survival of the pathogen under stressful environmental conditions (Boor, 2006, Kazmierczak et al., 2006, Liu et al., 2019). Gene expression in *L. monocytogenes* is controlled by transcriptional regulators, together with the alternative sigma factors and repressors (Liu et al., 2019). The stress conditions induce the activity of σ^B as indicated by enhanced transcription of the σ^B-dependent genes *opuCA* and *gadA* (Kazmierczak et al., 2006). According to Liu et al. (2019), strains of *L. monocytogenes* have diverse genomic characteristics, with differing σ^B regulons.

The complex and fine-tuned interplay between the regulatory circuits of σ^{B}, other transcriptional regulators and other sigma factors, is the key for successful survival and adaptation of the pathogen under different stresses (Liu et al., 2019).

2.5.2 Responses to heat stress

Heat is an established method of food preservation, known for its lethality, especially at elevated temperatures (Sibanda and Buys, 2017). Gahan et al. (2001) stated that exposing bacteria to mild hot temperatures leads to the induction of a heat shock response that enables the pathogen to withstand temperatures that are usually lethal. The authors further stated that *L. monocytogenes* could elicit a heat shock response and prompt the production of heat shock proteins *Dnak, DnaJ, GroES,* and *GroEL* (Gahan et al., 2001), which protect the bacteria from cell death due to heat (Hill et al., 2002).

Heat shock proteins are molecular chaperones and proteases, whose abundance swiftly rises upon exposure to stress (Roncarati and Scarlato, 2017). Heat shock proteins promotes protein folding and rescue proteins from degradation and deleterious aggregation (van der Veen et al., 2007, Roncarati and Scarlato, 2017). The molecular chaperone proteins *GroES* and *GroEL* are produced at raised levels by the bacteria under heat stress, *GroEL* in particular, is the most highly conserved protein in nature maintaining the protein integrity under harsh environmental conditions (Gahan et al., 2001).

Upon abrupt temperature surge, heat shock proteins rapidly accumulate in the cell to help it adapt to the new environment (Roncarati and Scarlato, 2017). *L. monocytogenes* has three specific heat shock response mechanisms, namely, the class I and III heat shock response, and the class II stress response (van der Veen et al., 2007).

Class I heat shock genes are controlled by the HrcA repressor, through binding to the CIRCE operator sequence (TTAGCACTC-N_9-GAGTGCTAA) preceding this class of genes. Class I heat shock genes are *dnaK, dnaJ, groES,* and *groEL* encoding chaperones (van der Veen et al., 2007). Class III heat shock genes encode chaperones and ATP-dependent *Clp* proteases, which destroy impaired proteins (van der Veen et al., 2007). The class *dis* regulated by the *CtsR* repressor, binds to a hepta-nucleotide in the promoter region

(A/GGTCAAANANA/GGTCAAA). Class II stress genes encode the general stress proteins, of which are regulated by the alternative sigma factor *SigB* (van der Veen et al., 2007).

Some proteins are also involved in thermo-tolerance, such as the family of *Clp* proteases, which are involved in heat tolerance of microorganisms (Hill et al., 2002). Hill et al. (2002) further stated that the gene encoding *ClpC* in *L. monocytogenes* was recognized in a transposon mutant exhibiting sensitivity to low iron conditions. Disturbance of *ClpC* leads to induced thermo-tolerance and increased sensitivity to high salt and low iron conditions (Hill et al., 2002). Several studies have revealed significant strain diversity in thermo-tolerance of *L. monocytogenes* (Buncic et al., 2001).

Table 2.4: Regulatory mechanisms controlling heat shock genes transcription (van der Veen et al., 2007)

Organism	**Transcriptional regulator**	**Regulated genes**
Listeria monocytogenes	HrcA	Class I: *groESL*, *dnaK*
	σ^B	Class II: general stress proteins
	CtsR	Class III: clpP, *clpB*, *hslU*

2.5.3 Responses to cold stress

Chilled storage is commonly used to slow bacterial growth in food; however, *L. monocytogenes* is a psychotolerant bacterium and can survive and grow at low temperatures (Bayles et al., 1996). It is thus vital to comprehend how the pathogen can multiply at low temperatures. Low temperatures induce the synthesis of some cellular proteins, as the bacteria adapt to the unfavourable temperature (Hébraud and Guzzo, 2000), the proteins maintain the cellular membrane fluidity, and macromolecules such as ribosomes (Wemekamp-Kamphuis et al., 2002).

In order to survive at low temperatures, bacteria produce cold shock proteins (*Csps*), and in contrast, during balanced growth at low temperatures cold acclimation proteins (*Caps*) are stimulated (Bayles et al., 1996, Hébraud and Guzzo, 2000). According to Schmid et al. (2009), *Csps* family comprises of minor nucleic acid binding proteins that regulates bacterial physiological processes. The proteins are broadly dispersed in prokaryotes, including *L. monocytogenes* (Schmid et al., 2009).

Csps are a subset of proteins, mostly identified as *7-kDa* proteins that act as RNA chaperones to minimise secondary folding (Hébraud and Guzzo, 2000, Wemekamp-Kamphuis et al., 2002). They act as transcription activators or anti-terminators hence stimulate the production of non-7-*kDa* cold-induced proteins (Wemekamp-Kamphuis et al., 2002). *Csps* assist in binding RNA and DNA to enable the cell processes such as replication, transcription, and translation (Schmid et al., 2009). Wemekamp-Kamphuis et al. (2002) stated that *Csps* can be induced by other environmental stresses and can protect the cells against freezing. This process is shown in Figure 2.3

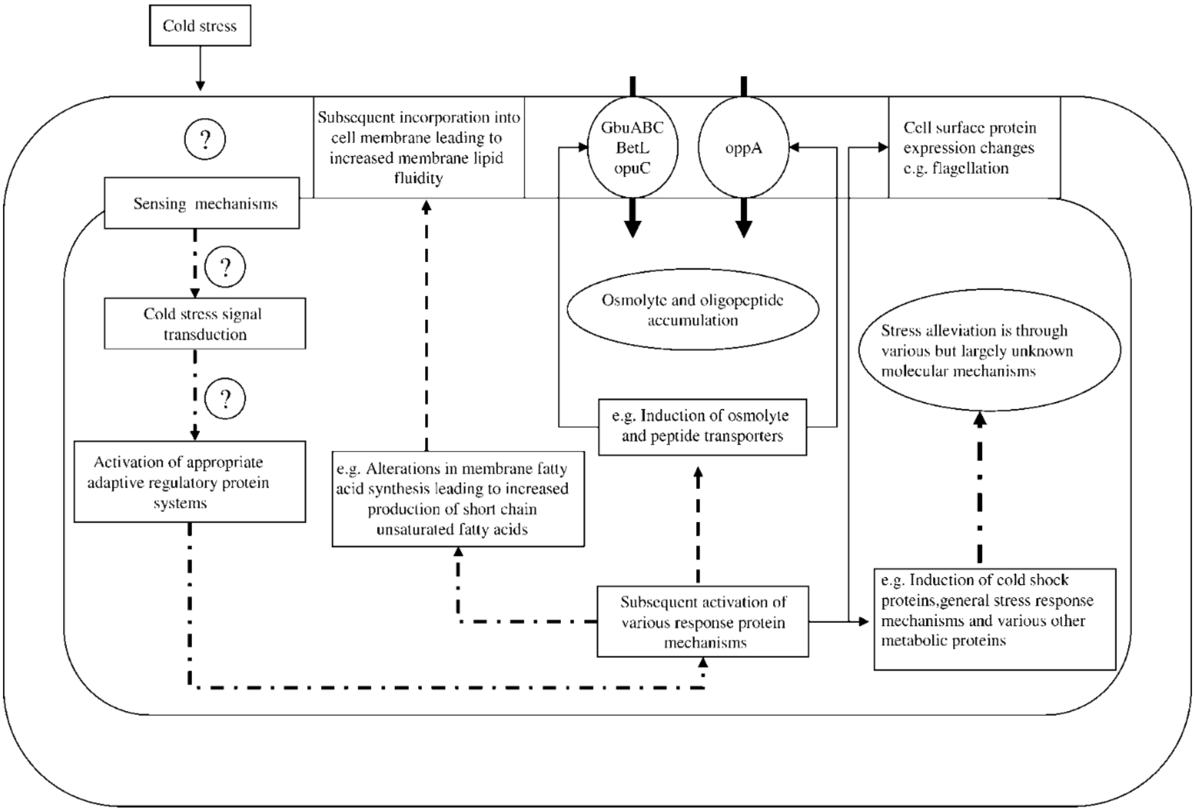


Figure 2.3: The outline of a sequence of molecular events during the cold adaptation process in *Listeria monocytogenes* (Tasara and Stephan, 2006)

According to Wemekamp-Kamphuis et al. (2002), adaptation in *L. monocytogenes* at refrigeration temperatures is also made possible by the modification of the splitting methyl end of the fatty acid from the *iso*-branching and by the reduction of the fatty acid chain length, which causes an increase in the ante-*iso* C15:0 fatty acids. Wemekamp-Kamphuis et al. (2002) further stated that the accumulation of the osmolytes betaine and carnitine stimulate growth at low temperatures.

2.5.4 Food safety implications of *L. monocytogenes* stress responses

Exposure of pathogens such as *L. monocytogenes* to some environmental and preservation stresses may compromise the safety of processed foods (Lou and Yousef, 1996) as the pathogens are capable of developing adaptive responses to prevent cell death. The adaptation responses are believed to be responsible for the frequent occurrences of deviations during heat treatments that are observed in the exponential model of microbiological inactivation

(Sergelidis and Abrahim, 2009). The responses also allow for resistance to the common food preservation techniques such as thermal processes and refrigeration and lead to enhanced survival in the food chain (Sibanda and Buys, 2017) causing a serious threat to food safety.

When bacterial cells are exposed to a moderate increase in temperature, their heat resistance is increased (Sergelidis and Abrahim, 2009). According to Sergelidis and Abrahim (2009), heating meat and meat products slowly to a final internal temperature also increase the microbial heat resistance in naturally contaminated foods. The bacterial population usually contain cells that have been impaired by sub-lethal injury due to prior exposure to adverse conditions of the processing environment (Jasson et al., 2007). These injured cells may repair and re-grow in food and gain pathogenicity thus posing a health hazard; however, the detection and enumeration of these cells are rather challenging and problematic (Jasson et al., 2007).

According to Jasson et al. (2007), injured bacterial cells are sensitive to agents to which they would otherwise show resistance. The conventional selective media used in food analysis contain selective ingredients such as bile salts and acriflavin that can be harmful to injured cells, this leads to underestimation as the cells fail to grow or even retarded growth due to them having a longer lag phase (Jasson et al., 2007). Injured cells, therefore, cause a risk of false-negative or underestimation of results which is a hazard to health (Jasson et al., 2007).

2.6 PREDICTIVE MICROBIOLOGY

Predictive microbiology is a scientific field that encompasses studying microbial growth in food niches as well as the development of mathematical equations that can be used to predict growth under specified conditions (González et al., 2019). According to Ross and McMeekin (1994), the principal foundation of predictive food microbiology is that microbial responses to environmental factors are reproducible, and from past observations, microbial responses in similar environments can be predicted. Predictive microbiology tries to estimate the numerical evolution of microorganisms over time; this is attained over the usage of models that comprise of tools governing microbiological population kinetics (Ferrer, 2009).

Predictive food models can be classified as primary, secondary, and tertiary models (González et al., 2019). Primary models use mathematics to describe the lag parameters, average growth rate, and initial concentration of cells (Perez-Rodriguez and Valero, 2013). They involve the selection and development of the most suitable mathematical functions that can describe microbiological response or observations (González et al., 2019). Approaches such as regression analysis and the Bayesian approach are usually used to develop primary models (González et al., 2019).

Secondary predictive models designate the response of factors of the primary model to changes in the environmental factors (Perez-Rodriguez and Valero, 2013) and the effect of environmental factors on growth and inactivation rate (McMeekin et al., 2013). Tertiary models are user-friendly software applications that were developed from primary and secondary models; the applications can be used by non-modellers.

Predictive models can be used in determining the food shelf life, as well as throughout the food value addition chain to identify critical points and to give an understanding of how environmental factors influence the growth kinetics of bacteria (Fakruddin et al., 2011). The crucial goal of predictive microbiology is to help the food technologist choose the most appropriate stress combinations to come up with a stable formulation (Brul et al., 2007).

When used objectively, bacteriological inactivation/growth predictions can be used to evaluate the processing effects and the operations involved in the food chain on the microbiological safety and quality of foods (Fakruddin et al., 2011). Application of this knowledge can provide cost-effective substitutes for traditional microbiological testing and when appropriately constructed and applied, can be viewed and used as the ultimate rapid method (McMeekin et al., 2008).

2.6.1 Computer Software applications in Predictive Microbiology

Advancements and research in predictive microbiology have in recent years yielded an increase in publications on predictive microbiology, intensive research in the field, and the development of user-friendly software tools (Tenenhaus-Aziza and Ellouze, 2015, González et al., 2019). According to González et al. (2019), the developed software tools are offering a wide range of solutions as they can be applied at any point in the food chain. Tamplin et al. (2003) also stated that the software programs have enhanced the use of microbiological models by the food industry stakeholders as they are well designed to allow users to define input parameters and easily observe model outputs in graphics formats

Predictive models in different software applications may be used to estimate the shelf life of RTE meat products (De Cesare et al., 2018). The models can accurately estimate the growth of *L. monocytogenes* in response to relevant stresses applicable to a specific food product (Mejlholm and Dalgaard, 2015). Seafood Spoilage and Safety Predictor software is recommended by the Danish Veterinary and Food Administration (DVFA) for use in predicting *L. monocytogenes* in food products. The Food Safety Spoilage Predictor (FSSP) model accounts for 12 environmental parameters and has been validated for use in different food types with different preserving parameters (Mejlholm and Dalgaard, 2015).

Application software permits specialists as well as people without expertise in the mathematics of predictive microbiology to acquire data from models quickly and more conveniently (Dalgaard et al., 2002). Some software tools can be used to study the relationship between product contamination and the manufacturing process as well as the possibility of the contaminated product to cause an illness when consumed (Tenenhaus-Aziza, 2015). At industrialized levels, the models can be used to authenticate new processing conditions and sampling plans (Tenenhaus-Aziza, 2015). More predictive models and their uses are detailed in Table 2.5 below.

Table 2.5: General information of the selected predictive microbiology computer software (Tenenhaus-Aziza and Ellouze, 2015)

Software	**Accessibility**	**Description**	**Targeted users**
ComBase	Free, internet access http://www.combase.cc	It describes the growth and inactivation of microorganisms in culture media and food with more than 50 000 records and 15 microorganisms	FBO, researchers, teachers, students, government
Food Spoilage and Safety Predictor	Free, downloadable http://fssp.food.dtu.dk	It predicts the growth of 3 pathogens, 3 spoilers and generic microorganisms in meat and seafood. The program is available in 18 languages and encompasses 12 environmental parameters.	FBO, researchers, teachers, students, government
Listeria Meat Model	Commercial, downloadable www.cpmf2.be	It predicts the growth of *Listeria* in meat and meat products	FBO, government
MicroHibro	Free, internet access www.microhibro.com	The program was developed at the University of Cordoba in Spain. It can be used to assess and validate primary model parameters based on experimental data.	FBO, researchers, teachers, students, government

According to González et al. (2019), the application of modelling outcomes in real case scenarios is a challenge due to limited knowledge and understanding of the interpretation of modelling outcomes. Effective use of predictive models is largely impacted by users' ability to retrieve and exchange information and through model evaluation by comparing predictions against observations from experiments (González et al., 2019).

Prior application of the models, it is important to determine their accuracy and performance concerning the food environment and microorganism of concern. Indices of model performance such as the bias factor (B_f), accuracy factor (A_f), root mean square error (RMSE) and coefficient of determination (R^2) are commonly used to determine the performance of the model through comparing observed bacterial data to predicted data (Ross and McMeekin, 1994, Ross et al., 2000, Mejlholm and Dalgaard, 2015).

Bias factor (B_f) refers to the average ratio of predicted versus experimental data; it shows how the predicted data lie above or below observed data (Ross, 1996, Heo et al., 2010). B_f of 1 indicates perfect agreement between predicted and observed value and a B_f of less than 1 shows a fail-dangerous prediction while B_f of more than 1 shows a fail-safe prediction. The following equation calculates it;

$$\left\lfloor B_f = 10\left\{\sum log\left(\frac{\frac{predicted}{observed}}{n}\right)\right\}\right]$$ **Equation 2.1**

Where; predicted is the predicted data by the model, observed is the observed data from challenge study and n is the number of observations.

The accuracy factor measures how close predictions are to observations (Ross, 1996). It shows how close predicted data is to experimental data, and the value is always equal or greater than 1, and the more the value gets higher than 1, the less precise the prediction (Heo et al., 2010).

$$\left\lfloor A_f = 10\left\{\sum log\left(\frac{\frac{predicted}{observed}}{n}\right)\right\}\right]$$ **Equation 2.2**

Where; predicted is the predicted data by the model, observed is the observed data from the challenge test and n is the number of observations.

The Root mean square error (RMSE) is the general goodness of fit that shows the average difference between the predicted and observed data (Ross, 1996).

$$RMSE = \sqrt{\sum \frac{(observed - predicted)^2}{n}}$$ **Equation 2.3**

Where; predicted is the predicted data by the model, observed is the observed data from the challenge study and n is the number of observations.

Coefficient of determination is the overall measure of the prediction and is calculated by the equation

$$\left\lfloor R^2 = 1 - \left(\frac{\sum e_i^2}{\sum (yi-y)^2}\right) \right\rfloor \qquad \textbf{Equation 2.4}$$

Where e_i^2 is the error of predicted data, y_i is predicted data and y is the average predicted data.

B_f and A_f of 1 show a perfect agreement between predicted and observed data. $B_f>1$ indicates that the predicted growth was longer than the observed growth and that the model over-predicted growth. The over-prediction suggests that prediction is fail-safe, meaning that the model predicted growth while there was no growth. B_f values of 0.87 to 1.43 are unacceptable and show that the model is not suitable for that particular prediction. The larger the A_f, the less accurate predictions are. RMSE value indicates the error of the estimate, and the larger the value, the more unsuitable the model is for the prediction. R^2 close to 1 indicates a good statistical fit of predicted to observed values (Ross, 1996).

2.7 CONCLUSION

The previous listeriosis outbreak in South Africa prompted the food industry to make food safety a priority through the implementation of more stringent food safety management systems. This involves ensuring that the current processing methods are sufficient to inactivate pathogens of concern, application of good sanitary practices, and pre-emptive pathogen detection procedures (Allam et al., 2018). In polony production, the industry has to ensure that *L. monocytogenes* inactivation is conducted in in-situ and in-vivo and minimise reliance on evidence from experiments conducted on laboratory media. Previous studies indicate that *L. monocytogenes* can survive processing hurdles and can survive chilled storage; however, strain heterogeneity affects survival and growth, hence the need to always account for strain variability in studies. Predictive microbiology models, when appropriately applied, can aid in pathogen detection. The models can accurately predict the growth rate and growth boundaries of *L. monocytogenes* in response to relevant stresses applicable to a specific food product (Mejlholm and Dalgaard, 2015). The models, when validated, can provide accurate information on microbiological growth kinetics. The food business operators can, therefore, use the models as they are quick and cheap as compared to traditional microbiological testing (Mejlholm and Dalgaard, 2015)

2.8 HYPOTHESES

2.8.1 Hypotheses 1

The survival of *L. monocytogenes* strains during heat treatment will depend on the inherent characteristics of the strains. The variability in thermal resistance is due to differences in the genetic sequences of the strains (Ferreira et al., 2014). Inheritable mutations due to *L. monocytogenes* strain response to sub-lethal conditions result in gene and protein profile changes (Edelson-Mammel et al., 2005, Ferreira et al., 2014). The gene and protein expression profiles of the cell are changed as the stress-responsive *sigB* (σ^B) is activated and consequently induces the activity of stress response proteins in the cell (Ferreira et al., 2014), making some cells more resistant than others.

2.8.2 Hypotheses 2

Predictive models will accurately estimate the growth of *L. monocytogenes* in polony. The models have been developed from scientific outputs and mathematical formulas, and the basis of the prediction is that microbial responses can be reproduced (Ross and McMeekin, 1994, Ross and McMeekin, 2003). Factors that affect microbial growth (intrinsic and extrinsic) are accounted for by the models, and they can accurately predict the growth rate and growth boundaries of *L. monocytogenes* in response to relevant stresses applicable to a specific food product (Mejlholm and Dalgaard, 2015).

2.9 OBJECTIVES

2.9.1 Objective 1

To determine the effect of polony processing on *L. monocytogenes* strains with the aim of enhancing the safety of the product.

2.9.2 Objective 2

To apply predictive models to estimate the growth of *L. monocytogenes* strains in polony during shelf life with the aim of enhancing the safety of the product.

CHAPTER 3: RESEARCH

3.1 A BRIEF INTRODUCTION TO THE RESEARCH STUDY

The study intended at determining the effectiveness of the conventional polony processing method in the inactivation of *L. monocytogenes* strains, and to apply suitable predictive models to estimate the growth of the pathogen during the product shelf life. The study findings are important to the polony industry as they provide food safety assurance data and can aid in the control and prevention of *L. monocytogenes* growth and consequently prevent the occurrences of listeriosis outbreaks. The study was divided into two segments; the first part was the pathogen inactivation challenge study in which raw polony emulsion was artificially contaminated with *L. monocytogenes* strains and processed to determine the effectiveness of processing in inactivating the strains of the pathogen. The second part of the study was the application of predictive microbiology tertiary models in estimating the growth of *L. monocytogenes* in polony during the product shelf life. This involved observing growth, applying models, and evaluating model performance. The phases are as follows;

i. Effect of polony processing on *L. monocytogenes* strains

ii. Application of predictive models in estimating the growth of *L. monocytogenes* strain in polony during the product shelf life

3.2 EFFECT OF POLONY PROCESSING ON *L. monocytogenes* STRAINS

ABSTRACT

The study investigated the effect of polony processing (heat treatment to a core temperature of 72°C and cooling to an internal temperature of 10°C) on three *L. monocytogenes* strains. The bacteriological quality (Total plate counts, lactic acid bacteria), safety (*Listeria* spp), and physicochemical characteristics (a_w, pH, moisture, and fat content) of polony emulsion and processed polony were determined. Raw polony emulsion was inoculated with *L. monocytogenes* (inoculation level 10^5 CFUg^{-1}) and processed. *L. monocytogenes* in the inoculated polony emulsion and inoculated processed polony was determined using the direct plate and MPN methods. It was confirmed that conventional polony processing effectively reduced *L. monocytogenes* to acceptable levels. All strains investigated were below the detection limit in inoculated and processed polony (log CFUg^{-1}). The effect of processing, as shown by MPN counts, varied among *L. monocytogenes* strains (p=0.0000). Strain 159/10 had the highest percentage log reduction (91%), followed by strain 732 (71%), and strain 69 had the lowest percentage log reduction (61%). The study highlights the importance of strain heterogeneity in pathogen inactivation studies for polony production as strains with low percentages of log reduction have a high re-growth potential and maybe a food safety risk.

3.2.1 INTRODUCTION

In many parts of the world, the occurrence of foodborne pathogens and their associated illnesses are frequently occurring and results in significant constraints due to mortality, morbidity, and economic losses (Olanya et al., 2019, Wang et al., 2020). The estimated annual burden of foodborne illnesses amounts to 76 million cases, 323 000 hospitalisations, and 5 000 deaths in the United States of America (Tauxe, 2002). The occurrence of many of these pathogens is a serious concern because of their wide prevalence in natural environments (Wang et al., 2020).

South Africa, in the years 2017 to 2018, experienced the world's largest listeriosis outbreak, with a total number of 1068 confirmed cases, 218 fatalities, and enormous economic losses as a result of a product recall, disposal, productivity losses, and hospitalization costs (Allam et al., 2018, Olanya et al., 2019, Smith et al., 2019). The cause of the outbreak was *L. monocytogenes* sequence type 6 (ST6) isolated from polony (Allam et al., 2018, Olanya et al., 2019, Smith et al., 2019).

Polony is a South African version of bologna sausage (Cluff et al., 2017). Similarly to most RTE meat products, heat processing is a critical step in its production aimed at inactivating *L. monocytogenes* (Lianou and Koutsoumanis, 2013, Wang et al., 2020). *L. monocytogenes* is ambiguous in nature, persistent in food processing environments, and can survive processing hurdles (Ferreira et al., 2014). According to McDermott et al. (2018), the pathogen can survive thermal processing depending on the temperature of processing, strain, culture history, the heating medium and the experimental design.

The resistance of *L. monocytogenes* to processing varies among strains due to differences in genetic lineages (Lianou and Koutsoumanis, 2013, McDermott et al., 2018). Stress responses to preservation processes induce changes in gene and protein expressions in the cells hence adaptation to the stress (Ferreira et al., 2014, Nowak et al., 2017). Strain heterogeneity is important and must be accounted for in-process calculations and risk assessments by producers (De Jesus and Whiting, 2003).

Thermal inactivation of *L. monocytogenes* has been studied; however, no studies have been made on the effect of the conventional polony processing technology on individual strains of *L. monocytogenes*. The traditional processing method is derived from data obtained from *L.*

monocytogenes studies done under laboratory conditions in culture media. According to McDermott et al. (2018), a process that kills *L. monocytogenes* in one product may not be sufficient to do so in another product due to the dynamic response of *L. monocytogenes* strains to changing environmental conditions, which affects the efficiency of conventional methods (Abee et al., 2016). More *in situ* and *in vivo* studies must be conducted to generate data that is as relevant as possible to real conditions in food and food-related environments (Lianou and Koutsoumanis, 2013, McDermott et al., 2018).

Quantifying responses of *L. monocytogenes* to conventional processing can provide information that can be used to realistically and adequately design processing treatments for the production of RTE meat products (Aryani et al., 2015). The data is also important in developing risk assessment strategies and predictive modelling (De Jesus and Whiting, 2003). During this study, we determined the effect of conventional polony processing on *L. monocytogenes* strains to enhance the safety of polony products.

3.2.2 MATERIALS AND METHODS

Polony processing

Polony emulsion was obtained, during three visits from a cold meats processing plant (Bavarian Cold Meats (PTY) LTD, Pretoria, RSA). The raw polony emulsion was transported in a cooler box with ice packs to the University of Pretoria. The emulsion was divided aseptically, 500 g each, into stomacher bags. Polony emulsion (500 g), (n=2) was transferred aseptically into a sterile (autoclaved) sausage filler (McCarter Equipment, Pretoria, RSA), and filled into 25 mm nylon polony casings (Freddy Hirsch (Pty) Ltd, Pretoria, RSA). The samples were linked and clipped to produce twenty 50 g polony samples.

The samples were cooked in an 85℃ water bath (Labotec, Midrand, RSA) to an internal temperature of 72℃ (SABS885, 2011). Cooking time was approximately 46±2 min, after which the samples were cooled in sterilised iced water to a core temperature of 10℃ for 30 min. The water and product temperatures were aseptically monitored using a thermometer (Thermocouple, Hanna Instruments, Rhode Islands, USA).

Bacterial strains

L. monocytogenes strains were used in this study; strains 69, 159/10 (Sibanda and Buys, 2017) and strain 732 isolated from polony (Department of Consumer and Food Sciences, University of Pretoria), confirmed as *L. monocytogenes* by matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry (Bruker, Germany). The stock cultures (100 µl) were inoculated of into 10 ml brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK) to activate them, and incubated at 37℃ for 24 h, and finally sub-cultured twice and streaked on BHI agar (Oxoid). To prepare a working culture, the bacterial colony was streaked on BHI agar at an incubation of 37℃ for 18 h, this was repeated twice. Individual colonies were suspended in sterile phosphate-buffered saline (Oxoid) to a cell density of 1.5×10^{8} CFUml^{-1} (McFarland 0.5) using a densitometer DEN-1/28712/2.01 (Grant Instruments, UK).

Inoculation of polony emulsion

Three Polony emulsion (500 g) (n=2) samples were inoculated with *L. monocytogenes* strains (159/10, 732, 69). Each polony emulsion was inoculated with 5 ml bacterial suspension of 1.5×10^{8} CFUml^{-1} cell density and stomached for 3 min (Stomacher 400, Art Medical Equipment (Pty) Ltd, Johannesburg, RSA). The inoculated samples were processed. For each strain, twenty 50 g samples were produced.

Bacteriological analysis of polony emulsion and processed polony

The polony emulsion and the processed polony were analysed for lactic acid bacteria and total plate counts (TPC), as well as for *Listeria* spp using direct plate counts and MPN techniques (Table 3.1). The inoculated polony emulsion and inoculated processed polony were enumerated for *L. monocytogenes* using the MPN technique and direct plate counts method.

The sample (25 g) was stomached for 3 min with 225 ml of 0.1% buffered peptone water (Oxoid) and plated on Plate count agar (Merck (Pty) LTD, Midrand, RSA) and incubated at 37℃ for 48 h (Khan et al., 2005), and on MRS agar (De Man et al., 1960), 30℃ for 48-72 h. The detection limit was 10 CFUg^{-1}. Detection of *Listeria* spp. was conducted following ISO standard 11290-1. 25 g of polony emulsion was weighed into 225 ml of half Fraser broth with supplement (Oxoid), incubated for 24 h at 30℃. After enrichment, 0.1 ml was transferred into 10 ml Fraser broth, with a supplement and incubated for 48 h at 37℃ and plated into Palcam agar (Oxoid), at 37℃ for 48 h.

For the inoculated emulsion and inoculated and processed polony, the MPN method was used to estimate *L. monocytogenes* with modifications suggested by Capita and Alonso-Calleja (2003). A 3 tube 2 steps MPN was used; 25 g of the test portion was added to half Fraser broth, stomached for 2 min and diluted to sample representatives of 1, 0.1, and 0.01 g, which were inoculated into MPN tubes containing half Fraser broth and incubated for 24 h at 30℃. The homogenate (0.1 ml) was transferred from each tube to another set of tubes containing Fraser broth, incubated at 37℃ for 24 h. Positive tubes were noted by the darkening of the broth. Ferric ammonium citrate and esculin are hydrolysed by *Listeria* and results in blackening. The number of positive to negative tubes at each dilution was used to get MPN counts from the MPN Index tables with 95% confidence limits. The counts were converted to $\log_{10}$ $MPNg^{-1}$ (Martin et al., 2004).

Table 3.1: Summary of detection and enumeration of bacteria in polony emulsion and processed polony, as well as inoculated (*Listeria monocytogenes* strains 159/10, 732, 69) polony emulsion and inoculated processed polony

Sample	**Bacterial analyses**	**Media**	**Incubation Time(h); Temperature (℃)**	**Reference**
Polony emulsion and processed polony	Total plate count	Plate Count Agar	48 h at 37℃	SABS:ISO4833 (2007)
	Lactic acid bacteria	MRS Agar (De Man et al., 1960)	48-72 h at 30℃	ISO15219 (1998)
	Listeria spp. (direct plate counts and MPN)	Half Fraser Fraser Palcam	24 h at 30℃ 48 h at 37℃ 48 h at 37℃	ISO 11290-1 (Scotter et al., 2001) (Capita and Alonso-Calleja, 2003)
Inoculated polony emulsion and inoculated processed polony	*L. monocytogenes* (Direct plate counts and MPN)	Half Fraser Fraser Palcam agar	24 h at 30℃ 48 h at 37℃ 48 h at 37℃	ISO 11290-1 (Scotter et al., 2001) (Capita and Alonso-Calleja, 2003)
	Lactic acid bacteria	MRS agar (De Man et al., 1960)	48-72 h at 30℃	ISO15219 (1998)

Physicochemical analysis

Processed polony samples were analysed for pH, water activity, fat content, and moisture content. pH was analysed using a pH meter (Instrulab Inc, Johannesburg, RSA). Water activity was analysed with a water activity meter (PawKit, Johannesburg, RSA).

The fat content of the polony samples was conducted using the Soxhlet method (AOAC 920.39-2002). The sample was dried in an oven at 102℃ for 4 h and cooled in a desiccator. 2

g of the sample was weighed into a thimble and extracted in the system using petroleum ether as a solvent. Crude fat (%) was calculated using the formula:

$$\% \text{ Fat} = \frac{\text{mass of thimble and fat (g)} - \text{mass of thimble (g)}}{\text{mass of sample (g)}} \times 100$$

Moisture content determination was conducted as per AOAC 934.01 method. 2 g of the sample was weighed into a pre-weighed crucible and dried overnight at 105℃. Dry samples were cooled in a desiccator and weighed, and the percentage moisture was calculated as:

$$\% \text{ moisture} = \frac{\text{mass of wet sample (g)} - [\text{mass of desiccator and dry sample (g)} - \text{mass of desiccator (g)}]}{\text{mass of wet sample (g)}} \times 100$$

Statistical analysis

All experiments were conducted in three independent replicates; all replicate analysis duplicated. Statistical analysis was done using the software Statgraphics Centurion XVII version 17.2.00 (Statpoint Technologies Inc.). Analysis of variance at 95% confidence level was used to test for the significant difference between *L. monocytogenes* strains in the inoculated polony before processing (log CFUg^{-1}). After processing, the pathogen was not detected (log CFUg^{-1}), making it impossible to statistically analyse the results to show the effect of processing in log CFUg^{-1} clearly. Before processing, the MPN counts were at an indefinite number according to the MPN Index table, >2.04 log MPNg^{-1}, also making it impossible to analyse results statistically. Statistical analysis was conducted on log MPNg^{-1} counts of inoculated processed polony to test for the significant difference between *L. monocytogenes* strains response as an effect of processing. Fisher's least significant difference (LSD) procedure was used.

3.2.3 RESULTS

Bacteriological and physicochemical qualities of polony emulsion and processed polony

The mean TPC and LAB of the polony emulsion were 5.50 and 4.64 log$_{10}$ CFUg^{-1}, respectively (Table 3.2). *Listeria* spp. was not detected, while *L. monocytogenes* was 1.18

$\log_{10}$ MPNg^{-1} in the polony emulsion (Table 3.2). In processed polony, the TPC, LAB and *Listeria* spp. were not detected, while *L monocytogenes* were reduced to 0.12 $\log_{10}$ MPNg^{-1} (Table 3.2). The maximum permissible level of TPC and LAB in processed meats standard in South Africa is 6 $\log_{10}$ CFUg^{-1} (SABS885, 2011). The standard further states that *L. monocytogenes* shall be absent (n=5) in processed meat products. TPC, LAB, and *Listeria* spp. in processed polony comply with the standard of the processed meat of South Africa.

The pH, a_w, and moisture content of processed polony was determined, as shown in Table 3.2. The mean pH, a_w, and moisture content of polony was 6.21, 0.95, and 68% respectively. The fat content of processed polony was 20%, the value that conforms to the standard of the processed meat in South Africa (SABS885, 2011) as outlined in Table 3.2.

Table 3.2: Effect of heat processing to an internal temperature of 72°C and cooling to 10°C on the bacteriological quality of polony samples and the polony physicochemical properties (n=6)

	Total plate counts **log$_{10}$ CFUg^{-1}**	**Lactic acid bacteria** **log$_{10}$ CFUg^{-1}**	*Listeria monocytogenes* **log$_{10}$ MPNg^{-1}**	*Listeria* **spp.** **log$_{10}$ CFUg^{-1}**	**a_w**	**pH**	**Fat%**	**Moisture %**
Before processing	5.50±0.24	4.64±0.19	1.18±0.88	nd				
After processing	nd	nd	0.12±0.01	nd	0.95±0.08	6.21±0.2	20±2	68±1.8
SANS 885	<6	<6	Absent (n=5)				≤30	

SANS 885 (SABS885, 2011), nd - not detected (*detection limit 10 CFUg^{-1}), sd - standard deviation

Effect of processing on *L. monocytogenes* strains in inoculated polony

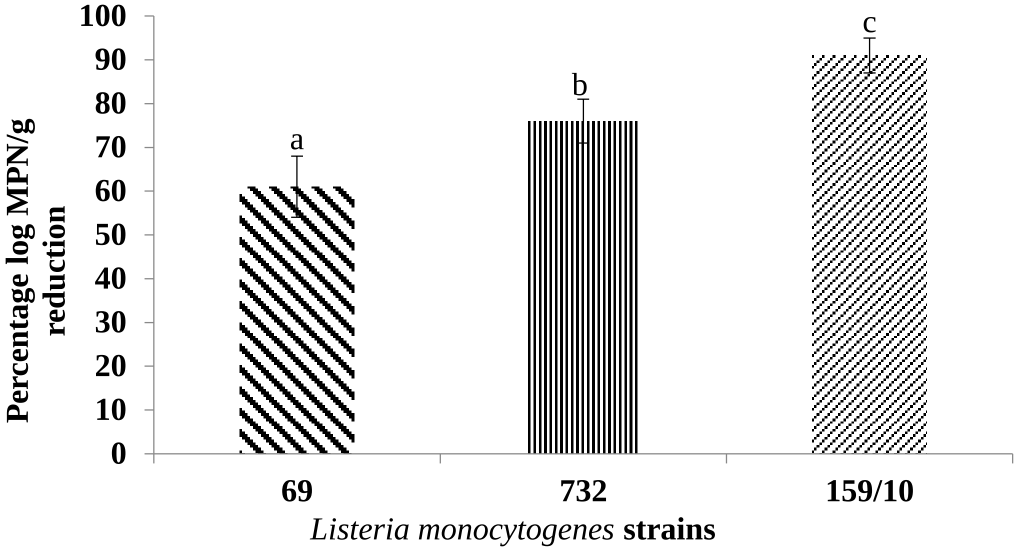
Table 3.3 shows the effect of processing on *L. monocytogenes* strains. The initial contamination levels of *L. monocytogenes* strains in inoculated polony emulsion was 5.72 $\log_{10}$ $CFUg^{-1}$, 5.83 $\log_{10}$ $CFUg^{-1,}$ and 5.79 $\log_{10}$ $CFUg^{-1}$ for strains 69, 732 and 159/10 respectively. There was no significant difference ($p=0.1556$) in the initial contamination level between the strains investigated, as shown in Table 3.3. Processing was able to reduce the counts ($CFUg^{-1}$) by more than 5 log, as all the strains were undetectable after processing (Table 3.3).

Using the MPN technique, the initial level of the three strains was >2.04 $\log_{10}$ $MPNg^{-1}$ as shown in Table 3.3. The value (>2.04 $\log_{10}$ $MPNg^{-1}$) is the maximum value as obtained from the USDA Laboratory Guidebook, MLG Appendix 2.05, MPN Table 2. The counts, as a result of processing, reduced to 0.15 $\log_{10}$ $MPNg^{-1}$, 0.48 $\log_{10}$ $MPNg^{-1}$, and 0.69 $\log_{10}$ $MPNg^{-1}$ for *L. monocytogenes* strain 159/10, 732, and 69, respectively (Table 3.3). There was a significant difference ($p=0.0000$) between the strains response, with strain 159/10 having the lowest counts depicting the highest sensitivity to processing and strain 732 and 69 showing similar processing response as shown in Table 3.3. Percentage log ($MPNg^{-1}$) reductions of *L. monocytogenes* strains inoculated in polony are shown in Figure 3.1. Processing had the least effect on strain 69, while strain 159/10 was the most affected ($p<0.05$).

Table 3.3: Effect of heat processing to a core temperature of 72°C and cooling to 10°C on the strains of *Listeria monocytogenes* in inoculated (5 log$_{10}$ CFUg^{-1}) polony (n=6)

	L. monocytogenes **strains (CFUg^{-1}) sd***			**SANS 885 requirements CFUg^{-1}**	*L. monocytogenes* **strains (MPNg^{-1})**		
Strain	**69**	**732**	**159/10**		**69**	**732**	**159/10**
Before processing	$5.72^{a}\pm0.13$	$5.83^{a}\pm0.09$	$5.79^{a}\pm0.08$	<100	>2.04	>2.04	>2.04
After processing	nd	nd	nd	Absent (n=5)	$0.69^{b}\pm0.3$	$0.48^{b}\pm0.10$	$0.15^{a}\pm0.1$

nd - not detected; *detection limit 10 CFUg^{-1}. Values with different letters in the same row indicate a significant difference ($p<0.05$) between the strains



Different letters of the alphabet indicate significance difference ($p<0.05$) in the percentage reductions of *L. monocytogenes* strains 69, 159/10 and 732

Figure 3.1: Effect of heat processing to an internal temperature of 72℃ and cooling to 10℃ on the percentage reduction of *Listeria monocytogenes* strains (69, 732, 159/10) in inoculated (5 $\log_{10}$ $CFUg^{-1}$) polony (n=6)

3.2.4 DISCUSSION

TPC and LAB in raw polony emulsion were at acceptable levels according to the standard for microbiological monitoring of meat, process hygiene, and cleaning-VPN/15/2010-01 of South Africa (DAFF, 2010). The low levels of TPC and LAB in raw polony emulsion indicated good hygiene during meat handling and processing, as well as proper implementation of food safety management systems. The same levels have been reported in raw sausage batter (Sukumaran et al., 2018).

As expected, polony processing was lethal to LAB and TPC- a more than 6 log reduction was achieved. Similar reductions have been reported during the processing of vienna sausages, ham, and other deli meats (Franz and Von Holy, 1996, Den Besten et al., 2018, Kharel et al., 2018). According to Franz and Von Holy (1996), LAB dominated the total counts of meat products and was inactivated at temperatures above 55℃. The reduction of TPC and LAB is an indication of the effectiveness of the conventional polony processing method. LAB contributes to spoilage usually indicated by slime formation, souring, and greening, the reduction of LAB is therefore, an important food quality issue (Subramaniam and Wareing, 2016).

The fat content of polony investigated was within limits set by the South African Bureau of Standards. The pH and a_w of the polony in this study were within the ranges that have been reported to support the growth of bacteria including LAB and *L. monocytogenes* (Buchanan et al., 2017, Hunt et al., 2018).

The conventional direct plating method showed that *L. monocytogenes* was not detected in the inoculated polony after thermal processing. The results indicated that processing achieved a more than 5 log CFUg^{-1} reduction of *L. monocytogenes* strains in polony. Currently, there is no published information on the reduction of *L. monocytogenes* in polony as a consequence of processing. In comparison, other authors found more than 6 log reductions of the pathogen in processed meats such as turkey bologna, salami, and ham (Zhu et al., 2005, McDermott et al., 2018).

According to Kharel et al. (2018), cooking food to an internal temperature of 70℃ for 2 min is sufficient to destroy vegetative cells of *L. monocytogenes*, this supports the current study findings as all strains of the pathogen were not detected after thermal treatment to the core temperature of 72℃ . The reduction of *L. monocytogenes* may have been due to the destructive ability of heat on the cells, causing protein denaturation, loss of cell solutes due to damage of membranes and consequently loss of cell viability and death (Russell, 2003).

The MPN method was able to detect *L. monocytogenes* in all the samples investigated due to its high sensitivity permitting enumeration of low numbers of bacteria. The MPN results showed a successful reduction of *L. monocytogenes* strains after polony processing. In a study conducted by Bersot et al. (2001) on mortadella; a Brazilian RTE meat product with almost similar ingredients and processing methods as polony, lower MPN counts (0.3 MPNg^{-1}) were reported than what was recorded in the current study. A possible explanation for the difference may be because, in the present study, high inoculation levels were used while in the mortadella study, low inoculation was used. The research, therefore, indicates that viable *L. monocytogenes* cells in polony could grow during the three months product shelf life hence pose a food safety risk.

The absence and, or low levels of *L. monocytogenes*, LAB and TPC in processed and inoculated processed polony indicate a good antibacterial effect provided by appropriate combination of the hurdles of salt, pH, water activity and heat treatment in polony production. This combination of hurdles evidently leads to the production of safe polony that may possibly have an extended shelf life due to microbial stability.

Varying percentage log reductions were achieved for *L. monocytogenes* strains. *L. monocytogenes* strain 159/10 had the highest percentage log reduction, while strain 69 had the lowest percentage log reduction. The results suggested that strain variability affects the response of the bacterium to processing. Several studies have also reported strain-specific response of *L. monocytogenes* to processing (Poimenidou et al., 2016, Zhu et al., 2017, Bucur et al., 2018, Den Besten et al., 2018). According to Den Besten et al. (2018), thermal response variations between strains maybe because of the presence or absence of some genes, as well as the difference between strains due to gene mutations. Bucur et al. (2018) stated that ClpL protease present in heat shock regulons of some strains of *L. monocytogenes* contributes to the strain's thermal processing resistance.

3.2.5 CONCLUSION

This study emphasised the importance of determination of the efficiency of the conventional polony processing in inactivating *L. monocytogenes* for the production of safe products for human consumption, and the importance of strain variability in pathogen inactivation studies. The conventional polony processing method was sufficient to inactivate all strains of *L. monocytogenes* investigated, this is a clear indication that the method, where properly and efficiently applied can yield safe polony hence prevent the occurrence of foodborne illnesses such as listeriosis. Strain variability during the pathogen inactivation studies, as shown by varying log reductions is a cause for concern, because as other strains are more resistant to processing, they may also have a better chance of survival hence a possibility of re-growth during storage. It is therefore important that producers ensure that processing is sufficient to inactivate all strains of *L. monocytogenes*.

3.3 APPLICATION OF PREDICTIVE MODELS IN ESTIMATING THE GROWTH OF *L. monocytogenes* STRAINS IN POLONY DURING THE PRODUCT SHELF LIFE

ABSTRACT

This study was aimed at determining the applicability of predictive models in predicting the growth of three strains (69, 732, and 159/10) of *L. monocytogenes* in polony. The growth of *L. monocytogenes* strains and lactic acid bacteria in polony was investigated during the product shelf life of 12 weeks at a storage temperature of 4℃. Specific maximum growth rate (μ_{max}) and lag phase duration (λ) of *L. monocytogenes* strains were determined. Three models were selected for the prediction of *L. monocytogenes* growth, and two for the prediction of LAB growth in polony. The models were selected based on the accessibility of the model and the bacteria of interest. The models used for the prediction of *L. monocytogenes* growth were; the "broth growth" model, the "growth of *L. monocytogenes* in RTE cured meats" model and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model. The models used for the prediction of LAB growth in polony were; "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" and the "growth of LAB in ground beef" models. Model performance was evaluated by comparing the predicted data and observed data. The coefficient of determination (R^2), bias factor (B_f), accuracy factor (A_f), and root mean square error (RMSE) were used as indices for the evaluation of the model performances. The "Growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model and the "Growth of *L. monocytogenes* in RTE cured meats model" gave acceptable predictions of the growth of all strains of *L. monocytogenes* investigated. The "broth growth" model gave unacceptable predictions as predicted data and observed data was further apart, making the model unsuitable for predicting the growth of *L. monocytogenes* in polony. The "Growth of *L. monocytogenes* and LAB in chilled seafood and meat products" and "Growth of *L. monocytogenes* in RTE cured meats" models can therefore be used for the prediction of the growth of *L. monocytogenes* strains in polony.

3.3.1 INTRODUCTION

Microbiological food safety is one of the major challenges in the global food market due to the re-occurrence of foodborne illness outbreaks (Dewey-Mattia et al., 2018, González et al., 2019). Of particular importance is RTE meat products, as they have been implicated in listeriosis outbreaks worldwide (Balamurugan et al., 2018, Horita et al., 2018). South Africa has had a fair share of the outbreaks, with the world's largest listeriosis outbreak in 2017-2018, in which a bologna-type sausage known as polony was the culprit (Allam et al., 2018, Olanya et al., 2019, Smith et al., 2019).

The safety of polony relies predominantly on heat processing, followed by refrigeration to preserve the product and attain a longer shelf life. However, *L. monocytogenes* as a psychrotolerant bacterium and can grow in refrigerated temperatures, allowing it to reach high numbers before the time of consumption and be a health hazard (De Oliveira et al., 2011, Lund, 2015). The South African polony industry also relies on end-product testing to assure product testing, and this has been reported to be ineffective (González et al., 2019).

The occurrence of listeriosis, coupled with risks associated with the capability of the pathogen to grow during storage, and reliance of the industry on end-product testing calls for more research and strengthening of the industry's food safety strategies. One strategy that the industry can adopt is the use of predictive microbiology models. The models are developed to estimate the numerical evolution of microorganisms over time, considering environmental factors (Ferrer, 2009, Perez-Rodriguez and Valero, 2013). The models are available in user-friendly software packages and can readily predict pathogens such as *L. monocytogenes* in food throughout the food chain (Tenenhaus-Aziza, 2015, González et al., 2019).

The application of predictive models in estimating bacterial growth in food have been explored and recommended for use in developed countries such as New Zealand and Australia (Mejlholm and Dalgaard, 2015) and companies such as Unilever (Membré and Valdramidis, 2016). Regulatory authorities such as the Danish Veterinary and Food Administration and European Commission Regulation (EC) 2073/2005 have endorsed the application of predictive models to document the growth of foodborne pathogens and spoilage organisms (Mejlholm and Dalgaard, 2007, Mejlholm et al., 2015, Mejlholm and Dalgaard, 2015, Membré and Valdramidis, 2016).

Predictive modelling is a fairly new technology in South Africa, and no work is documented on the application of the models to estimate *L. monocytogenes* growth in polony. The use of the models does not require any intense expertise, is cost-effective and robust, and when coupled with end-product testing can provide important information for food safety risk assessments (McMeekin and Ross, 2002). This could be beneficial to the polony industry, and help in the production of safe food and possibly reduce the risk of listeriosis outbreaks. The study is, therefore aimed at observing the growth of *L. monocytogenes* in polony, and determining the applicability of predictive tertiary models to predict the growth of the pathogen in polony.

3.3.2 MATERIALS AND METHODS

Polony processing

Polony emulsion was obtained, during 3 visits from a cold meats processing plant (Bavarian Cold Meats (PTY) LTD, Pretoria, RSA). The raw polony emulsion was transported in a cooler box with ice packs to the University of Pretoria. The emulsion was divided aseptically, 500 g each, into stomacher bags. Polony emulsion (500 g), (n=2) was transferred aseptically into a sterile (autoclaved) sausage filler (McCarter Equipment, Pretoria, RSA), and filled into 25 mm nylon polony casings (Freddy Hirsch (Pty) Ltd, Pretoria, RSA). The samples were linked and clipped to produce twenty 50 g polony samples.

The samples were cooked in an 85℃ water bath (Labotec, Midrand, RSA) to a core temperature of 72℃ . Cooking time was approximately 46±2 min, after which the samples were cooled in sterilised iced water to an internal temperature of 10℃ for 30 min. The water and product temperatures were aseptically monitored using a thermometer (Thermocouple, Hanna Instruments, Rhode Islands, USA).

Bacterial strains

L. monocytogenes strains were used in this study; 69, 159/10 (Sibanda and Buys, 2017), and strain 732 isolated from polony (Department of Consumer and Food Sciences, University of Pretoria), confirmed as *L. monocytogenes* by MALDI-ToF (Bruker, Germany). 100 µl of stock culture was inoculated into 10 ml brain heart infusion (BHI) broth (Oxoid, Basingstoke, UK) to activate the culture, and incubated at 37°C for 24 h. The bacterial culture was sub-cultured twice and streaked on BHI agar (Oxoid). To prepare a working culture, the bacterial colony was streaked on BHI agar and incubated at 37°C for 18 h, this was repeated twice. Individual colonies were suspended in sterile phosphate-buffered saline (Oxoid) to a cell density of 1.5×10^{8} CFUml^{-1} (McFarland 0.5) using a densitometer DEN-1/28712/2.01 (Grant Instruments, UK).

Inoculation of polony emulsion

Three Polony emulsion (500 g) (n=2) samples were inoculated with *L. monocytogenes* strains (159/10, 732, 69). Each polony emulsion was inoculated with 5 ml bacterial suspension and stomached for 3 min (Stomacher 400, Art Medical Equipment (Pty) Ltd, Johannesburg, RSA). The inoculated samples were processed as described in section 3.3.2. For each strain, twenty 50 g samples were produced.

Bacteriological analysis of polony emulsion and processed polony

Processed polony and processed inoculated polony were stored at 4°C for 12 weeks and analysed at day 0 (immediately after processing) and every fortnight. The processed polony and processed inoculated polony were analysed for lactic acid bacteria and *L. monocytogenes* (Table 3.1). The sample (25 g) was stomached for 3 min with 225 ml of 0.1% buffered peptone water (Oxoid) and plated on MRS agar (De Man et al., 1960), 30°C for 48-72 h. The detection limit was 10 CFUg^{-1}.

The MPN method was used to estimate *L. monocytogenes* with modifications suggested by Capita and Alonso-Calleja (2003) . A 3 tube 2 steps MPN was used; 25 g of the test portion was added to half Fraser broth, stomached for 2 min and diluted to sample representatives of 1, 0.1, and 0.01 g, which were inoculated into MPN tubes containing half Fraser broth and incubated for 24 h at 30°C. The homogenate (0.1 ml) was transferred from each tube to another set of tubes containing Fraser broth, incubated at 37°C for 24 h. Positive tubes were noted by the darkening of the broth. Ferric ammonium citrate and esculin are hydrolysed by *Listeria* and results in blackening. The number of positive to negative tubes at each dilution was used to get MPN counts from the MPN Index tables with 95% confidence limits. The counts were converted to $\log_{10}$ MPNg^{-1} (Martin et al., 2004).

Application of predictive models to estimate *L. monocytogenes* and LAB growth in polony

The growth of different strains of *L. monocytogenes* in polony was predicted using three predictive models available in different software packages; the “Broth growth” model, “Growth of *L. monocytogenes* in RTE cured meats” model, and the “Growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model. The growth of LAB in polony was predicted using two models; the “growth of LAB in ground beef” and the “growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model.

The input data used for the predictions were a_w (0.95), pH (6.21), storage temperature (4°C), moisture content (68%); all these characteristics as determined in the polony. Inhibition factor (γ) of LAB on *L. monocytogenes* based on the Jameson effect at γ=1 (de Carvalho et al., 2006) was also used as input for the predictions. The initial levels of *L. monocytogenes* strains used in the prediction were averages of 0.69 $\log_{10}$ MPNg^{-1}, 0.48 $\log_{10}$ MPNg^{-1}, 0.15 $\log_{10}$ MPNg^{-1} for strain 69, 732, and 159/10 respectively, and 0.12 $\log_{10}$ MPNg^{-1} for *L. monocytogenes* in the control sample.

The physiological state of the cells of *L. monocytogenes* strains was used as inputs for the prediction in ComBase. The value was calculated using the formula; Phys. State = $10^{(-\lambda \times \mu)}$, where λ is the lag phase duration, and μ is the maximum specific growth rate (www.combase.cc). The physiological state of the *L. monocytogenes* strains used in this study was 0.44, 0.28, and 0.58 for strain 69, 732, and 159/10, respectively, and 0.79 for the control sample. The initial levels of LAB used for the prediction were 10 CFUg^{-1} and 200 CFUg^{-1}.

Prediction models;

a. The Broth growth model

The broth model in ComBase software was used for the prediction of the growth of different strains of *L. monocytogenes* in polony. The model was developed from laboratory outputs observed in culture media. The primary mathematical function used to develop the model is

Baranyi and Roberts (1994) formula which describes microorganism cell concentration and growth. The secondary function explains the influence of environmental factors on the primary function of the model.

The inputs for the ComBase broth model prediction were temperature, a_w, pH, initial level, and physiological state of the cells.

b. The "growth of *L. monocytogenes* in RTE cured meats" model

The software application MicroHibro, version 2.5.1 (www.microhibro.com) was used for the prediction. The model was developed from outputs of experimental data conducted on processed meat products (Seman et al., 2002). The primary model used for the growth of *L. monocytogenes* in RTE cured meats are the Baranyi and Roberts (1994) and the secondary function is Seman et al. (2002). The input data used for prediction of *L. monocytogenes* growth were the initial level, physiological state of the cells (denoted by *m* in the model); lag duration, NaCl, and moisture content.

c. Model of the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products"

The model is available in Food Safety and Spoilage Predictor software (FSSP) version 4.0, freely downloadable at http://fssp.food.dtu.dk. The model was used to predict the growth of the pathogen accounting for the effects of salt, nitrites, pH, and the inhibition potential of lactic acid bacteria. The model was developed from laboratory outputs conducted by Mejlholm and Dalgaard (2007) and has been validated for use in the prediction of simultaneous growth of *L. monocytogenes* and LAB in salmon, trout, and brined shrimp (Mejlholm and Dalgaard, 2007, Mejlholm and Dalgaard, 2015).

The primary and secondary functions used for the model development are the Gimenez and Dalgaard (2004) logistic model and the simplified cardinal parameter type model. Inputs of predictions were pH, inhibition factor, initial LAB counts, and *L. monocytogenes* counts.

d. Model of the "growth of LAB in ground beef"

The model is available in MicroHibro software and was used to predict the growth of LAB in polony. The model was developed from laboratory outputs of experiments conducted on ground meat. The primary function of the model is Baranyi et al. (1999), and the secondary function is Koutsoumanis et al. (2006).

The input data used for the predictions were pH, storage temperature, and initial LAB counts.

Evaluation of model performance

To evaluate the applicability of the predictive models for the growth of *L. monocytogenes* in polony, the data observed during the challenge study was compared to the predicted data. Indices of performance (bias factor, accuracy factor, root mean square error and the coefficient of determination) were used to evaluate the performance of the models. The indices assess the level of confidence in model predictions (Ross, 1996).

Bias factor (B_f) refers to the average ratio of predicted versus observed data, it shows how the predicted data lie above or below observed data (Ross, 1996, Heo et al., 2010). The following equation calculates it;

$$\left\lfloor B_f = 10\left\{\sum log\left(\frac{\frac{predicted}{observed}}{n}\right)\right\}\right| \qquad \textbf{Equation 3.1}$$

Where; predicted is the estimated growth data by the model, observed is the experimental data from the challenge study and n is the number of observations.

The accuracy factor (A_f) measures how close predictions are to observations (Ross, 1996). It shows how close predicted data is to experimental data (Heo et al., 2010).

$$\left\lfloor A_f = 10\left\{\sum log\left(\frac{\frac{predicted}{observed}}{n}\right)\right\}\right| \qquad \textbf{Equation 3.2}$$

Where; predicted is the estimated data by the model, observed is the experimental data from the challenge test, and n is the number of observations.

The Root mean square error (RMSE) is the general goodness of fit that shows the average difference between the predicted and observed data (Ross, 1996)

$$RMSE = \sqrt{\sum\frac{(observed-predicted)^2}{n}} \qquad \textbf{Equation 3.3}$$

Where; predicted is the estimated data by the model, observed is the experimental data from the challenge study and n is the number of observations.

Coefficient of determination is the overall measure of the prediction, and is calculated by the equation;

$$\left\lfloor R^2 = 1 - \left(\frac{\sum e_i^2}{\sum (yi - y)^2}\right) \right\rfloor \qquad \textbf{Equation 3.4}$$

Where e_i^2 is the error of predicted data, y_i is predicted data and y is the average predicted data.

Determination of kinetic growth parameters of *L. monocytogenes* in polony

To determine the kinetic growth of observed *L. monocytogenes* strains in polony, observed data in $\log_{10}$ MPNg^{-1} were fitted into the Baranyi and Roberts (1994) growth model in the DMFit online package in ComBase software. Maximum growth rate (μ_{max}) and lag phase duration (λ) were calculated by the software from the fitted data.

Statistical Analysis

All experiments were conducted in three independent replicates, all duplicated. Statistical analysis was done using the software Statgraphics Centurion XVII version 17.2.00 (Statpoint Technologies Inc.). Analysis of variance, at 95% confidence level was applied to determine the effect of storage time and strain on the growth of *L. monocytogenes* in the inoculated processed polony during its shelf-life storage. Fisher's least significant difference (LSD) procedure was used, $P<0.05$.

3.3.3 RESULTS

Growth of *L. monocytogenes* in polony

Growth of *L. monocytogenes* strains in polony was observed in the twelve weeks of product storage, as shown in Figure 3.2. The initial level (at day 0) of the strains was 0.15 $\log_{10}$ MPNg^{-1}, 0.48 $\log_{10}$ MPNg^{-1} and 0.69 $\log_{10}$ MPNg^{-1} for strain 159/10, 732 and 69 respectively. The control sample had an initial level of 0.12 $\log_{10}$ MPNg^{-1}. Of the three strains studied, *L. monocytogenes* strain 159/10 had the lowest initial count while strain 69 had the highest initial level.

At week 2, there was an insignificant increase in the counts of strain 159/10 and *L. monocytogenes* in the control sample; the counts were 0.17 $\log_{10}$ MPNg^{-1} and 0.16 $\log_{10}$ MPNg^{-1} respectively. Strain 732 had a slight decrease in counts to 0.45 $\log_{10}$ MPNg^{-1}, while strain 69 increased to 0.93 $\log_{10}$ MPNg^{-1} (Figure 3.2).

The growth of *L. monocytogenes* in the control sample and that of strain 159/10 was almost equal at week 6, with counts of 0.33 $\log_{10}$ MPNg^{-1} and 0.34 $\log_{10}$ MPNg^{-1} respectively. An increase in the counts of strain 69 and 732 was also observed at week 6, (1.18 $\log_{10}$ MPNg^{-1} and 0.59 $\log_{10}$ MPNg^{-1} respectively) as shown in Figure 3.2.

The increase of counts of all strains of *L. monocytogenes* continued through to week 8, 10, and 12. At week 8, strain 69 counts were 1.31 $\log_{10}$ MPNg^{-1}, and it increased to 1.42 $\log_{10}$ MPNg^{-1} and finally 1.54 $\log_{10}$ MPNg^{-1} by the end of the polony storage period. Strain 732 counts were 0.69 $\log_{10}$ MPNg^{-1}, 0.92 $\log_{10}$ MPNg^{-1} and finally 1.29 $\log_{10}$ MPNg^{-1} at week 8, 10 and 12. The counts of strain 159/10 were 0.5 $\log_{10}$ MPNg^{-1}, 0.7 $\log_{10}$ MPNg^{-1} and 0.88 $\log_{10}$ MPNg^{-1} at week 8, 10 and 12 (Figure 3.2).

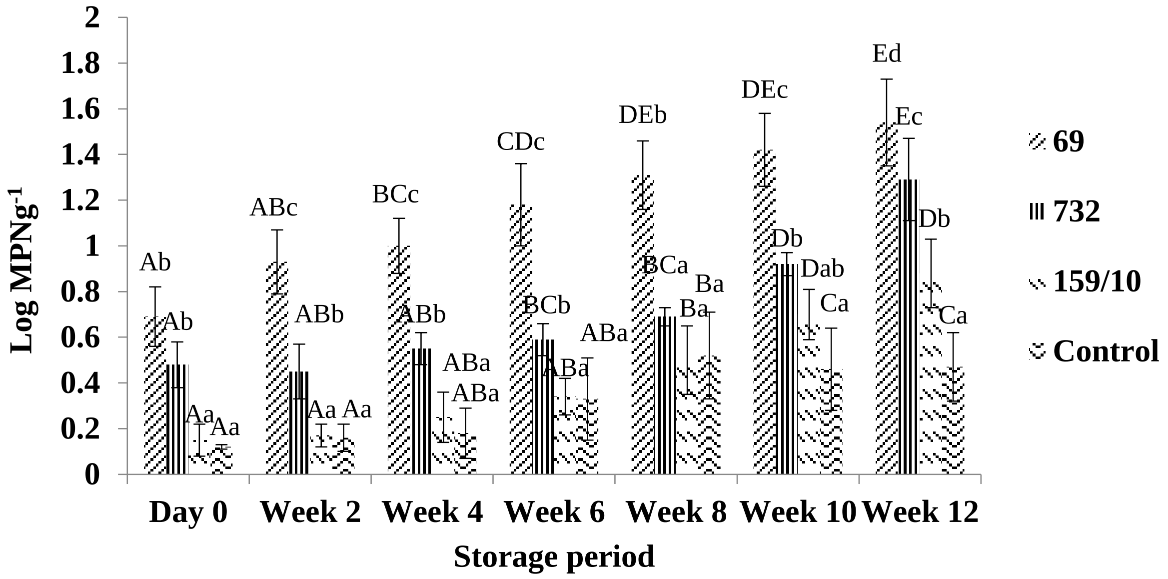
An overall increase in counts of *L. monocytogenes* strains in polony was observed throughout storage from day 0 until twelve weeks, and strain 69 had the highest counts while strain 159/10 had the lowest counts in polony after 12 weeks of storage (Figure 3.2)

Strain variability was found to have a significant effect (p=0.0000) in the growth of *L. monocytogenes*. Storage period was also found to have a significant effect (p=0.0000) in the growth of the pathogen; however, the interaction of the two had no significant difference (p=0.0783) on the growth of *L. monocytogenes* (Table 3.4).

Table 3.4: Analysis of variance of the effect of storage time and strain on the growth of *Listeria monocytogenes* in polony (n=168)

Source	Df	P-value
Main effects		
A: Strain (69, 732, 159/10, Control)	3	0.0000
B: Storage period	6	0.0000
Interactions		
AB	18	0.0783

Df - degrees of freedom



L. monocytogenes strains are 69, 159/10, and 732 and *L. monocytogenes* in the control sample. Each bar represents the mean and its corresponding standard deviation. Bars with different lower case alphabetical letters indicate significant difference (p<0.05) in the strain responses. Bars with different upper case alphabetical letters indicate a significant difference (p<0.05) in the growth of *L. monocytogenes* as a function of time or storage period

Figure 3.2: Effect of storage time and strain on the growth of *Listeria monocytogenes* in polony (n=168) stored at 4℃ for 12 weeks

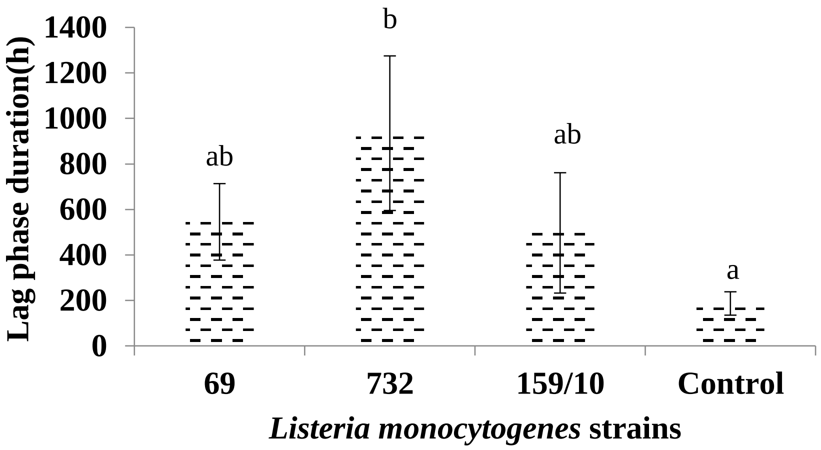
Kinetic growth of *L. monocytogenes* strains in polony

The lag phase duration (λ) and maximum growth rate (μ_{max}) of observed *L. monocytogenes* strains growth in polony are shown in Figures 3.3 and 3.4, respectively. *L. monocytogenes* in the control sample had the lowest λ and μ_{max}, with values of 187 h and 0.003 $MPNg^{-1}h^{-1}$, respectively. Strain 69 had λ of 546 h and μ_{max} of 0.009 $MPNg^{-1}h^{-1}$. Strain 732 had the longest λ of 935 h and μ_{max} of 0.0064 $MPNg^{-1}h^{-1}$. Strain variability was found to have a significant effect in the λ (p=0.0243) and μ_{max} (p=0.0167) of *L. monocytogenes* during storage (Table 3.5).

Table 3.5: Analysis of variance of the effect of strain variation on the lag phase duration and specific growth rate of *Listeria monocytogenes* in polony (n=168)

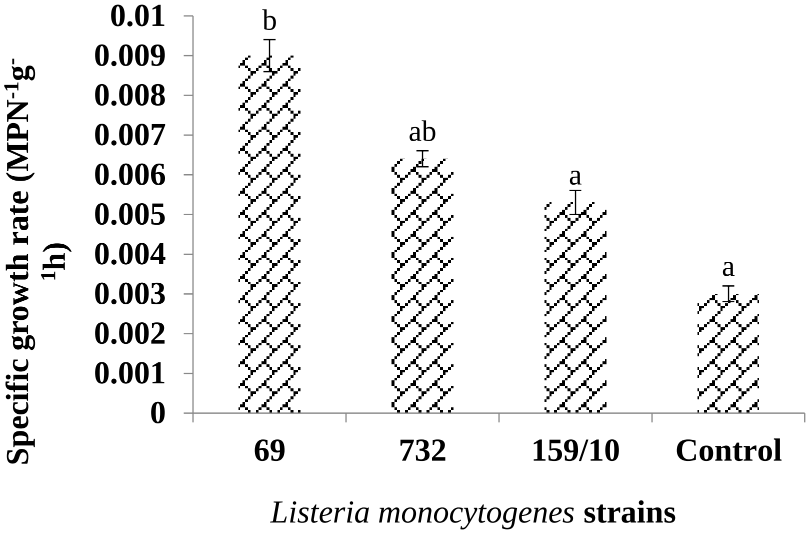
Source	**Df**	**P-value**
Lag phase duration, λ	3	0.0243
Specific maximum growth rate, μ_{max}	3	0.0167

Df - degrees of freedom



L. monocytogenes strains; 69, 159/10. and 732. Each bar represent mean of the three independent replicates and their standard deviation. Bars with different alphabetical letters indicate significant difference (p<0.05) between lag phase durations of the strains

Figure 3.3: Effect of strain variability on the lag phase duration of *Listeria monocytogenes* in polony (n=168) during storage (12 weeks) at 4℃



L. monocytogenes strains; 69, 159/10 and 732. Each bar represents the mean of the three independent replicates and their standard deviation. Bars with different alphabetical letters indicate significant differences ($p<0.05$) of the specific growth rates of *L. monocytogenes* strains

Figure 3.4: Effect of strain variability on the maximum growth rate of *Listeria monocytogenes* in polony (n=168) during storage (12 weeks) at 4℃

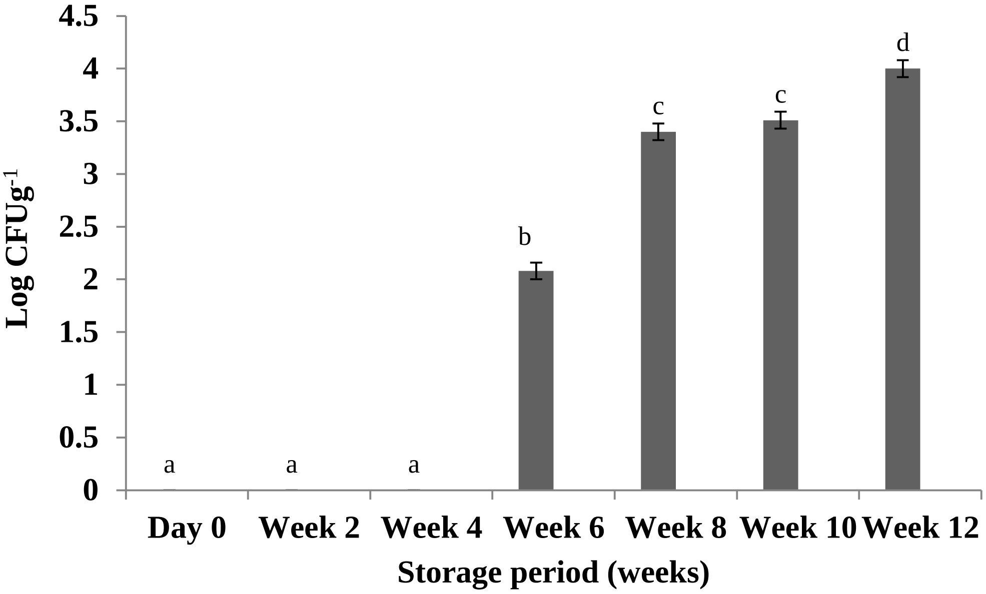
Growth of Lactic acid bacteria in polony

The growth of LAB was observed in polony, as shown in Figure 3.5. LAB was not detected (detection limit 10 CFUg^{-1}) from day 0 until week 4 of storage. From week 6 to week 8 the counts increased by approximately 1 log, from 2.08 log$_{10}$ CFUg^{-1} and 3.4 log$_{10}$ CFUg^{-1} at week 8. At week 10, the counts had increased to 3.51 log$_{10}$ CFUg^{-1} and finally 4 log$_{10}$ CFUg^{-1} at the end of the storage period. Storage period was found to significantly affect (p=0.0000) the growth of LAB in polony (Table 3.6), with the highest counts observed at week 12.

Table 3.6: Analysis of variance of the effect of storage time on the growth of lactic acid bacteria (n=42)

Source	**Df**	**P-value**
Lactic acid bacteria	6	0.0000

Df - degrees of freedom



Each bar represents the mean and standard deviation of the three replicates. Bars with different alphabetical letters indicate a significant difference ($p<0.05$) in the growth of lactic acid bacteria during the storage period

Figure 3.5: Effect of storage period (12 weeks) on the growth of lactic acid bacteria in polony samples (n=42) stored at 4°C

Application of predictive models to estimate *L. monocytogenes* growth in polony

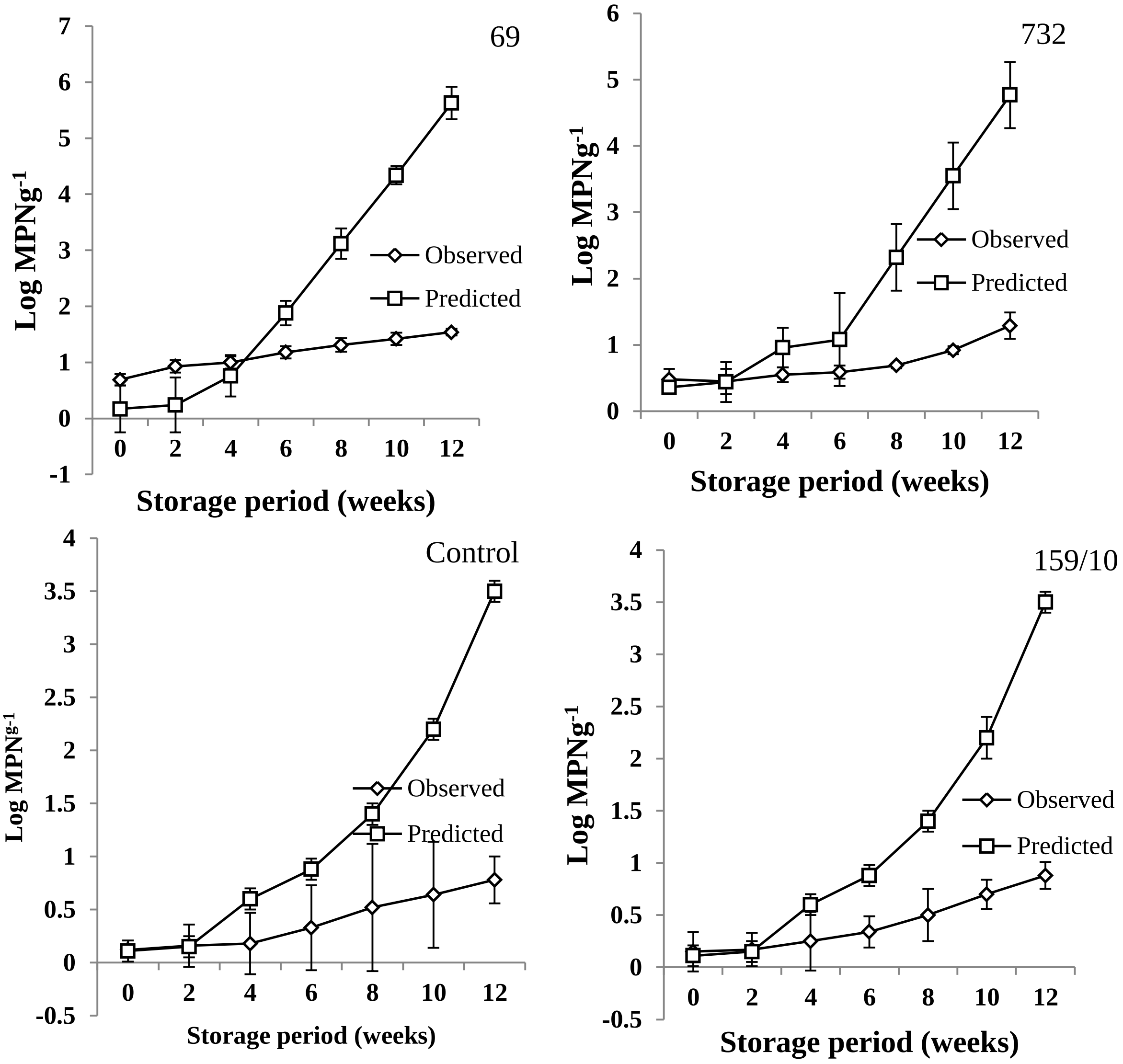
a. Broth growth model predictions

Results of the predictions of *L. monocytogenes* during storage at 4^{o}C, using the broth growth model is shown in Figure 3.6. The initial observed count of strain 69 was 0.69 $\log_{10}$ MPNg^{-1} while the predicted initial count was 0.17 $\log_{10}$ MPNg^{-1}. At week 2 and 4, the model under-predicted the growth of strain 69 with a predicted value of 0.24 $\log_{10}$ MPNg^{-1} and 0.76 $\log_{10}$ MPNg^{-1} versus the observed growth values of 0.93 $\log_{10}$ MPNg^{-1} and 1 $\log_{10}$ MPNg^{-1}, respectively for the two weeks. From week 6 until the end of the storage period, the model over-estimated the growth of *L. monocytogenes* strain 69 in polony. The predicted growth value at week 6 was 1.88 $\log_{10}$ MPNg^{-1} versus the observed growth of 1.18 $\log_{10}$ MPNg^{-1}. The growth of the strain was predicted to be 3.12 $\log_{10}$ MPNg^{-1}, 4.34 $\log_{10}$ MPNg^{-1}, and 5.63 $\log_{10}$ MPNg^{-1} while the observed growth values were 1.31 $\log_{10}$ MPNg^{-1}, 1.42 $\log_{10}$ MPNg^{-1}, and 1.54 $\log_{10}$ MPNg^{-1}, respectively at week 8, 10, and 12.

Predictions of strain 732 by the broth growth model are shown in Figure 3.6. The initial observed counts for the strain was 0.48 $\log_{10}$ MPNg^{-1}, while the predicted value was slightly lower (0.36 $\log_{10}$ MPNg^{-1}). Predicted and observed growth counts were the same at week 2 (0.44 $\log_{10}$ MPNg^{-1}). The broth model over-estimated the strain from week 4 until the end of the polony storage period. The predicted counts were 0.96 $\log_{10}$ MPNg^{-1}, 1.08 $\log_{10}$ MPNg^{-1}, 2.32 $\log_{10}$ MPNg^{-1} and 3.55 $\log_{10}$ MPNg^{-1} versus the observed growth values of 0.55 $\log_{10}$ MPNg^{-1}, 0.59 $\log_{10}$ MPNg^{-1}, 0.69 $\log_{10}$ MPNg^{-1} and 0.92 $\log_{10}$ MPNg^{-1}, respectively for week 4, 6, 8 and 10. At the end of the storage period, the broth growth model predicted count to be 4.77 $\log_{10}$ MPNg^{-1} versus the observed growth value of 1.29 $\log_{10}$ MPNg^{-1}.

The growth broth model over-estimated the growth of *L. monocytogenes* strain 159/10 in polony. The initial observed count of the strain was 0.15 $\log_{10}$ MPNg^{-1} versus the predicted growth value of 0.11 $\log_{10}$ MPNg^{-1}. By week 2, the observed growth had increased slightly to 0.17 $\log_{10}$ MPNg^{-1} while the predicted value was 0.15 $\log_{10}$ MPNg^{-1}. An over-estimation of the strain was observed from week 4 until the end of the storage period. The predicted counts were 0.6 $\log_{10}$ MPNg^{-1}, 0.88 $\log_{10}$ MPNg^{-1}, 1.4 $\log_{10}$ MPNg^{-1} and 2.2 $\log_{10}$ MPNg^{-1} while the observed growth counts were 0.25 $\log_{10}$ MPNg^{-1}, 0.34 $\log_{10}$ MPNg^{-1}, 0.5 $\log_{10}$ MPNg^{-1} and 0.7 $\log_{10}$ MPNg^{-1}, respectively for week 4, 6, 8 and 10. The final counts of strain 159/10 in polony were 0.88 $\log_{10}$ MPNg^{-1} observed versus 3.5 $\log_{10}$ MPNg^{-1} predicted (Figure 3.6).

The growth curves of observed and predicted growth of *L. monocytogenes* in the control sample is shown in Figure 3.6. The broth growth model generally overestimated the growth of *L. monocytogenes* in the polony. The initial observed (0.12 $\log_{10}$ MPNg^{-1}) and predicted (0.11 $\log_{10}$ MPNg^{-1}) levels were almost similar. By week 2, predicted and observed data was the same. Over-estimation of the pathogen growth was observed from week 4 until the end of polony storage period. Predicted counts were 0.6 log$_{10}$ MPNg^{-1}, 0.88 log$_{10}$ MPNg^{-1}, 1.4 log$_{10}$ MPNg^{-1}, 2.2 log$_{10}$ MPNg^{-1}, and 3.5 log$_{10}$ MPNg^{-1} at week 4, 6, 8, 10, and 12, respectively. The observed growth counts were 0.18 log$_{10}$ MPNg^{-1}, 0.33 log$_{10}$ MPNg^{-1}, 0.52 log$_{10}$ MPNg^{-1}, 0.64 log$_{10}$ MPNg^{-1} and 0.78 log$_{10}$ MPNg^{-1} at week 4, 6, 8, 10 and 12, respectively.



Broth growth model predictions of *L. monocytogenes* strains (69, 732,159/10) and *L. monocytogenes* in the control sample

Figure 3.6: Growth curves of predicted and observed growth of *Listeria monocytogenes* strains in polony (n=168) during the product shelf life (12 weeks) at 4°C

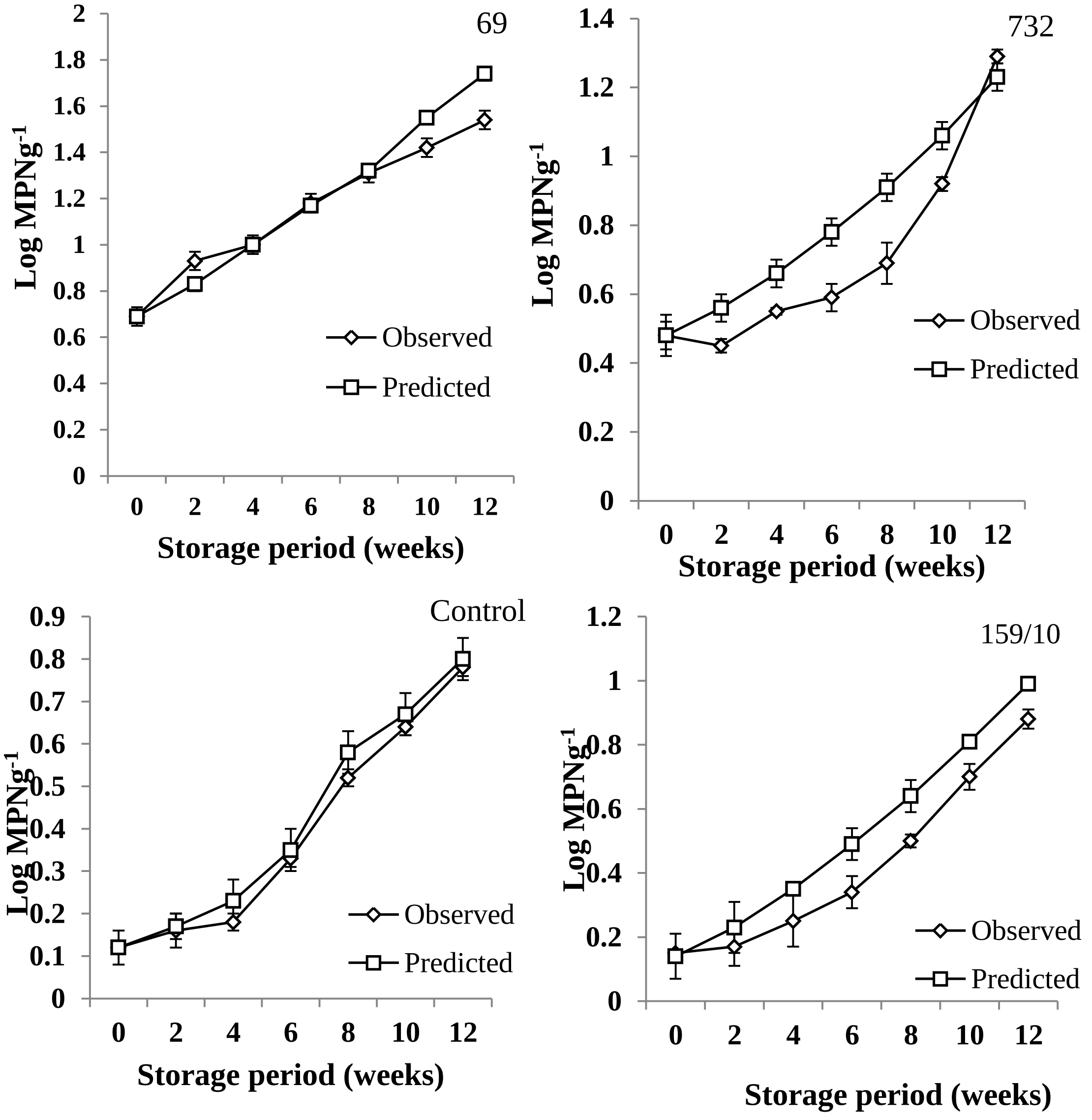
b. "Growth of *L. monocytogenes* in RTE cured meats" model predictions

Growth curves of observed compared to the predicted data of the model are shown in Figure 3.7. Predictions of strain 69 growth were close to the observed data. The initial and observed counts were both 0.69 $\log_{10}$ MPNg^{-1}. At week 2, the model under-estimated the growth of the strain, with a predicted value of 0.83 $\log_{10}$ MPNg^{-1} versus the observed growth value of 0.93 $\log_{10}$ MPNg^{-1}. Prediction and observed growth counts for the strain were almost similar at weeks 4, 6 and 8. By week 10 and 12, the model over-estimated growth of strain 69 in polony with predicted values of 1.55 $\log_{10}$ MPNg^{-1} and 1.74 $\log_{10}$ MPNg^{-1} versus the observed counts of 1.42 $\log_{10}$ MPNg^{-1} and 1.54 $\log_{10}$ MPNg^{-1} at week 10 and 12, respectively.

Growth curves of observed and predicted data of strain 732 are shown in Figure 3.7. The initial and observed count of the strain was 0.48 $\log_{10}$ MPNg^{-1}. An over-estimation of the model predictions was observed from week 2 until week 10 of polony storage period. The predicted counts were 0.56 $\log_{10}$ MPNg^{-1}, 0.66 $\log_{10}$ MPNg^{-1}, 0.78 $\log_{10}$ MPNg^{-1}, 0.91 $\log_{10}$ MPNg^{-1} and 1.06 $\log_{10}$ MPNg^{-1} at week 2, 4, 6, 8 and 10, respectively. The observed growth counts were at week 2, 4, 6, 8 and 10 were 0.45 $\log_{10}$ MPNg^{-1}, 0.55 $\log_{10}$ MPNg^{-1}, 0.59 $\log_{10}$ MPNg^{-1}, 0.69 $\log_{10}$ MPNg^{-1} and 0.92 $\log_{10}$ MPNg^{-1}, respectively. At the end of the storage period, the predicted count of strain 732 was 1.23 $\log_{10}$ MPNg^{-1} versus the observed count of 1.29 $\log_{10}$ MPNg^{-1}.

The growth of strain 159/10 in polony was generally over-estimated by the model. The initial predicted and observed counts were 0.14 $\log_{10}$ MPNg^{-1} and 0.15 $\log_{10}$ MPNg^{-1}, respectively. Predicted counts at week 2, 4, 6, 8, 10 and 12 were 0.23 $\log_{10}$ MPNg^{-1}, 0.35 $\log_{10}$ MPNg^{-1}, 0.49 $\log_{10}$ MPNg^{-1}, 0.64 $\log_{10}$ MPNg^{-1}, 0.81 $\log_{10}$ MPNg^{-1} and 0.99 $\log_{10}$ MPNg^{-1}, respectively (Figure 3.7).

The initial *L. monocytogenes* in the control sample was 0.12 $\log_{10}$ MPNg^{-1} for both the observed and predicted data. At week 4, the predicted count was 0.23 $\log_{10}$ MPNg^{-1} versus 0.18 $\log_{10}$ MPNg^{-1} observed count. The model predicted *L. monocytogenes* counts to be 0.35 $\log_{10}$ MPNg^{-1}, 0.58 $\log_{10}$ MPNg^{-1}, 0.67 $\log_{10}$ MPNg^{-1} and 0.88 $\log_{10}$ MPNg^{-1} at week 6, 8, 10 and 12 respectively (Figure 3.7).



Prediction outputs of the “growth of *Listeria monocytogenes* in RTE cured meat products” model. *L. monocytogenes* strains (69, 732, 159/10) and *L. monocytogenes* in the control sample were predicted

Figure 3.7: Growth curves of observed versus predicted *Listeria monocytogenes* strains (69, 732, and 159/10) in polony (n=168) stored at 4℃ for 12 weeks

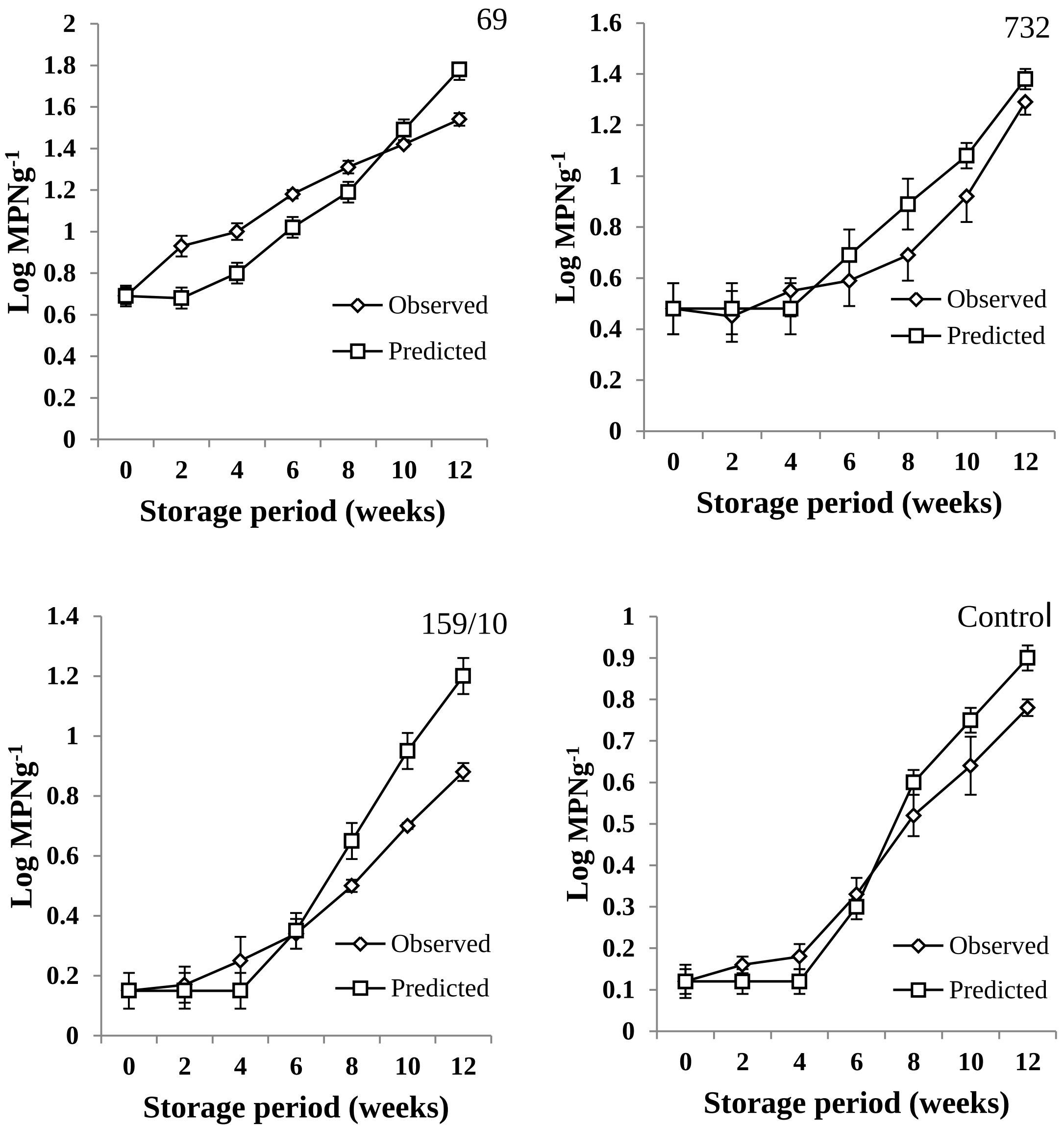
c. **“Growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model predictions**

The growth curves of observed and predicted growth of *L. monocytogenes* strains in polony using the model are shown in Figure 3.8. The initial (at day 0) observed and predicted level of strain 69 was 0.69 $\log_{10}$ MPNg^{-1}. The model under-estimated strain 69 for 4 consecutive weeks as shown in Figure 3.8. Predicted counts of the strains were 0.68 $\log_{10}$ MPNg^{-1}, 0.8 $\log_{10}$ MPNg^{-1}, 1.02 $\log_{10}$ MPNg^{-1} and 1.19 $\log_{10}$ MPNg^{-1} at weeks 2, 4, 6 and 8, respectively. The final predicted count for the strain at week 12 was 1.78 $\log_{10}$ MPNg^{-1} versus the observed count of 1.54 $\log_{10}$ MPNg^{-1}.

Growth curves of observed and predicted growth of *L. monocytogenes* strain 732 in polony are shown in Figure 3.8. The initial observed and predicted counts were 0.48 $\log_{10}$ MPNg^{-1}. The model over-estimated the growth of the pathogen from week 2 until the end of the storage period. At week 2 and week 4 the predicted counts of the strain were 0.48 $\log_{10}$ MPNg^{-1} versus the observed values of 0.45 $\log_{10}$ MPNg^{-1} and 0.55 $\log_{10}$ MPNg^{-1} for the two weeks respectively. The model predictions were 0.69 $\log_{10}$ MPNg^{-1}, 0.89 $\log_{10}$ MPNg^{-1}, 1.08 $\log_{10}$ MPNg^{-1} and 1.38 $\log_{10}$ MPNg^{-1} at weeks 6, 8, 10 and 12, respectively for strain 732 (Figure 3.8).

The observed and predicted data of strain 159/10, using the “growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model is shown in Figure 3.8. The initial and predicted levels of the strain were equal. From week 2, the model under-estimated the strain with predictions of 0.15 $\log_{10}$ MPNg^{-1} versus the observed counts of 0.17 $\log_{10}$ MPNg^{-1} and 0.25 $\log_{10}$ MPNg^{-1}. The model predictions were 0.65 $\log_{10}$ MPNg^{-1}, 0.95 $\log_{10}$ MPNg^{-1} and 1.2 $\log_{10}$ MPNg^{-1} at weeks 8, 10 and 12, respectively.

The observed and predicted data of *L. monocytogenes* in the control sample, using the “growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model is shown in Figure 3.8. The initial and predicted levels of the pathogen were 0.12 $\log_{10}$ MPNg^{-1}. From week 2, the model under-estimated the pathogen with predictions of 0.12 $\log_{10}$ MPNg^{-1} versus the observed counts of 0.16 $\log_{10}$ MPNg^{-1} and 0.18 $\log_{10}$ MPNg^{-1}. The model predictions were 0.60 $\log_{10}$ MPNg^{-1}, 0.75 $\log_{10}$ MPNg^{-1} and 0.90 $\log_{10}$ MPNg^{-1} at weeks 8, 10 and 12, respectively.



Predictions of the growth of *Listeria monocytogenes* strains 69, 732, 159/10 and *L. monocytogenes* in the control sample according to the "Growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model

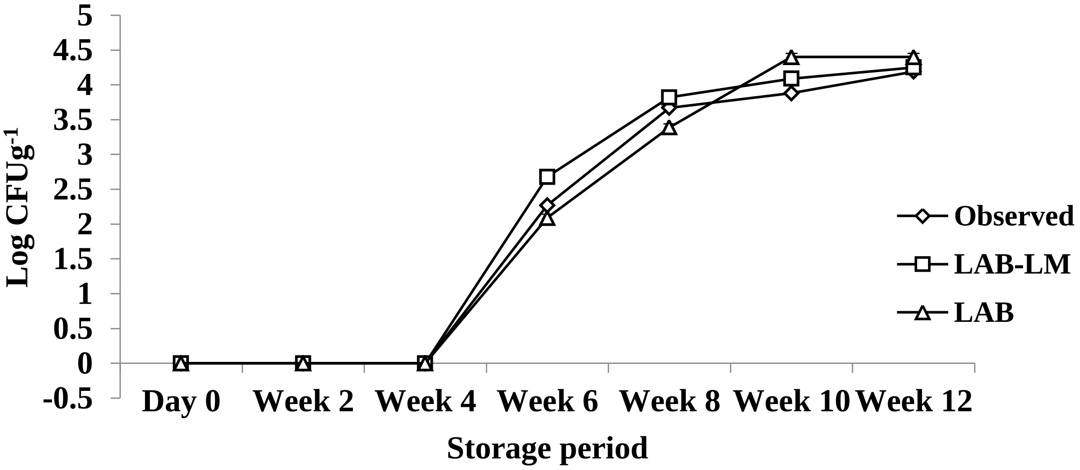
Figure 3.8: Growth curves of observed versus predicted growth of *Listeria monocytogenes* strains (69, 732, 159/10) in polony (n=168) stored at 4℃ for 12 weeks

Prediction of lactic acid bacteria growth in polony

Two models were used for the prediction of LAB in polony as shown in Figure 3.9 LAB was not detected (detection limit 10 CFUg^{-1}) from day 0 until week 4. At weeks 6, 8 and 10 the observed LAB growth in polony was 2.27 log$_{10}$ CFUg^{-1}, 3.82 log$_{10}$ CFUg^{-1} and 3.88 log$_{10}$

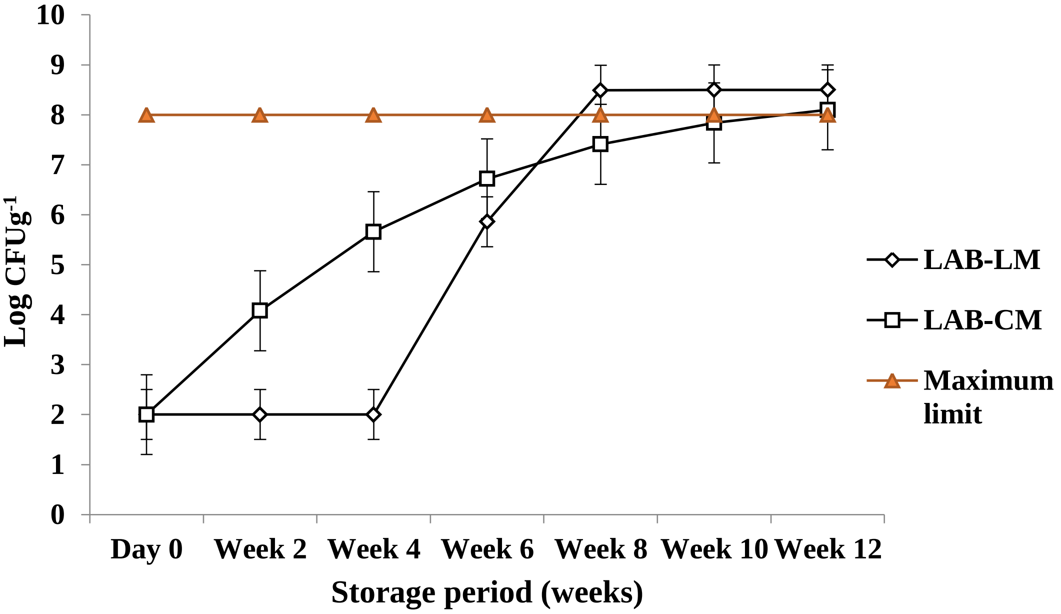
CFUg^{-1}, respectively. At day 0 until week 4, both models predicted no detection (detection limit 10 CFUg^{-1}) of LAB in polony. The growth of LAB was shown by both models at week 6 where the "growth of LAB in ground meat" model predicted growth of 2.09 $\log_{10}$ CFUg^{-1} and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model predicted growth of 2.68 $\log_{10}$ CFUg^{-1}. By the end of the polony storage period, the "growth of *L. monocytogenes* and LAB in chilled meat products" model predicted final counts of 4.25 log10 CFUg^{-1}. In comparison, the "growth of LAB in ground beef" model predicted counts of 4.4 $\log_{10}$ CFUg^{-1} versus the observed growth counts of 4.19 $\log_{10}$ CFUg^{-1}. By the end of the product storage period, both models gave close predictions of growth of LAB in polony.

Figure 3.10 shows the predicted growth curves using the two models at an initial level of 2 $\log_{10}$ CFUg^{-1}. The "growth of *L. monocytogenes* and LAB in chilled seafood and meat" indicates that LAB counts in polony, with the prediction set at an initial level of 2 $\log_{10}$ CFUg^{-1}, the LAB counts will surpass their maximum set limit in RTE meats at week 8. The "growth of LAB in ground beef" model shows that at the same conditions, LAB counts will reach maximum levels by the end of the storage period.



LAB-LM-"growth of lactic acid bacteria and *Listeria monocytogenes* in chilled seafood and meats" model, LAB - the "growth of LAB in ground meat" model

Figure 3.9: Growth curves of observed versus predicted growth of lactic acid bacteria in polony (n=42) stored at 4℃ for 12 weeks



Maximum limit - lactic acid bacteria limit in RTE meat products (Horita et al., 2018), LAB - LM-“growth of lactic acid and *Listeria monocytogenes* in chilled seafood and meat products” model predictions, LAB - CM- the “growth of lactic acid bacteria in RTE cured meats” model predictions

Figure 3.10: Growth curves of predicted lactic acid bacteria growth in polony samples at an initial contamination level of 2 $\log_{10}$ CFUg^{-1} stored at 4℃ for 12 weeks

Performance evaluation of predictive models for *L. monocytogenes* growth in polony

Prediction models used in the study were evaluated for performance, as shown in Table 3.7.

a. The “growth of *L. monocytogenes* in RTE cured meats” model

The model predicted initial and final counts of strain 69 to be 0.69 $\log_{10}$ MPNg^{-1} and 1.78 $\log_{10}$ MPNg^{-1} while observed growth was 0.69 $\log_{10}$ MPNg^{-1} and 1.54 $\log_{10}$ MPNg^{-1}, respectively. The model under-estimated strain 69 by 8%, with predictions close to observed data (A_f-1.15), the deviation of predicted from observed data was low (RMSE-0.17), and the predictions depicted a good statistical fit (R^2-0.97).

The initial and final predicted counts were 0.48 $\log_{10}$ MPNg^{-1} and 1.38 $\log_{10}$ MPNg^{-1} for *L. monocytogenes* strain 732. Observed initial and final counts were 0.48 $\log_{10}$ MPNg^{-1} and 1.29 $\log_{10}$ MPNg^{-1}, respectively. The model over-estimated strain 732 by 8% (B_f-1.08). A_f of the prediction was 1.13, showing that predictions are closer to observed growth. A good statistical fit (R^2-0.98) and low error (RMSE-0.11) was recorded for the prediction (Table 3.7).

The model also predicted initial and final counts of 0.48 $\log_{10}$ MPNg^{-1} and 1.2 $\log_{10}$ MPNg^{-1} respectively for the growth of *L. monocytogenes* strain 159/10 in polony. The observed initial and final counts of strain 159/10 were 0.15 $\log_{10}$ MPNg^{-1} and 0.88 $\log_{10}$ MPNg^{-1}, respectively. The model over-estimated 159/10 by 4% (B_f-1.04), with A_f of 1.13 showing that the predictions are close to observed growth. RMSE of the prediction was 0.11, and R^2 was 0.98. *L. monocytogenes* in the control sample was predicted to have an initial and final value of 0.12 $\log_{10}$ MPNg^{-1} and 0.19 $\log_{10}$ MPNg^{-1}. In comparison, the observed counts were 0.12 $\log_{10}$ MPNg^{-1} and 0.88 $\log_{10}$ MPNg^{-1} respectively. The model overestimated the growth of *L. monocytogenes* in the control sample by 38%, with B_f and A_f of 1.38, RMSE of 0.07 and R^2 of 0.90 (Table 3.7).

b. The "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model

The model performance was evaluated for the prediction of growth of *L. monocytogenes* strains in polony, as shown in Table 3.7.

The model predicted 0.69 $\log_{10}$ MPNg^{-1} initial levels and 1.74 log10 MPNg^{-1} final counts of *L. monocytogenes* strain 69 in polony. An almost perfect agreement between the observed and predicted data was found, with B_f of 1.01 and A_f of 1.05. Strain 69 predictions were of a good statistical fit (R^2-0.99) with a low level of error (RMSE-0.10). Predictions of strain 732 gave an initial level of 0.48 $\log_{10}$ MPNg^{-1} and final counts of 1.28 $\log_{10}$ MPNg^{-1}. The model over-estimated the strain by 8% with a B_f of 1.08. Predictions of the strain were, however, close to observed data as A_f was 1.13. RMSE was 0.14 while R^2 was 0.98.

L. monocytogenes strain 159/10 was predicted to have initial and final counts of 0.14 $\log_{10}$ MPNg^{-1} and 0.99 $\log_{10}$ MPNg^{-1}, respectively. The model overestimated the growth of 159/10 in polony by 23% (B_f-1.23), the predicted data was, however, closer to observed growth, with low error level and good statistical fit (A_f-1.25, RMSE-0.11and A_f-1.25). *L. monocytogenes* in the control sample was predicted to be 0.12 $\log_{10}$ MPNg^{-1} and 0.80 $\log_{10}$ MPNg^{-1} initial and final counts, respectively. The model overestimated the growth of *L. monocytogenes* by

8%; however, the predicted growth was closer to the observed growth (B_f-1.08). The predicted growth had a good statistical fit to the observed, with R^2 of 0.99 and a low error level of 0.03 (Table 3.7).

c. The "broth growth" model

The broth model predicted the initial and final counts of *L. monocytogenes* 69 in polony to be 0.17 $\log_{10}$ MPNg^{-1} and 5.63 $\log_{10}$ MPNg^{-1} versus observed counts were 0.69 $\log_{10}$ MPNg^{-1} and 1.54 $\log_{10}$ MPNg^{-1}, respectively. A 71% over-estimation was observed (B_f-1.71), and A_f, RMSE, and R^2 of prediction were 1.71, 2.06, and 0.37, respectively (Table 3.7).

Strain 732 had a predicted initial and final count of 0.36 $\log_{10}$ MPNg^{-1} and 4.77 $\log_{10}$ MPNg^{-1}, respectively. Observed initial and final counts of strain 732 were 0.48 $\log_{10}$ MPNg^{-1} and 1.29 $\log_{10}$ MPNg^{-1}, respectively. An 89% over-estimation of the strain was recorded, with B_f of 1.89, A_f of 1.90, R^2 of 0.30 and RMSE of 1.78 (Table 3.7).

Similar to other strains, 159/10 was also overestimated by the model (B_f-1.90). The A_f, RMSE and R^2 of prediction were 1.90, 1.21 and 0.30, respectively. Observed initial and final counts of the strain were 0.15 $\log_{10}$ MPNg^{-1} and 0.88 $\log_{10}$ MPNg^{-1} while predicted initial and final counts were 0.11 $\log_{10}$ MPNg^{-1} and 3.5 $\log_{10}$ MPNg^{-1}, respectively (Table 3.7).

L .monocytogenes in the control sample was predicted to have an initial and final level of 0.11 $\log_{10}$ MPNg^{-1} and 3.5 $\log_{10}$ MPNg$^{-1,}$ respectively, while it's observed counts were 0.12 $\log_{10}$ MPNg^{-1} and 0.47 $\log_{10}$ MPNg^{-1} initial and final counts, respectively. The B_f and A_f, RMSE, and R^2 of prediction were 1.90, 1.21, and 0.30, respectively, showing an over-prediction of the pathogen in polony (Table 3.7).

Table 3.7: Performance evaluation of selected models in the prediction of *Listeria monocytogenes* strains in polony (n=168) during its shelf life at 4℃ storage

Polony samples	**Indices of performance**	**Observed growth**	**Models**		
			Growth of *L. monocytogenes* in RTE cured meats	Growth of *L. monocytogenes* and LAB in chilled seafood and meat products	Broth growth
69	y_o (Log MPNg^{-1})	0.69	0.69	0.69	0.17
	y_f (Log MPNg^{-1})	1.54	1.78	1.74	5.63
	B_f	na	0.92	1.01	1.71
	A_f	na	1.15	1.05	1.71
	RSME	na	0.17	0.10	2.06
	R^2	na	0.97	0.99	0.37
732	y_o (Log MPNg^{-1})	0.48	0.48	0.48	0.36
	y_f (Log MPNg^{-1})	1.29	1.38	1.28	4.77
	B_f	na	1.08	1.08	1.89
	A_f	na	1.13	1.13	1.90
	RSME	na	0.11	0.14	1.78

Table continues in the next page

	R^2	na	0.98	0.98	0.30
159/10	y_o (Log MPNg^{-1})	0.15	0.15	0.14	0.11
	y_f (Log MPNg^{-1})	0.88	1.2	0.99	3.5
	B_f	na	1.04	1.23	1.90
	A_f	na	1.25	1.25	1.90
	RSME	na	0.17	0.11	1.21
	R^2	na	0.91	0.96	0.30
Control	y_o (Log MPNg^{-1})	0.12	0.12	0.12	0.11
	y_f (Log MPNg^{-1})	0.47	0.90	0.80	3.5
	B_f	na	1.38	1.08	1.90
	A_f	na	1.38	1.08	1.90
	RSME	na	0.07	0.03	1.21
	R^2	na	0.90	0.99	0.30

y_0 - initial count, y_f - final count, A_f - Accuracy factor, B_f - Bias factor, RMSE - root mean square error, R^2 - coefficient of determination, na - not applicable

Evaluation of models for the prediction of lactic acid bacteria growth in polony

The "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" and the "growth of LAB in ground beef" models were evaluated for their performance in predicting LAB in polony (Table 3.8).

Both models predicted that LAB was not detected in the first 3 weeks of storage. The "growth of LAB in ground beef" model predicted a final count of 4.40 $\log_{10}$ CFUg^{-1}, with a perfect agreement between predicted and observed growth (B_f-1.00, A_f-1.05). The RMSE and

R^2 of predictions were 0.25 and 0.99, respectively, showing a low level of error and good statistical fit of predicted growth to observed growth. The "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model predicted final counts of 4.25 $\log_{10}$ $CFUg^{-1}$. The model showed a 4% over-estimation of LAB growth in polony (B_f-1.04), with A_f of 1.04, RMSE of 0.18, and R^2 of 0.99 (Table 3.8).

Table 3.8: Performance evaluation of selected models for the prediction of lactic acid bacteria in polony (n=42) during storage at 4℃

Indices of performance	**Observed growth**	**Models**	
		Growth of *L. monocytogenes* and LAB in chilled seafood and meat products model	Growth of lactic acid bacteria in ground beef
y_o (Log $CFUg^{-1}$)	nd	nd	nd
y_f (Log $CFUg^{-1}$)	4.19	4.25	4.40
B_f	na	1.04	1.00
A_f	na	1.04	1.05
RSME	na	0.18	0.25
R^2	na	0.99	0.99

nd - not detected (detection limit 10 CFU/g), na -not applicable, y_o - initial count, y_f - final count, B_f - bias factor, Af - accuracy factor, RSME - root mean square error, R^2 - coefficient of determination

3.3.4 DISCUSSION

The main objective of the study was to determine the applicability of predictive models in estimating the growth of *L. monocytogenes* strains in polony during the product's refrigerated storage (twelve weeks at 4℃). Pre-emptive pathogen detection is important in the production of safe food, and the study is important to the polony industry as it provides for an alternative method that can be used to detect the pathogen growth more rapidly and cheaply.

The assessment of the growth of three *L. monocytogenes* strains (69, 732, and 159/10) in artificially contaminated and processed polony was conducted, and the results of the assessment indicated that all the strains were able to grow at refrigerated storage. This implies that refrigeration storage does not prevent the multiplication of the pathogen, and can allow the pathogen to reach levels that can pose a health hazard during storage.

Several studies have reported the ability of *L. monocytogenes* to grow at chilled temperature (Bayles et al., 1996, Hingston et al., 2017, Ye et al., 2017, Zhu et al., 2017, Ziegler et al., 2019). The ability of the pathogen to grow in chilled storage is attributed to adaptation mechanisms that involve a synthesis of cellular proteins for the maintenance of cellular membrane integrity and fluidity and cell macrostructure preservation, e.g. ribosomes (Hébraud and Guzzo, 2000, Wemekamp-Kamphuis et al., 2002).

As expected, the product storage period was a crucial factor in the growth of *L. monocytogenes* strains. The results highlighted a significant increase in all the strains of *L. monocytogenes* throughout the storage period. The counts of all the investigated strains increased significantly ($p < 0.05$) between day 0 and week 12, with the highest counts recorded at week 12. Studies have identified storage time as one of the most important factors that significantly impact the growth of *L. monocytogenes* in chilled food products (Uyttendaele et al., 2016, Balamurugan et al., 2018, Ziegler et al., 2019).

The increase in the growth of *L. monocytogenes* as an effect of time has been observed by (Guyer and Jemmi, 1991, Uyttendaele et al., 2016) in salmon fillets and cooked roast beef respectively. An overall increase of 2.5 $\log_{10}$ MPNg^{-1} was observed in salmon stored for 10 days at 4℃ by Guyer and Jemmi (1991) while Uyttendaele et al. (2016) reported more than 2 orders of magnitude increase in the growth of *L. monocytogenes* in cooked roast beef.

Strain variability affected the growth of *L. monocytogenes*; this is shown by the high counts for some strains (69 and 732) than others under similar growth conditions. The significant effect of the strain on the growth of *L. monocytogenes* is shown in the study results. The highest growth was observed for strain 69, followed by 732 and lastly 159/10 with the lowest counts throughout the storage period. Initial contamination level influence the growth of bacterial species (Uyttendaele et al., 2016), the results show that strain 69 had high initial counts and consequently, high final counts. The high initial counts for the strain is a consequence of resistance to processing (Table 3.3, Section 3.2), and is supported by findings from a study conducted by Sibanda and Buys (2017) where it was discovered that strain 69 recovered well in broth at 4℃ after being subjected to heat stress, and consequently, had more regrowth potential than 159/10.

The significant difference between the growth of the strains is further explained by a clear indication of strain variability influence on the lag phase duration (λ) and a specific maximum growth rate (μ_{max}). The long lag phase duration and low maximum growth rate of 159/10 explain the low counts throughout storage. Strain 69 had the highest maximum growth rate and moderate lag duration, hence the highest observed growth. According to Guillier et al. (2005), long lag periods of *L. monocytogenes* in chilled food products is influenced by the response of the cell to cold stress, which is ultimately controlled by the phenotypic and genotypic characteristics of the cells hence variability in strain responses.

LAB has been largely reported to have inhibition effects on *L. monocytogenes* in several meat products through the concept of bacterial antagonism (De Martinis and Franco, 1998, Amézquita and Brashears, 2002, de Carvalho et al., 2006, da Costa et al., 2018). The possibility of such an inhibition prompted for the simultaneous determination of LAB and *L. monocytogenes* growth in polony in this study. The results of the study showed significant growth of LAB as a function of storage time, even with an undetectable initial level. These findings are supported by a study conducted in ham by Uyttendaele et al. (2016). The authors state that *L. monocytogenes* is inhibited at high LAB counts (>10^7 CFUg^{-1}); this could explain why *L. monocytogenes* in the study continued to multiply throughout storage as LAB counts were low.

The last phase of the study involved the application of predictive models in predicting the growth of *L. monocytogenes* strains. Performance indices (B_f, A_f, RMSE, R^2) were used to compare predicted and observed data and consequently determine the model that is most suitable for predicting the growth of *L. monocytogenes* strains and LAB growth in polony.

B_f and A_f of 1 show perfect agreement between predicted and observed data. $B_f>1$ indicates that the predicted growth was longer than the observed growth and that the model over-predicted growth. The over-prediction suggests that prediction is fail-safe, meaning that the model predicted growth while there was no growth. B_f values between 0.87 and 1.43 are acceptable and show that the model is suitable for that particular prediction. B_f values out of this range show unacceptable predictions. The larger the A_f, the less accurate predictions are. RMSE value indicates the error of the estimate, and the larger the value, the more unsuitable the model is for the prediction. R^2 close to 1 indicates a good statistical fit of predicted to observed values (Ross, 1996, Mellefont et al., 2003, Mejlholm et al., 2010).

The “broth growth” model was found to be unsuitable for the prediction of growth of all strains of *L. monocytogenes* in polony as B_f was more than the maximum acceptable limit of 1.43. All the strains were over-predicted by more than 70%, even though predictions were all fail-safe, high level of errors was recorded, with a poor statistical fit of predicted to observed growth making the model unacceptable for the prediction of *L. monocytogenes* strains in polony. The prediction of the growth of *L. monocytogenes* strains in polony using the broth model was therefore unacceptable.

The “broth growth” model gave high unacceptable over-predictions. Models that have been developed based on laboratory outputs in culture media may yield predictions that are not valid in food environments (Ross, 1996, Pin et al., 1999, Panagou and Nychas, 2008, Mejlholm et al., 2010). Pin et al. (1999) further states that bacterial cells may behave differently in media than in structured food; hence broth models may give predictions with high positive bias and error. The broth models have been found to yield accurate predictions of bacterial growth in liquid-based food; however, the use of the models in solid foods over-estimates growth (Schvartzman et al., 2010) as observed in this study.

The “growth of *L. monocytogenes* in RTE cured meats” model was also evaluated for the prediction of *L. monocytogenes* in polony. Acceptable predictions (B_f all less than 1.43) were observed for strain 69, 732, 159/10 as well as for *L. monocytogenes* in the control sample. A low level of error was observed for all the predictions as well as a good statistical fit of predictions to observed data. The model over-predicted the growth of strain 732 and 159/10 by 8% and 4%, respectively, and both predictions were fail-safe. Strain 69 was under-predicted by 8%, making the prediction fail-dangerous.

The use of the "growth of *L. monocytogenes* in RTE cured meats" model yielded good and acceptable predictions of *L. monocytogenes* strains growth in polony. One desirable feature of the model, which is not catered for in other models, is cell variability brought by prediction inputs of the lag period (from observed growth) as well as the physiological state of the cells, hence close predicted and observed data. The model also accounts for bacterial responses to the food matrix composition and has been validated for use in the prediction of *L. monocytogenes* in RTE meats (Seman et al., 2002); hence the accurate predictions observed.

The "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model was evaluated for the prediction of *L. monocytogenes* and lactic acid bacteria in polony. Overall, the model showed good and acceptable predictions of the strains of *L. monocytogenes* in polony. Over-predictions of 1%, 8%, and 4% were observed for strains 69, 732, and 159/10, respectively. All the predictions were deemed fail-safe and acceptable, and low level of errors and good statistical fit to observed data was recorded. The model also showed a good prediction of LAB growth in polony (4% over-prediction). A_f of prediction was 1.04 with a low level of error and good statistical fit. The prediction was fail-safe.

The "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model yielded accurate, fail-safe predictions of the growth of LAB and *L. monocytogenes* in polony. According to Dalgaard and Mejlholm (2019), accurate predictions can be attributed to the model's ability to account for the response of the bacterial cells to the product characteristics, storage period, and temperature. A desirable feature that is unique to this model is its ability to simultaneously predict LAB and *L. monocytogenes*, and account for the effect of the interaction of the two using the Jameson effect phenomenon (Mejlholm and Dalgaard, 2007, Mejlholm and Dalgaard, 2015, Dalgaard and Mejlholm, 2019). The model also allows the user to select growth with lag time, making predictions more realistic (Mejlholm and Dalgaard, 2015).

The "growth of LAB in ground beef" model yielded perfect prediction of LAB in polony. Perfect agreement between predicted and observed was shown by the model, with A_f of 1.05, low level of error, and good statistical fit. The perfect predictions were due to the model's ability to predict growth as a function of product characteristics, storage temperature as well as the lag duration (of observed data) and physiological state of the cells.

In summary, the two models that were developed from outputs of experiments conducted on real food environments yielded acceptable, fail-safe predictions. However, only the "growth of *L. monocytogenes* in RTE cured meats" model accounted for strain variation due to the user-defined inputs of lag duration (observed data) and the physiological state of the cell. Fail-safe predictions pose no food safety risk as they alert for growth while there is no growth, fail-safe predictions of over 43% over-estimation are however not acceptable as they show extreme deviation from the actual growth, and can be disadvantageous as the prediction can lead to premature product disposal hence food waste and economic losses. Fail-dangerous predictions predict no growth while there is observed growth, thus pose food safety risks.

3.3.5 CONCLUSION

All the models investigated were able to give fail-safe predictions of *L. monocytogenes* growth in polony. However, the "broth growth" model gave an overly fail-safe prediction, which is unacceptable, making the model unsuitable for predicting the growth of *L. monocytogenes* in polony. Predictions of the "growth of LAB and *L. monocytogenes* in chilled seafood and meat products" model gave good fail-safe predictions but could not account for the effect of strain variability on the growth of the pathogen. The model was nevertheless accurate in predicting the growth of all strains of *L. monocytogenes*. The "growth of *L. monocytogenes* in RTE cured meats" model gave accurate, fail-safe predictions for most strains of *L. monocytogenes*. The model embraces the effect of strain variability in the growth of the pathogen. The study highlights acceptable predictions by two models; the "growth of LAB and *L. monocytogenes* in chilled seafood and meat products" model and the "growth of *L. monocytogenes* in RTE cured meats" model. The South African polony industry can therefore apply the two models in the prediction of growth of *L. monocytogenes* in polony. The study highlights the importance of developing predictive models from outputs of the real food environment and shows the need for the development of models that are based on the growth of *L. monocytogenes* in polony.

CHAPTER 4: GENERAL DISCUSSION

4.1 METHODOLOGY REVIEW

The study was conducted on polony; a common RTE meat product in South Africa. Polony was selected for this study due to its recent association with the world's largest listeriosis outbreak. Studies have also linked listeriosis to RTE meat products, highlighting the risk of contamination of RTE meat products by *L. monocytogenes* (Notermans et al., 1993, Spanu et al., 2014, Uyttendaele et al., 2016, Desai et al., 2019). For the production of polony, raw emulsion samples were obtained from a polony manufacturing facility; this was done to get a formulation that is as close as possible to what is already in the market.

Physiochemical characteristics of polony were determined, as well as TPC and LAB. Food properties such as water activity, pH, and fat content affect the survival and growth of *L. monocytogenes* (McCormick et al., 2003, Den Besten et al., 2018, Abel et al., 2020, Verheyen et al., 2020). Background bacteria such as LAB have been reported to have inhibition effects on *L. monocytogenes*, hence the need to quantify them with the pathogen simultaneously. Microbial analysis was conducted using the MPN technique as the method allows for the enumeration of low numbers of bacteria due to its high sensitivity (Capita and Alonso-Calleja, 2003).

Challenge testing was used to determine the effect of the conventional polony processing method on *L. monocytogenes* strains, as well as to monitor the growth of the strains during the polony shelf life. Challenge tests involve the artificial contamination of food with the organism of interest to determine the efficacy of a lethality treatment or to determine if the food can support the growth of the particular organism (Álvarez-Ordóñez et al., 2015). For this study, the challenge test was designed to determine the efficacy of polony processing and the growth of *L. monocytogenes* during the product shelf life. The tests were conducted in three batches, and each experiment was duplicated.

Three strains of *L. monocytogenes* were used in the study; the strains were selected based on availability and on the fact that they were isolated from food processing environments. Strain 69 and 159/10 were isolated from an avocado processing plant (Sibanda and Buys, 2017) and strain 732 was previously isolated from polony in the Department of Consumer and Food Sciences, University of Pretoria. The inoculation level used was 10^6 CFUg^{-1}, and approximately 1% of the inoculum was used, leading to a final inoculation level of 10^5 CFUg^{-1} in the polony emulsion. It is recommended that for validation of lethality treatments, high inoculation levels (10^5 to 10^7 CFUg^{-1}) should be used, and the level of contamination should be confirmed by testing after inoculation (Notermans et al., 1993, Spanu et al., 2014, Álvarez-Ordóñez et al., 2015).

Processing of the samples was conducted as per the South African standard on Processed Meats (SABS885, 2011) to imitate the real processing conditions of the product. The controls of the study were the un-inoculated samples, as recommended by Spanu et al. (2014). After processing counts, at day 0 were determined, and after every fortnight until the end of the twelve weeks storage period. The period was decided on based on the 10 weeks product shelf life determined by the manufacturer (Bavarian Cold Meats), with an additional two weeks. Álvarez-Ordóñez et al. (2015) recommended that the storage period must at least be equivalent to the shelf life of the product.

Tertiary predictive models were used to estimate the growth of *L. monocytogenes* strains and LAB in polony. The models were selected based on their accessibility, availability (all available freely online), and their ability to predict the microorganisms of interest. The ComBase “broth growth” model was used to estimate the growth of *L. monocytogenes* strains in polony. According to Nyhan et al. (2018), the broth model can accurately predict the growth of most food pathogens in response to intrinsic and extrinsic characteristics.

The “growth of *L. monocytogenes* in RTE cured meats” model was also used to estimate the pathogen growth, the model estimates response to product characteristics and have been validated for use in predicting the pathogen growth in RTE meats (Seman et al., 2002). The growth of *L. monocytogenes* and LAB in polony was also estimated using the “growth of *L. monocytogenes* and LAB in chilled seafood and meat products” model available in FSSP software. The model accounts for microbial interactions of the two bacteria, based on the Jameson effect (Mejlholm and Dalgaard, 2007, Mejlholm et al., 2010, Mejlholm et al., 2015, Mejlholm and Dalgaard, 2015). The “growth of LAB in ground beef” model available in MicroHibro software was also used to predict LAB growth in polony.

All the models were evaluated using A_f, B_f, RMSE, and R^2 (Ross, 1996, Ross et al., 2000, Mejlholm et al., 2015, Dalgaard and Mejlholm, 2019).

4.2 MAIN RESEARCH FINDINGS

The study determined the effect of polony processing in inactivating *L. monocytogenes* strain, as well as the determination of the applicability of predictive models in estimating the growth of different strains of *L. monocytogenes* in polony during storage.

Based on the outcomes of the process lethality challenge study, polony processing (heat treatment to an internal temperature of 72℃ followed by cooling) was sufficient to inactivate all strains of *L. monocytogenes* investigated. LAB and TPC were also sufficiently inactivated to undetectable levels. Polony processing relies primarily on heating to inactivate pathogenic and spoilage bacteria. The lethality of high temperatures to bacteria is reported to be due to the destruction of major cell components such as the cytoplasmic membrane, ribosomal RNA, proteins, and DNA (Russell, 2003).

The effect of polony processing on *L. monocytogenes* was found to be strain-specific, with percentage $MPNg^{-1}$ log reductions of 61%, 76%, and 91% for strains 69, 732, and 159/10, respectively. The results indicate the importance of strain variability in polony processing lethality studies. The strain-specific response of *L. monocytogenes* to processing has been reported in several studies (Poimenidou et al., 2016, Zhu et al., 2017, Bucur et al., 2018, Den Besten et al., 2018). The response involves the induction of heat shock proteins in the cell to protect the cell components from damage (Russell, 2003). The genetic make-up of the cells affects the response, and the presence of ClpL protease in some *L. monocytogenes* strains contributes to heat resistance (Bucur et al., 2018).

L. monocytogenes strains were able to grow at refrigerated storage, and growth was strain-dependent. The ability of *L. monocytogenes* to grow at chilled temperatures have been widely reported (Zhu et al., 2005, Hingston et al., 2017, Ye et al., 2017, Zhu et al., 2017, Saraiva et al., 2018, Ziegler et al., 2019). Survival and growth at chilled temperatures were due to cell adaptation mechanism, aimed at maintaining the cell membrane integrity (Hébraud and Guzzo, 2000, Wemekamp-Kamphuis et al., 2002). At low temperatures, cold shock proteins are induced, and they change gene profiles of the cell leading to a change in membrane lipid composition hence maintenance of cell fluidity, solute transport, and enzymatic activity (Saraiva et al., 2018). Variability in strain response was also due to phenotypic and genotypic characteristics, which affect lag phase duration and growth (Saraiva et al., 2018).

Two models gave acceptable (B_f less than 1.43 and more than 0.87), fail-safe prediction; the "growth of *L. monocytogenes* in RTE cured meats" model and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model. Low levels of error were recorded for both models predictions as predicted data was closer to observed growth. Both models have been validated for use in the prediction of *L. monocytogenes* growth in RTE meat products (Seman et al., 2002, Mejlholm and Dalgaard, 2007, Mejlholm et al., 2015, Mejlholm and Dalgaard, 2015).

The "growth of LAB in ground beef" model and the "growth of *L. monocytogenes* and LAB in chilled seafood and meat products" model also gave acceptable fail-safe predictions of LAB growth in polony. According to Dalgaard and Mejlholm (2019), models that have been developed from laboratory output of experiments conducted on real-food give the most realistic growth estimations as they account for the effect of the food matrix on bacterial growth.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

The results of the study highlight the importance of effective inactivation of *L. monocytogenes* strains in polony processing, and the relevance of predictive food microbiology in pathogen detection as a preventative measure against listeriosis outbreaks. The conventional polony processing method is sufficient to inactivate all strains of *L. monocytogenes* investigated in the study. Correct implementation of the processing method can lead to the production of polony products that are safe for human consumption. Production of safe food does not end at the processing stage but extends throughout storage until consumption; it is therefore important that polony products are stored at the required temperature (chilled) to reduce bacterial growth. The polony production industry can also explore predictive modelling to detect pathogen growth, as the application of these models were found to be accurate and can provide a cost-effective and robust alternative to traditional testing. To effectively apply predictive models, the South African polony industry should embark on an intensive research on predictive modelling, validate the already existing models against their product and possibly try to develop models based on polony. The industry should however avoid using the models as the ultimate method, as there are several limitations attached to them. Most models predict the growth at constant intrinsic and extrinsic factors, while in real food environments, the factors are not stable. Also, the models are mostly developed using data from laboratory outputs conducted on media, hence high possibilities of over-predictions. It is therefore important that models are validated and the best performing chosen for use by the South African polony production industry.

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