Application of predictive food microbiology to reduce food waste

By

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DECLARATION

I Olaonipekun Basirat Arinola hereby declare that this dissertation submitted for the degree MSc. Food Science, at the University of Pretoria, is my own original work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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DEDICATION

This dissertation is dedicated to God Almighty for giving me the grace to come this far, I owe it to you Allah. Also to my husband, thank you for your unwavering support financially, morally and physically. I appreciate and love you.

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ABSTRACT

Application of predictive food microbiology to reduce food waste

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Co-Supervisor: **Dr. R. Coorey**

Universal food insecurity continue to be a challenge that needs attention from all stakeholders. The problem of food waste however is highly important as it slows down the effort to improve food security, most especially in the world's poorest countries. Conservative shelf life estimation of RTE foods by food producers is one of the major contributor to food waste.

After a survey was carried out on the different RTE food products (n=195) available on the shelf of 3 supermarkets in Hatfield, with their set shelf life and storage instructions. Microbiological quality (Total viable count, LAB, Enterobacteriaceae, yeasts and moulds, and *Pseudomonas* spp.) and safety (*E. coli*, *Staphylococcus aureus*, *Listeria* spp. and *Salmonella* spp.) was conducted on selected RTE products (used as a reference point) during storage at ± 5^{o} C. This wass to evaluate the validity of the set shelf life of beef lasagne (3 days), egg noodles (3 days), pre-cut mango (4 days) and pre-cut papaya (4 days) by food producers.

Challenge test study was also conducted on representative RTE food products (beef lasagne, egg noodles, and pre-cut mango) with relevant food borne pathogens (*L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli*) during storage for 12 days at ± 5^{o}C. Growth potential (δ) of these pathogens in the RTE foods were calculated using the concept of EU-CRL technical guidance on shelf life for *L. monocytogenes* on RTE foods as δ values can be very useful in potential food safety risk evaluation.

Performance of 4 different types of software (ComBase, PMP, MicroHibro & FSSP) was evaluated for use in shelf life estimation of these selected RTE foods. These software were selected based on different criteria (User-friendly, accessibility and availability and types of pathogens for its application). The predicted growth from these software were compared to observed growth (generated from experimental data got from challenge test) of *L. monocytogenes* in beef lasagne and egg noodles. Indices of performance; Coefficient of determination (R^2), root mean square error (*RMSE*), bias factor (B_f) and accuracy factor (A_f) were used to evaluate the performance of these software.

All the RTE food products reviewed had no specific refrigeration storage temperature instruction on the product package. Storage test study indicated that some of these RTE foods (beef lasagne, pre-cut mango and papaya) could have longer shelf life (5, 13 and 5 days respectively), while egg noodles could be a potential public health risk due to the presence of food borne pathogens right from day of purchase.

However, the challenge test results also confirmed the conservative shelf life estimation by food producers in that the shelf life of all the products evaluated can be extended (Beef lasagne by 6 days, Egg noodles by 6 days and pre-cut mango by 9 days) with no food safety risk associated with the extension. On the other hand. RTE egg noodles and beef lasagne may support the growth of *L. monocytogenes* ($\delta > 0.5$ $\log_{10}$ cfu/g) if present in the food while egg noodles may not support the growth of *S.* Typhimurium ($\delta \leq 0.5$ $\log_{10}$ cfu/g). Beef lasagne and pre-cut mango may also not support the growth of *E. coli* ($\delta \leq 0.5$ $\log_{10}$ cfu/g).

Growth of *L. monocytogenes* predicted by ComBase, PMP, MicroHibro & FSSP in beef lasagne and egg noodles was in agreement with the observed growth from the challenge test study, with a fail-safe prediction. However, ComBase predictor had the closest prediction to the observed growth. Hence, it had overall best performance for prediction compared to the other software. Notwithstanding, all the software evaluated in this study can be applied in shelf life prediction of RTE food products.

Predictive microbiology is a field of food microbiology that can be looked into and implemented by the authorities. Its use by the South African food industry to scientifically estimate the shelf life of RTE food products is thereby encouraged. This will assist in decision making with regards to food quality and safety, thereby reducing the problem of food waste as result of product shelf life and at the same time protect public health.

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CHAPTER 1: PROBLEM STATEMENT

Global food insecurity, particularly in Sub-Saharan Africa (SSA) remain a problem that is demanding increasing attention from all stakeholders. Based on most recent estimate, approximately 795 million people amounting to about one in nine of the world's population were chronically undernourished between 2014 and 2016 with insufficient food for an active and healthy life. The vast majority of these undernourished people live in developing countries, with an estimate of 220 million in SSA, which is the highest of any region in the world (FAO, 2015). It was reported that 70% of poor urban households in South Africa (SA) live under severe food insecurity (Frayne *et al*., 2009) with a 5.2 % prevalence of undernourishment between 2014 and 2016 amounting to about 3.2 million people (FAO, 2015). One of the major underlying cause of food insecurity in this region is the inability to have access to food and food unavailability (FAO, 2014) which can be associated to food waste and losses throughout the food supply chain (Gustavsson *et al*., 2011, Oelofse & Nahman, 2013).

The problem of food waste and losses is of high importance in the efforts to combat hunger, raise income and improve food security (Action Contre la Faim (ACF), 2014; High Level Panel of Experts (HLPE), 2014; Vanessa, 2014) in the world's poorest countries. Globally, about one-third of the edible parts of food produced for human consumption gets lost or is wasted, and this is estimated to be about 1.3 billion ton per year (Gustavsson *et al*., 2011). It is estimated that food waste in SA is approximately 9.04 million tonnes per year which tends to aggravate the problem of food insecurity, hence having a negative impact on food and nutritional security in SA (Oelofse & Nahman, 2013). This estimation of food waste excludes the waste incurred by the informal sector which are mainly the Small and Medium Enterprises (SMEs).

In Southern African, towns and cities lack access to food. The informal food economy plays an essential role in making food available to the urban poor households (Crush & Frayne, 2011) as well as the rural regions that are particularly at risk in terms of food insecurity due to food waste. The lack of postharvest expertise to serve the needs of developing country's production and supply chain has led to greater waste and loss at the SME level (The World Bank, 2011). This food wastage stalls the effort to improve global food security. Hence, support mechanisms need to be adapted to the specific needs of food processing SMEs that can be geared to assisting these enterprises in overcoming their financial and technical challenges as they have limited understanding of food handling, storage, distribution, and processing essentials.

A survey conducted on SME processors in SA (mainly Gauteng province) showed that SME companies recognised the need for a system of quality control and one of the technical challenges

affecting the SMEs is the unscientific determination of 'use-by' dates of food products (Mather, 2005), which negatively affects consumer perception of the use-by-date labelling leading to unnecessary substantial food loss and waste (Newsome *et al*., 2014). SMEs however need support and opportunity to optimise on shelf life.

The world population is projected to reach 9.7 billion by 2050 in which Africa is expected to account for more than half of this figure between 2015 and 2050 (UN, 2015). To feed this growing population, ACF, (2014) proposed food production increase by 70%. Making food available for the growing populations can be achieved by reduction in the amount of food lost and wasted (Lundqvist, de Fraiture, & Molden, 2008; Gustavsson *et al*., 2011). This can be achieved by accurate determination of shelf life of food (Newsome *et al*., 2014) with the use of predictive models (McMeekin *et al*, 2002; McKellar & Lu, 2004; Valero, Carrasco & García-Gimeno, 2012; Pérez-Rodríguez &Valero, 2013).

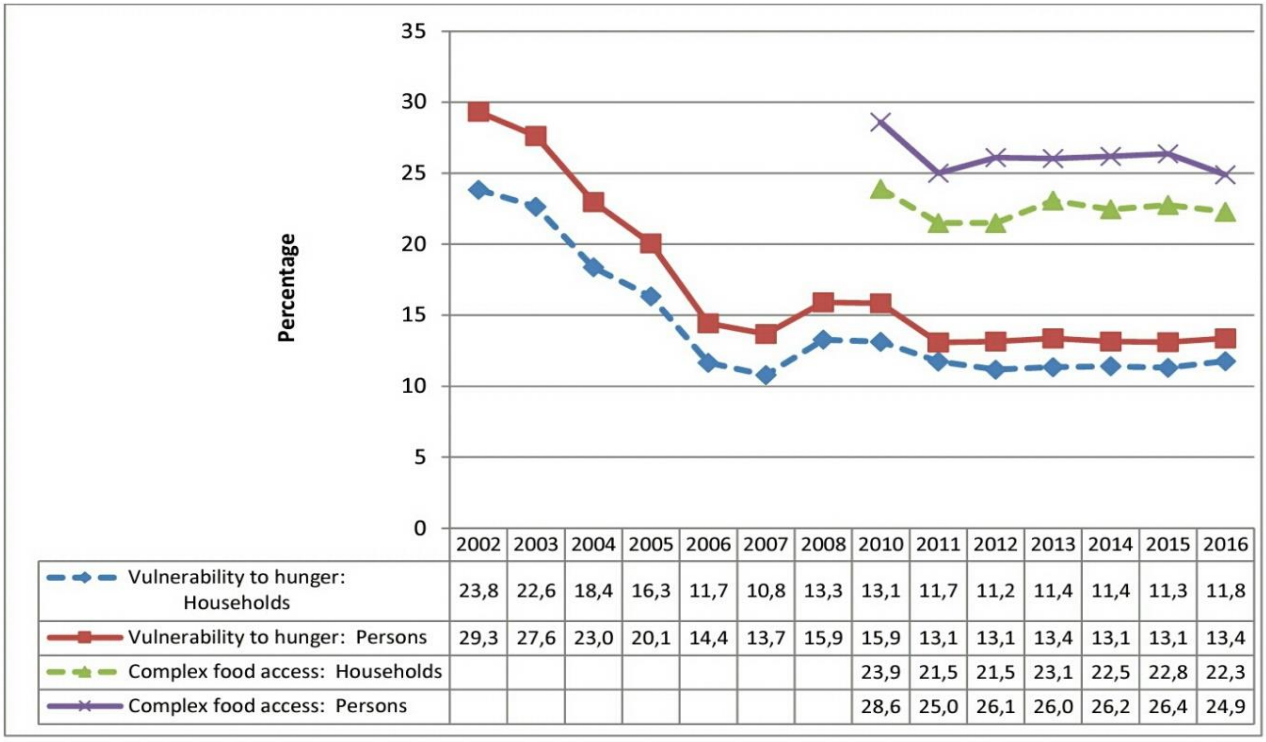
Ready to eat (RTE) foods are perishable by nature and changes will naturally take place during storage by the processor, retailer and the consumer. For microbiological spoilage or quality of food, predictive microbiology is recognized as a scientific-based reliable tool for providing an estimation of the course of the bacteria in the foods, estimating shelf-life of the product in cases where the cause of food spoilage or unacceptability is known to be microbiological (Kilcast & Subramaniam, 2000; Pérez-Rodríguez & Valero, 2013). This is a research area within food microbiology intended to provide mathematical models to accurately predict microbial behaviour in food environments (McMeekin *et al*, 2002; McKellar & Lu, 2004; Valero *et al*., 2012; Pérez-Rodríguez &Valero, 2013). The establishment of validated models for the determination of food shelf life is currently demanded by food industries (Valero *et al*., 2012; Pérez-Rodríguez &Valero, 2013), especially small scale food producers. Coupled with 'user friendly' software and the development of expert systems, these models are providing powerful new tools for rapidly estimating the effects of formulation and storage factors on the microbiological relations in foods (Pérez-Rodríguez &Valero, 2013). However, no work has been done on the application of predictive models for shelf life determination to reduce food waste in SA.

Therefore, the objective of this study is to apply predictive microbiology using tertiary shelf life models (software) in shelf life estimation of RTE food products with the aim of reducing food waste. Resulting in a shelf life determination tool that can be understood and employed by SMEs to accurately estimate shelf life of RTE food products.

CHAPTER 2: LITERATURE REVIEW

2.1 An overview of food security issues in developing countries

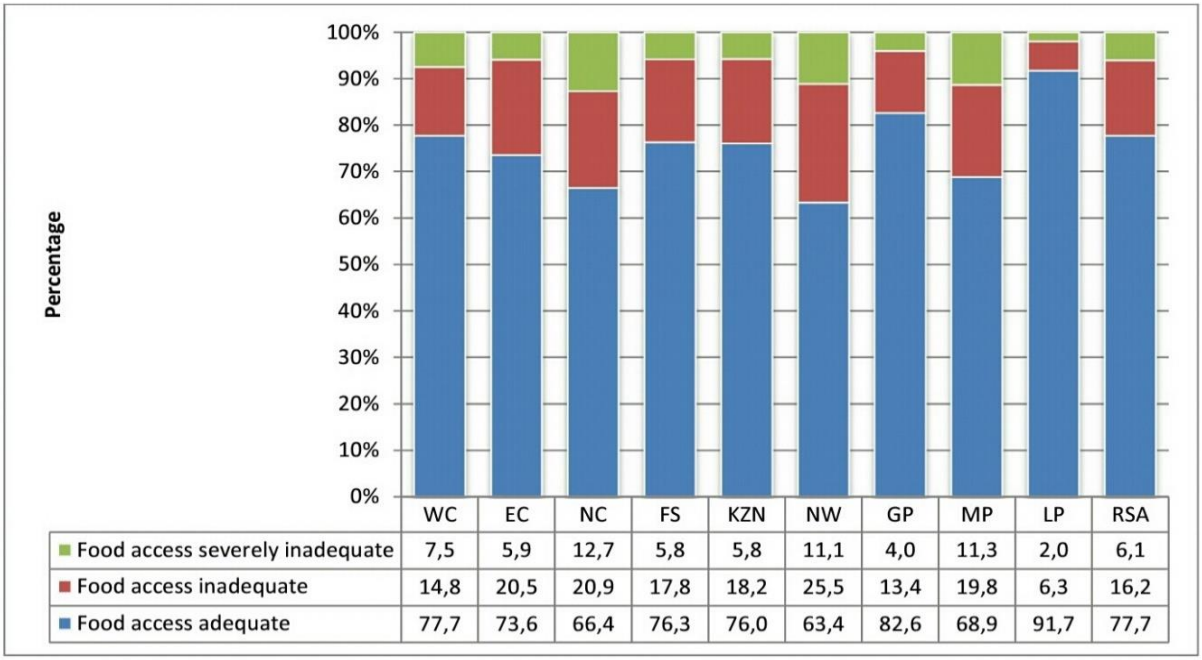
Food security exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life (FAO, 1996 World Summit; Pereira, 2014) and is established in four dimensions: availability, access, stability and utilization. Food availability captures the quantity, quality and diversity of food while food access comprises indicators of physical access, infrastructure. The stability of food covers factors that measure exposure to food security risk and also focuses on the incidence of shocks such as domestic food price volatility, fluctuations in domestic food supply, and political instability. Food utilization encompasses variables that determine the ability to utilize food and also focuses on outcomes of poor food utilization, i.e. nutritional failures of children under five years of age, such as wasting, stunting and underweight (FAO, 2014) with all the dimensions having their indicators. The issue of food insecurity has been critical in many parts of the world including SA (Misselhorn, 2005). There is no specific and accepted measure of food security in SA, and currently there are no regularised ways of monitoring it (Altman, Hart & Jacobs, 2009). SA may be food secure as a country with less than 5% of undernourished population (FAO, 2015) as the percentage of South African households with inadequate or severely inadequate access to food decreased from 23.9% in 2010 to 22.3% in 2016 (Fig. 1). The percentage of households that experienced hunger decreased from 23.8% to 11.8% while the percentage of individuals who experienced hunger decreased from 29.3% to 13.4% over the same period (STAT SA, 2016). It has however been reported that large populations of South African households, mainly concentrated in rural areas, face food insecurity and poverty (Rooyen & Sigwele, 1998; Altman *et al*., 2009). Achieving food security in its totality continues to be a challenge not only for the developing nations, but also for the developed world. The difference lies in the magnitude of the problem in terms of its severity and proportion of the population affected.



	2002	2003	2004	2005	2006	2007	2008	2010	2011	2012	2013	2014	2015	2016
Vulnerability to hunger: Households	23,8	22,6	18,4	16,3	11,7	10,8	13,3	13,1	11,7	11,2	11,4	11,4	11,3	11,8
Vulnerability to hunger: Persons	29,3	27,6	23,0	20,1	14,4	13,7	15,9	15,9	13,1	13,1	13,4	13,1	13,1	13,4
Complex food access: Households								23,9	21,5	21,5	23,1	22,5	22,8	22,3
Complex food access: Persons								28,6	25,0	26,1	26,0	26,2	26,4	24,9

Figure 1: Number of household and persons vulnerable to hunger in SA from year 2010 to 2016 with their access to food within the year 2010 and 2016. Source; Stats SA

In 1990, the international community and national governments set a target of achieving Millennium Development Goals (MDGs) by 2015 for food security. Although a number of countries are currently within track, achieving these targets remains a challenge for many others most especially the developing countries (FAO, 2014). Regardless of the progress made in achieving the MDG 1c goal, considerable efforts are still needed to reach the MDG hunger target by 2015 and beyond, especially in developing countries. Globally, about 795 million people are still estimated to be chronically undernourished between 2014 and 2016, with about one in every nine people in the world having insufficient food for an active and healthy life (FAO, 2015). The vast majority of these undernourished people live in developing countries, where an estimated 779.9 million are chronically hungry, with SSA having the highest prevalence (23.2%) of undernourishment (FAO, 2015). In SA, between 18% (Department of social development & Department of Agriculture Forestry and Fisheries (DAFF), 2013) to 35% (Kirsten, 2012) of the population experience hunger.



	WC	EC	NC	FS	KZN	NW	GP	MP	LP	RSA
Food access severely inadequate	7,5	5,9	12,7	5,8	5,8	11,1	4,0	11,3	2,0	6,1
Food access inadequate	14,8	20,5	20,9	17,8	18,2	25,5	13,4	19,8	6,3	16,2
Food access adequate	77,7	73,6	66,4	76,3	76,0	63,4	82,6	68,9	91,7	77,7

Figure 2: The severity in percentage of inadequate access to food in all province within SA in 2016. Source: Stats SA

According to DAFF, (2011), food security was reprioritised as one of the top priorities for South African government (State of Nation Address, 2010) in the 2010/2011 financial year which is in line with SA's MDG. The aim of this is reducing the proportion of people who go hungry over the period of 1990 and 2015 by half in which one of the critical components in meeting that objective is household food security. Household food insecurity is a major determinant of under nutrition (Rose & Charlton, 2001) and both are complex problems that cannot be solved by a single stakeholder or sector. A variety of actions are required to deal with the immediate and underlying causes of hunger and malnutrition.

2.1.1 Food waste and loss as a major contributor to food insecurity

There are numerous causes of food insecurity. These include population growth, rising cost of food, transportation and agricultural amenities and post-harvest losses (The World Bank, 2011; HLPE, 2014; The Rockefeller Foundation, 2015) as well as distribution and efficiency of food system. SA ranks among the countries with the highest rate of income inequality in the world and compared to other middle income countries, it has extremely high levels of absolute poverty (Altman *et al*., 2009). In this region, lack of access to food is key to the food insecurity of poor households which is caused by the absence of a sustained or reliable income source (Crush & Frayne, 2011) as a high percentage of their income is devoted to food (The World Bank, 2011; Nahman, *et al*., 2012). Food that could feed these hungry set of people has however been put to waste.

The relationship between food wastage and food security is a complex one and in spite of the popularity of food wastage interventions, the number of studies and documents on the relationship between food

wastage interventions and food security has been found to be limited (Tielens & Candel, 2014). In October, 2012, FAO Director-General Jose Graziano da Silva suggested that in order to achieve zero hunger, elimination of food wastes and losses among other measures is essential. This statement was also supported by the UN after a meeting of 13 UN organizations held in September, 2013 where it was suggested that food waste and loss reduction is one of the most effective ways of improving global food supply thus contributing to enhanced food and nutrition security (Tielens & Candel, 2014). At the consumer end of the supply chain, some actors for instance, European Federation of Food Banks (FEBA) work on the direct link between food waste prevention and food security by collecting food that consumers or retailers would otherwise throw away and donate it to those in need and this is similar to the concept of South African food bank. It is also assumed that reduction of post-harvest losses through interventions aiming at a more efficient supply chain in developing countries has a direct impact on food security as it increases the amount of food available for poor small holder food producers and increases the general availability of food at community or regional level (Tielens & Candel, 2014). Food crisis and chronic food shortages lead to compromised human wellbeing, hunger and under nutrition which is developed when nutrient intakes are insufficient to meet nutrient requirements (Rose & Charlton, 2001), posing serious challenges to governmental and non-governmental institutions and formal and informal policy and decision makers at all levels (Misselhom, 2005). Hence, in an effort to combat hunger and alleviate the problem of food insecurity in poor countries, food waste and loss is an important factor to be considered (Gustavsson *et al*., 2011; Oelofse & Nahman, 2013).

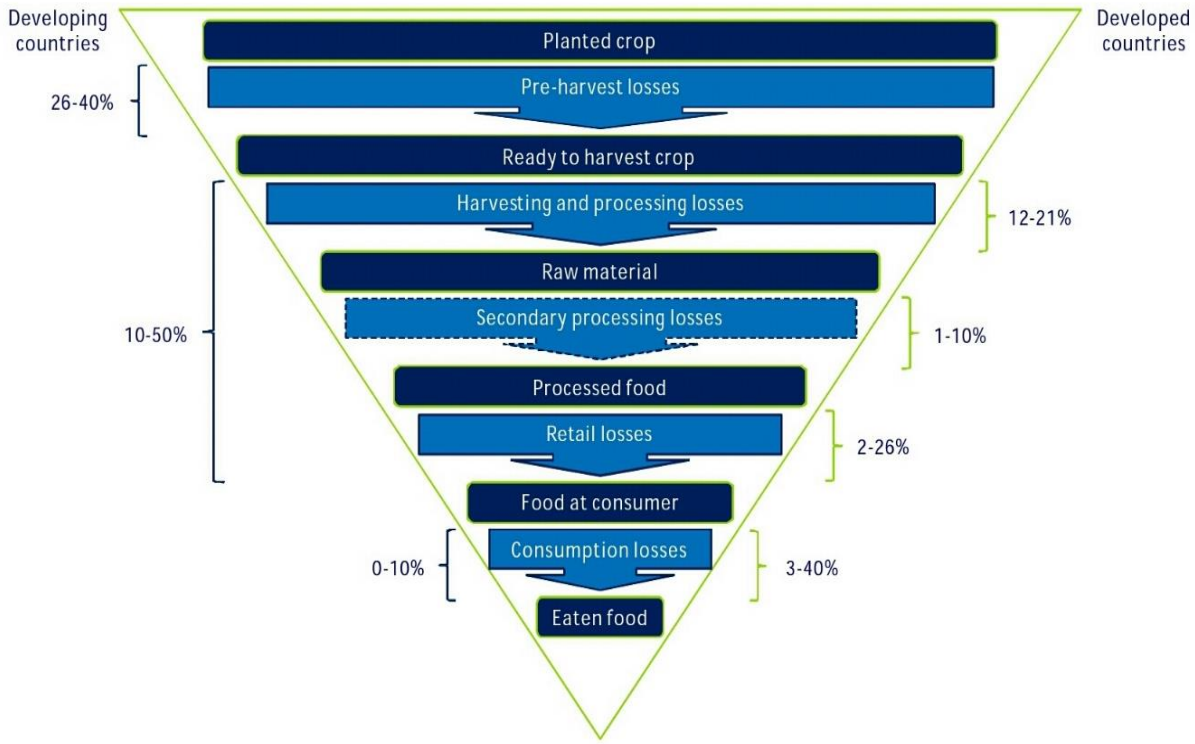


Figure 3: Comparing food loss in developed and developing countries along the value chain. Source: World Economic Forum, 2009.

2.2 Food waste and loss across the value chain

Food losses occur at all stages in the food supply chain (FSC), from initial agricultural production to final household consumption (Gustavsson *et al*., 2011), including during food storage, transportation, food processing, at retailers, and in the kitchens of restaurants, hotels and households (Lungqvist *et al*., 2008). Causes of food waste and losses vary among developed and developing countries (Fig 3) with each facing different challenges, with more waste downstream from the consumer in the industrialized world due to consumer behaviour and lack of coordination between different actors in the FSC (Oelofse & Nahman, 2013) and more spoilage upstream from production line in the developing world (Rutten, 2013). There are different causes of food waste throughout the FSC. Post-harvest losses are influenced by available technologies and the extent to which markets for agricultural produce have developed (Parfitt, Barthel & Macnaughton, 2010) while at household level, food waste vary considerably from one area to another, as a result of cultural practices, climate, diet and socio-economic factors (e.g. household size, household income and frequency of eating out) (European Commission (EC), 2010). In low-income countries, food waste and losses are influenced by financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in difficult

climatic conditions, infrastructure, packaging and marketing systems (Parfitt *et al*., 2010). Food waste and loss occur at all levels of the food system (Table 1), from farming, processing, and retailing, through to the final consumers in both developed and developing countries (Oelofse & Nahman, 2013).

Table 1: Percentage estimated food waste for food commodity group in each step of the FSC for SSA (Gustavsson *et al*., 2011)

Commodity group	**Agricultural production (%)**	**Post-harvest handling and storage (%)**	**Processing and packaging (%)**	**Distribution (%)**	**Consumption (%)**
Cereal	6	8	3.5	2	1
Roots and tubers	14	18	15	5	2
Oil seeds and pulses	12	8	8	2	1
Fruits and vegetables	10	9	25	17	5
Meat	15	0.7	5	7	2
Fish and seafood	5.7	6	9	15	2
Milk	6	11	0.	10	0.1

Taking imports and exports into account, recent study by Nahman & de Lange, (2013), on the total estimated quantity of food waste along the value chain in SA amounted to 10.2 million tonnes per annum compared to the 9.04 million tonnes per annum estimated by Oelofse & Nahman, (2013) who only estimated food waste from local food production. Overall, fruits and vegetables (44%) contributes the highest quantity of food waste, followed by cereals (26%) with oil seeds and pulses contributing the lowest (4%), while across the value chain, food waste ranged between 5 and 26% with the least food wastage (5%) at the consumption stage. This study estimates that about 9 million tonnes of the 28.79 million tonnes per annum of food produced between 2007 and 2009 is wasted (Table 2). Significant household food waste has been generated which has made this aspect of food waste to be an increasingly discussed topic (Lebersorger & Schneider, 2011). Several studies has been carried out on food wastage at household level in the developed countries, little or nothing is known about the level of food waste at household level in developing countries and most especially in SA.

Table 2: Average estimate of food waste generated per annum in SA between 2007 and 2009 (Oelofse & Nahman, 2013)

Commodity group	Average production between 2007-2009 (1000 tonnes)	Agricultural production (waste per 1000 tonnes)	Post-harvest handling and storage (waste per 1000 tonnes)	Processing and packaging (waste per 1000 tonnes)	Distribution (waste per 1000 tonnes)	Pre-consumer waste (waste per 1000 tonnes)	Consumption (waste per 1000 tonnes)	Total waste commodity group (waste per 1000 tonnes)
Cereal	13154	789.3	989	398	220	2396	108	2504
Roots and tubers	2017	282.4	312	213	60	869	23	892
Oil seeds and pulses	453	54.4	32	29	7	122	3	126
Fruits and vegetables	8230	823	667	1685	859	4034	210	4244
Meat	1587	238.1	9	67	89	404	24	427
Fish and seafood	224	12.8	13	18	27	71	3	74
Milk	3119	187.1	323	3	261	773	2	775
Total per stage of the FSC	28785	2387	2344.6	2413.4	1523	8668.2	372.7	9040.9

Definition of food waste/loss differ widely. According to Nahman & de Lange, (2013), food waste includes both the edible and inedible portion of the food waste and it can be described at the pre-consumer stage to include losses that are incurred before food reaches the final consumer while food waste at the post-consumer stage is the food that is discarded by the final consumers. The outcome of the study carried out by Nahman & de Lange, (2013) suggests that food waste alleviation in SA can be aimed at all stages of meat and fruit and vegetables (Oelofse & Nahman, 2013) value chain which include the post-consumer stage targeted at household level.

2.3 Small Medium Enterprises (SMEs) and food security in South Africa

The importance of SMEs in the economy expresses itself in their contribution to the GDP and employment, which is likely to be as high as the large enterprises' contribution. Mather, (2005), interviewed 30 SME food processors mainly in Gauteng province in SA and it was reported that 26 SME food processors tend to be more involved in supplying black South Africans in townships. Means of supplying are through spaza shops, networks of hawkers, commuter stations and local markets as there is direct link between them and other businesses that do not involve supermarket chains. The

informal food economy thus plays an essential role in the provision of food for urban households and in making food available to the urban poor. These are important issues for urban food security since the informal economy is an important income source for many urban households (Crush & Frayne, 2011). Hence, SME food processors can have impact on alleviating food insecurity. It is important to recognize, however, that access to food through any of these informal channels contributes to the availability of food to the poor households. The survey of SME food processors carried out by Mather, (2005) suggests that support mechanisms geared at overcoming technical (quality) challenges need to be confronted as these companies are not normally audited nor have they implemented an internationally accredited facility audit to improve the quality of their products (Mather, 2005).

2.4 Microbiological quality of Ready-To-Eat (RTE) foods products

Over the years, consumption of RTE such as RTE meals, RTE meat products, minimally processed fruits and vegetables has increased and this increase can be attributed to demand for convenient and healthy food products. However, they are highly perishable with short shelf life (Gutierrez *et al*., 2008). Foods are dynamic systems that experience changes in pH, atmosphere, nutrient composition and microflora over time (Valero *et al*., 2012). They are not only nutritious to consumers, but are also excellent source of nutrients for microbial growth. Spoilage is characterised by any change in a food product that renders it unacceptable to the consumer from a sensory point of view. Food spoilage is a complex process and excessive amounts of foods are lost due to microbial spoilage even with modern day preservation techniques (Huis in't Veld, 1996; Gram *et al*., 2002). Hence, microbial spoilage is an area of great concern. It is estimated that as much as 25% of all food produced is lost post-harvest due to microbial activity that has been identified as a significant threat to food security (Barth *et al*., 2009).

During harvesting, processing and handling operations, food may become contaminated with a wide range of microorganisms. In foods, microbial degradation manifest itself as changes in sensory properties of the food product rendering it unsuitable for consumption due to the formation of metabolic substances such as amines, sulphides, alcohols, aldehydes, ketones, and organic acids with unpleasant and unacceptable off-flavours, which is product specific (Gram & Huss, 1996; Gram & Dalgaard, 2002). Factors affecting microbial spoilage are classified into intrinsic and extrinsic factors. Intrinsic parameters are the physical, chemical and structural properties inherent in the food itself and the most important intrinsic factors are water activity, acidity, redox potential, available nutrients and natural antimicrobial substances. Extrinsic parameters are factors in the environment in which a food is stored, notably temperature, humidity and atmosphere composition. To a large extent, intrinsic

factors and type of packaging of food determine the expected shelf life of RTE food products (Huis in't Veld, 1996; Barth *et al*., 2009).

Microbial spoilage can be detected by organoleptic, microbiological and chemical methods. At the point of sensory rejection (spoilage), the spoilage microflora is composed of both microorganisms that have contributed to the spoilage called specific spoilage organism (SSO) and microorganisms that have grown but not caused unpleasant changes of the product, foods that are spoilt have microbial counts of greater than 10^6cfu/g (Patsias *et al*., 2006; Barth *et al*., 2009). Many flora of spoilage bacteria have an effect on the shelf-life of refrigerated food products (Lebert, Begot & Lebert, 1998). For example, *Brochothrix thermosphacta* in precooked chicken (Patsias *et al*., 2006), gram negative bacteria in fresh-cut minimally processed fruits and vegetables (Rico *et al*., 2007; Christison, Lindsay & Van Holy, 2008), *Erwinia* spp. and *Pseudomonas* spp. in RTE vegetable products (Lund, 1992). The microbial flora that colonizes a particular food or beverage depends highly on the characteristics of the product, the way the food is processed and stored (Huis in't Veld, 1996; Gram *et al*., 2002; Valero *et al*., 2012).

Microbial safety is also an aspect of food shelf life that must be considered when determining the shelf life of food products. RTE food products has been a major source of contamination and foodborne illnesses due to proliferation of foodborne pathogens (Harris *et al*., 2003; Hwang & Tamplin, 2005; Soto *et al*., 2007; Uyttendaele *et al*., 2009; Vermeulen *et al*., 2011; Sant'Ana *et al*., 2011, 2012a; Scolforo *et al*., 2016; CDC, 2016a; CDC, 2017). Microbial safety on the other hand, cannot be detected by organoleptic or sensory rejection. In SA however, an outbreak of salmonellosis was reported in Kwazulu-Natal province (Niehaus *et al*., 2011) while *E. coli* was isolated in biltong (Naidoo & Lindsay, 2010).

Listeria spp. especially *Listeria monocytogenes* are a major concern in refrigerated RTE foods because this pathogen can persist and often proliferate in contaminated foods under a wide range of antimicrobial conditions. They can be easily found in quite a number of environments and substrates and this makes it easy for the organism to contaminate food products especially during production, processing and packaging (Mejlholm, Bøkæs & Dalgaard, 2005; Pouillot *et al*., 2015). *L. monocytogenes* is a food borne pathogen of concern, leading to a fatal disease known as listeriosis (CDC, 2015; CDC, 2016b). Virulent strains have been found to cause serious illnesses including death albeit the infectious levels based on data from animal models are reported to be > 8 $\log_{10}$ cfu/g for healthy individuals and between 2 – 3 $\log_{10}$ cfu/g for immunocompromised (infants, elderly and pregnant) individuals (Takeuchi *et al*., 2006; Williams *et al*., 2007; Warriner & Namvar, 2009).

Survival and growth potential of *L. monocytogenes* depends on storage temperature, product type, environment, sanitary practices and product composition (Vermeulen *et al*., 2011; Pouillot *et al*., 2015; Sahu *et al*., 2017).

Salmonella spp. are facultative pathogens belonging to the Enterobacteriaceae family. The genus consists of two species: *S. enterica* and *S. bongori* (ICMSF, 1996; Forshell & Wierup, 2006). *Salmonella enterica* Serovar Typhimurium is among the 10 serovars most commonly associated with human infections and are of public health concern as they have been associated with significant illness and death in those infected with the pathogens (Forshell & Wierup, 2006; Foley, Lynne & Nayak, 2008; Batz, Hoffmann & Morris, 2012; Jackson *et al*., 2013; CDC, 2017). Salmonellosis is the second leading cause of bacterial foodborne illness in the United States. The majority of infections due to salmonellosis are associated with the consumption of products such as meat, poultry, egg products, pasta, noodles, milk, seafood and fresh produce contaminated with Salmonella (Foley *et al*., 2008; Batz *et al*., 2012; Jackson *et al*., 2013; Li *et al*., 2014; Fang & Huang, 2014; CDC, 2017).

E. coli in food is an indicator microorganism for faecal contamination and it belongs to the family Enterobacteriacae. Non-pathogenic *E. coli* strains are typically found in the gastrointestinal tracts of warm blooded animals and humans (Forsythe & Hayes, 1998; Ryan & Drew, 2010; Baylis *et al*., 2011) and can be shed into the environment via faeces. All species are opportunistic pathogens and cause disease in humans, most especially immunocompromised individuals such as infants, the elderly or HIV positive individuals (Ryan & Drew, 2010). *E. coli* are of particular concern due to their potential for growth on fresh-cut fruit prior to consumption as well as their low infectious dose (Beuchat, 2002). Enterohaemorrhagic *E. coli* (EHEC) which also have animal source has often been associated with foodborne infections and illnesses in RTE food products (Baylis *et al*., 2011; CDC, 2013).

2.4.1 Traditional methods of shelf life determination of RTE food products by SMEs

Product "shelf life" can be defined as "the time or period a product may be stored before a specific element of the product makes it unsuitable for use or consumption" which could be of biological or physicochemical nature (Valero *et al*., 2012). Different procedures have been reported for the establishment of shelf-life, mainly based on the detection of microbial alteration, as well as physico-chemical and sensorial changes. The traditional approach is done experimentally by the storage of food product at different temperatures, performance of microbial analysis and the assessment of spoilage by sensorial testing. In the case of foods whose shelf-lives might be conditioned by the presence and proliferation of pathogenic microorganisms, experiments also involve challenge testing with the target organism prior to storage (Labuza & Taoukis, 1990; New Zealand government, 2014; Valero *et al*.,

2012). A cut-off point referred to as quality or safety limit is set along the storage period at the time when any of the measured attributes exceeds a pre-established limit. This method is usually labour-intensive and expensive. It is advisable to validate the established shelf life of a food as well as to repeat the analyses in several occasions to include shelf life variability due to effects of different environmental variables (Valero *et al*., 2012).

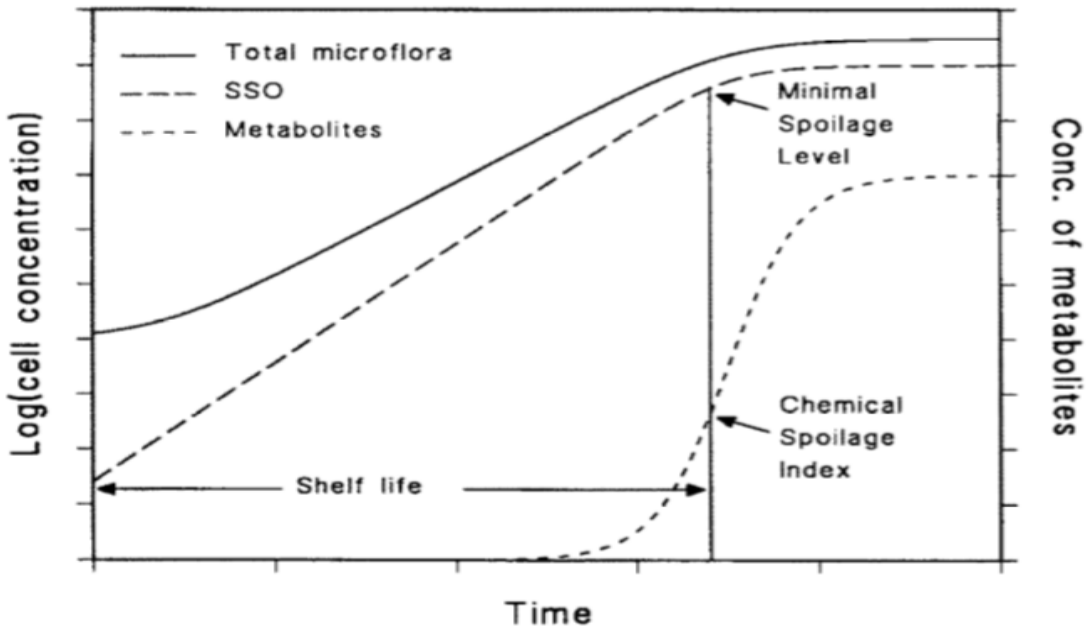


Figure 4: Microbial spoilage process, SSO concept. The minimal spoilage level and the chemical spoilage index are, respectively, the numbers of SSO and the concentration of metabolites determined at the time of sensory rejection. Source: Dalgaard, 1993

2.5 Predictive microbiology and its applications

Predictive microbiology is a broad scientific branch of food microbiology that make use of modeling to quantitatively assess the microbial behaviour in food environments to derive adequate mathematical models (Valero *et al*., 2012; Pérez-Rodríguez & Valero, 2013). Predictive modelling has been in existence since the early 20th century (McMeekin *et al*., 2006) where models were used to describe the inactivation kinetics of food-borne pathogens during thermal processing of foods. In general, a model simplifies a system by using a combination of descriptions, mathematical functions or equations, and specific starting conditions (McKellar & Lu, 2004). Predictive microbiology has applications in both microbial safety and quality of foods (Pérez-Rodríguez & Valero, 2013) and has been used as an alternative concept in shelf life determination (Valero *et al*, 2012) as the determination of microbial

growth with traditional microbiological challenge tests is expensive and time-consuming (McKellar & Lu, 2004; Valero *et al*., 2012; Bruckner *et al*., 2013).

Predictive models are useful tools for improving food safety and quality, which can be applied to different facets of the food sector which includes Hazard Analysis and Critical Control Points (HACCP), Risk Assessment and Risk Management, shelf life studies, Innovation and Development of a new product, Hygienic measures and Temperature integration, Education, and Experimental design (Pérez-Rodríguez & Valero, 2013;). According to (McMeekin & Ross, 2002; McKellar & Lu, 2004), there are two general classes of models, depending on the basis of information used to construct the model: descriptive and explanatory. The explanatory (mechanistic or deterministic) models is composed of analytical and numerical models while descriptive (empirical or probabilistic) models are data driven which are classified by approaches such as polynomial functions, artificial neural nets and principal component analysis.

Predictive microbiology models describing kinetic processes are categorized into the following: (i) Primary models describes microbial population, their interactions through time with the purpose of describing growth and activation phases (growth or death curve). This is done by estimating kinetic parameters such as maximum growth rate, lag phase and inactivation rate. (ii) Secondary models describes how kinetic parameters of the primary models depend on the environmental conditions such as pH, temperature, NaCl, water activity etc. (Whiting, 1995; Ross, Dalgaard & Tienungoon, 2000; McMeekin *et al*., 2006). (iii) Tertiary models are applications of both the primary and secondary model in computer tools intended to provide predictions that allow model inputs to be entered. Estimates are observed through simplified graphical outputs (Whiting & Buchanan, 1994; McKellar & Lu, 2004; Pérez-Rodríguez & Valero, 2013). The use of this classification depends mainly on the purpose and type of predictions to be generated.

2.5.1 Modeling microbial responses in food environment

The scientific basis of predictive microbiology are that microbial responses in foods are in a way reproducible against several extrinsic and intrinsic environmental factors (Ross *et al*., 2000). This responses can be translated into mathematical models that estimate microbial growth/inactivation/toxin production/probability of growth, and so on (Pérez-Rodríguez & Valero, 2013). A basic model is structured as;

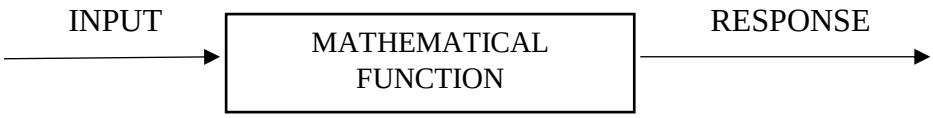


Figure 5: A basic model structure (Adapted from Pérez-Rodríguez & Valero, 2013)

As seen in fig 5, mathematical model estimates the response of the represented system or process based on the values of the input variables. Therefore, mathematical models are composed of two components, a deterministic part describing the deterministic relationship between explanatory variables and the response variable and a stochastic part corresponding to the observed data variability that cannot be explained by the deterministic part (Pérez-Rodríguez & Valero, 2013). Basically, models predicting microbial responses can be split up into three groups: survival/inactivation models, boundary (growth/no growth) models, and growth models (Pérez-Rodríguez & Valero, 2013). The use of a model to accurately predict the behaviour of microorganisms over a range of conditions may often lead to misleading shelf-life predictions which can be due to each product having specific microflora which is determined by intrinsic and extrinsic factors of the product, within the limited range at which the microbial model can be applied (Koutsoumanis & Nychas, 2000). Hence, in order to achieve accurate predictions of shelf-life, it is essential to choose and apply a model based on the spoilage process of the specific product in question (Dalgaard, 1997).

According to Dalgaard, (1995), certain information is required for shelf-life predictions that include microorganisms responsible for spoilage of the specific food product SSO, the environmental conditions over which a specific SSO is responsible for spoilage (Spoilage domain (SD), and the population level of SSO at which spoilage occurs (Spoilage level). Shelf-life can thereafter be easily predicted using one of the existing models for the SSOs within their SD (Dalgaard, 1997). Due to the variation in food products, initial population of SSO is also an important information needed in shelf-life prediction using microbial models (Koutsoumanis & Nychas, 2000). Models that recognize and account for uncertainty or variability in an experimental system are called stochastic or probabilistic models (McKellar & Lu, 2004; Koutsoumanis & Angelidis, 2007).

2.5.2 Predictive microbiology in food as a tool for shelf life estimation

Determination of shelf life is one of the most likely applications of predictive microbiology in food industries, rendering a reliable and economic tool for obtaining rapid estimations of microbial responses to food environment (McMeekin *et al*., 2002). Predictive microbiology used as a tool in shelf life determination can predict the growth of specific food spoilers and pathogens (Fakruddin,

Mazumder & Mannan, 2011; Pérez-Rodríguez & Valero, 2013). Predictive models are mostly based on observations obtained from experiments on food developed under controlled conditions. The different types of models allows prediction of growth, inactivation, growth boundaries and no growth of bacteria in foods under different environmental conditions and considering additional factors such as the physiological state of cells or interaction with other microorganisms (Baranyi & Roberts, 1994; Pérez-Rodríguez & Valero, 2013). Data accumulation on behaviour of microorganisms in food is quite laborious and expensive and can describe microorganism's response in food, although they provide little insight into the relationship between physiological processes and growth or survival. One way this link can be made is through the use of mathematical models (McKellar & Lu, 2004), which means a simple mathematical description of a process (Fig 5). Different approaches to shelf-life estimation has been used and in all cases, models should be validated as reliability of predictions should be compared with real data in foods (Dalgaard, Mejlholm & Huss, 1997; Pérez-Rodríguez & Valero, 2013) because accurate prediction of shelf-life is particularly important to ensure product quality and safety.

Primary, secondary and tertiary models can be used in model application. Primary model phase can assume, a storage phase, processing, and/or thermal treatment and aim to describe the four phases of a typical microbial population as seen in fig 4. Primary models commonly used include (1) Growth model which uses different mathematical equations/functions to fit generated microbial growth data. They include, sigmoidal functions (Gibson, Bartchetll & Roberts, 1987) e.g. modified logistic and the gompertz functions, mechanistic functions (Baranyi, Roberts & McClure, 1993; Baranyi, *et al*., 1995; Baranyi & Roberts, 1999), logistic and linear functions (Rosso *et al*., 1996). (2) Inactivation models include, Bigelow Model (Linear Model), Weibull Model, Shoulder/Tail Models. Secondary models are mathematical expressions and commonly used secondary models are, Polynomial models, Square Root-Type models, The Gamma Concept and the Cardinal Parameter Model (McKellar & Lu, 2004; Pérez-Rodríguez & Valero, 2013). Microbial growth models can be used to predict the effect of various time-temperature combinations on shelf life of food products (Koutsoumanis & Nychas, 2000); while tertiary models (Whiting & Buchanan, 1994) are mathematical models incorporated into software packages and are described in the next section below.

2.5.3 Computer Software applications for shelf life prediction

As a result of advancement in computer and statistical software packages, the use of modeling in food microbiology has grown to the point of recognition (McKellar & Lu, 2004; Pérez-Rodríguez & Valero, 2013; Valero *et al*., 2012). However, wider use of predictive models in industry, research and teaching

depends on availability of application software that allows different users to obtain information from models in a rapid and convenient way (McMeekin *et al*., 2006). Many predictive modeling software has been developed in order to provide predictions of microbial responses in foods by controlling environmental and physicochemical factors and/or food additives (Baranyi & Tamplin, 2004; McMeekin *et al*., 2006). Whiting & Buchanan (1994) called the integrated software models-based "tertiary models". Incorporation of predictive microbiology models in software packages is important in order to facilitate their use by the food industry, regulatory authorities, academics and interested parties. The use of predictive modeling software makes it better to understand microbial behaviour in foods.

The software intended to be used by both expert and non-expert users may be a valuable decision support tool for the food industry, assisting in the management of foods based on their actual shelf-life and microbial safety, thereby limiting the deterministic 'best-by' practice for determination of shelf-life (Psomas *et al*., 2011). Table 3 gives an overview of the different types of software applied for shelf life modeling. However, some examples of software used in predictive modeling include; GInaFiT (Geeraerd, Valdramidis & Van Impe, 2005), where different inactivation models are available DMFit (Baranyi & Roberts, 1994), with implementation of a dynamic growth primary model. Pathogen Modeling Program (PMP) (Buchanan, 1993), incorporates a variety of models of different pathogens in broth culture and foods. Food Spoilage and Safety Predictor (FSSP) (Dalgaard, Buch & Silberg, 2002), offers models for specific spoilage microorganisms and for *L. monocytogenes* in seafood and meat products.

ComBase (Baranyi & Tamplin, 2004) is a predictive tool for important foodborne pathogenic and spoilage microorganisms. Sym´Previus (Leporq *et al*., 2005) is a tool with a collection of models and data to be applied in the food industry context, for example strengthening HACCP plans, developing new products, quantifying microbial behaviour, determining shelf life and improving safety. Microbial Responses Viewer (MRV) (Koseki, 2009), consist of microbial growth/no growth data derived from ComBase. It also model the specific growth rate of microorganisms as a function of temperature, pH and a_w. *E. coli* fermented meat model (Ross & Shadbolt, 2004) describes the rate of inactivation of *E. coli*, due to low a_w or pH or both, in fermented meats. Most of these software packages are available for free (Table 3) and developed for specific microorganisms in specific foods. European Union launched a project for development of software tool for shelf life prediction of RTE foods such as fruit, vegetables and salads under the SOPHY project (www.sophy-project.eu/project.html) which can be used by SMEs.

Table 3: Different types of software available in the field of predictive microbiology for prediction of food safety and quality.

Software	Accessibility	Link
Baseline 1.0	Free internet access	www.baselineapp.com
ComBase	Free internet access	www.combase.cc
DMFit	Free to be downloaded	www.combase.cc http://www.ifr.ac.uk/safety/dmfit/
Escherichia coli fermented meat model	Free to be downloaded	http://foodsafetycentre.com.au/fermenter.php
Filtrex	Free to be downloaded	http://w3.jouy.inra.fr/unites/miaj/public/logiciels/filtrex
FISHMAP	Free to be downloaded	http://azti.es/fishmap
Sym'Previus	Commercial internet access	www.symprevius.org
Shelf Stability Predictor	Free internet access	http://meathaccp.wisc.edu/ST_calc.html
Prediction of Microbial Safety in meat Products (DMRI model)	Free internet access	www.dmripredict.dk
Corbion Listeria Control model	Commercial internet access	https://clcm.corbion.com/
Unified Growth Prediction Model (UGPM)	Free to be downloaded	http://www.aua.gr.psomas
Pathogen Modeling Program (PMP)	Free to be downloaded	http://www.ars.usda.gov/Services/docs.htm?docid=11550
Frisbee cold chain prediction software	Free to be downloaded	http://frisbee-wp2.chemeng.ntua.gr/coldchaindb/?go=download_software
Growth predictor and perfringes predictor	Free internet access	http://www.ifr.ac.uk/Safety/GrowthPredictor
Food Spoilage and Safety Predictor (FSSP)	Free to be downloaded	www.fssp.food.dtu.dk
FoodProcess-Lab	Free to be downloaded	https://sourceforge.net/projects/foodprocesslab
Campden BRI Forecast	Not freely accessible (information@)campdenbri.co.uk or gail.betts@campdenbri.co.uk)	http://www.campdenbri.co.uk/services/predictive-microbiology.php
Therm 2.0	Free internet access	http://www.meathaccp.wisc.edu/pathogen_modeling/therm.html
OptiPa	To be obtained from author for free	www.biw.kuleuven.be/biosyst/mebios/downloads/optipa/OrderOptipa
GroPin	Free to be downloaded	www.aua.gr/psomas/gropin
Sweetshelf	Commercial (a.vermeulen@UGent.be)	www.cpmf2.be/software.php
IPMP	Free to be downloaded	https://www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/docs/ipmp-2013/
GInaFit (Add-in for Microsoft excel)	Free to be downloaded	www.cit.kuleuven.be/biotec/downloads.php
MicroHibro	Free internet access	www.microhibro.com
PredOxyPack	Commercial	http://predoxypack.be/
Microbial Response Viewer (MRV)	Free internet access	www.mrviewer.info
PMM-Lab	Free to be downloaded	www.sourceforge.net/projects/pmmlab

Despite the existence of a relatively high numbers of predictive models that can potentially help the food industry to reliably predict microbial responses and estimate shelf-life of food products, the application of these models is limited. In SA for instance, this field is yet to be explored while in some developed countries, such as in Europe, predictive microbiology has been explored for various food applications and it has been reported that accurately and scientifically predicting shelf life of food products can be achieved using predictive food microbiology (Fakruddin *et al*., 2011; Plaza-Rodríguez *et al*., 2015). Exploring this area for shelf life estimation of RTE food products of microbiological concern in SA is thereby encouraged as Food Business Operators (FBOs) in SA are conservative in estimating shelf life of RTE food products leading to avoidable food wastes and losses. It is well known that predictive models and particularly complex mathematical functions in predictive microbiology are not accessible for non-expert users. Computing sciences and software engineering allows the development of models with an applicability component, converting models not only in suitable function describing observation, but also in tools that can be applied by end users in the form of software (Pérez-Rodríguez & Valero, 2013).

2.6 HYPOTHESES

2.6.1 Hypotheses 1

Shelf life estimation of food products by FBOs are inaccurate due to the unscientific methods used for shelf life estimation and contribute to food waste because, traditional shelf life determination is based solely on practical observations and has high uncertainty which in turn may result in the rejection of large quantities of unspoiled or safe foods (Psomas *et al*., 2011).

2.6.2 Hypotheses 2

RTE food products with prolonged shelf life will not pose a food safety concern. FBOs formulate RTE food products in a way not to support pathogen growth with the use of safe combinations as with hurdle technology. Depending on the type of product which guarantees no growth of pathogens and if present at moderate levels (1-10 cfu/g), compliance with the 100 cfu/g limit during the product shelf life is enabled (Uyttendaele *et al*., 2009; Ceuppens *et al*., 2016).

2.6.3 Hypotheses 3

Application of predictive models for shelf life determination will accurately estimate shelf life of RTE food products because; the premises behind the scientific basis of predictive microbiology are that microbial responses in foods are reproducible against several extrinsic and intrinsic environmental

factors (Ross *et al*., 2000; McMeekin *et al*., 2006). This behaviour can be translated into diverse mathematical models that estimate microbial growth/inactivation/toxin production/probability of growth (Pérez-Rodríguez & Valero, 2013) which are not considered in unscientific traditional shelf life determination methods.

2.7 OBJECTIVES

2.7.1 Objective 1

To estimate and verify the shelf life of selected RTE food products during storage at ± 5^{o}C with the aim of investigating the shelf life estimation of RTE foods by FBOs as a contributing factor to food wastes in SA.

2.7.2 Objective 2

To determine the food safety implications of extending the shelf life of selected RTE food products with the aim of reducing food waste in SA.

2.7.3 Objective 3

To observe the kinetic behaviour of *L. monocytogenes*, *E. coli* and *Salmonella* Typhimurium in these selected RTE food products if shelf life of these selected RTE foods are extended, with the aim of food waste reduction and predict their growth using applicable software with the aim of applying well performed tertiary models (software) for shelf life prediction of RTE foods.

CHAPTER 3: RESEARCH

3.1 BRIEF INTRODUCTION TO RESEARCH STUDY

This study aimed at finding methods of reducing food waste suggested to be caused by unscientific and conservative shelf life estimation of RTE food products by FBOs. For this reason, it was proposed that the use of predictive models in the form of software which are built on scientific principles can be applied in the accurate estimation of shelf life of RTE food products can be explored to reduce food waste caused by the conservative shelf life estimation. This study was however divided into three phases. The first phase was carried out to estimate and verify the shelf life of selected RTE food products estimated by FBOs, while the second phase involved challenge test studies on these selected RTE food products to evaluate the safety implications of these products if their shelf life is extended for the purpose of reducing food waste. The third phase involved the performance evaluation of some selected modeling software, carried out by applying and evaluation best performed software for accurate shelf life prediction. The three phases of this study are divided thus:

(i) Shelf life estimation of RTE food products: A contributor to food waste and losses in SA

(ii) Evaluation of shelf life and food safety implications of extended shelf life of RTE food products with the aim of reducing food waste

(iii) Performance evaluation of tertiary predictive models for application in shelf life estimation of RTE food products with the aim of reducing food waste in SA.

3.2 SHELF LIFE ESTIMATION OF RTE FOOD PRODUCTS: A CONTRIBUTOR TO FOOD WASTE AND LOSSES IN SOUTH AFRICA

ABSTRACT

Conservative shelf life estimation of RTE foods by FBOs could be one of the major contributor to avoidable food waste. Different RTE food products (n=195) available on the shelf of 3 supermarkets in Hatfield, SA was investigated, set shelf life and storage instructions of these products were also reviewed. In addition, microbiological quality (Total viable count, LAB, Enterobacteriaceae, yeasts and moulds, and *Pseudomonas* spp.) and safety (*E. coli*, *Staphylococcus aureus*, *Listeria* spp. and *Salmonella* spp.,) of selected RTE products (used as a reference point) during storage at ± 5^{o}C was also studied to evaluate the validity of the set shelf life of beef lasagne (3 days), egg noodles (3 days), pre-cut mango (4 days) and pre-cut papaya (4 days). It was observed that all RTE food products had no specific refrigeration storage temperature instruction. Microbiological quality study indicated that some of these RTE foods (beef lasagne, pre-cut mango and papaya) could have longer shelf life (5, 13 and 5 days respectively) while egg noodles indicated potential health risk due to the presence of food borne pathogens right from day of purchase. FBOs in SA should scientifically estimate the shelf life of RTE food products as it would minimise unwarranted disposal of wholesome food and the risk that consumer will buy a product that is unsafe or already spoilt.

3.2.1 INTRODUCTION

About one-third to half of all food produced for human consumption is wasted globally (Lundqvist *et al*., 2008; Gustavsson *et al*., 2011; Oelofse & Nahman, 2013). SSA contributes about 120-170 kg/annum to the global food waste (Gustavsson *et al*., 2011). SA is not exempted from this global menace as about 10.2 million tonnes per annum of food is wasted and this has negative impact on the economy as a whole as it worsens the problem of food insecurity (Lungqvist *et al*. 2008; Kristen, 2012; Nahman & de Lange, 2013; Oelofse & Nahman, 2013). According to Nahman & de Lange, (2013), this waste is valued at 7.7 billion dollars per annum, which equates to 2.1% of SA's gross domestic product (GDP). Food waste and losses have separate meanings and varying definitions have been given to these terms. Nevertheless, food waste in the context of this study relates to behaviour issues and occurs at the retail and consumer levels where food meant to be consumed is discarded (Parfitt *et al*., 2010). Food losses on the other hand relates to infrastructure and technical issues and can be referred to as a decrease in food quantity and quality, which makes it unfit for human consumption (Grolleaud, 2002). Food is lost or wasted throughout the food supply chain, from initial agricultural production down to final household consumption including during food storage, transportation, food processing, at retailers, and in the kitchens of restaurants, hotels and households due to weather, poor infrastructure and spoilage (Lundqvist *et al*., 2008; FAO, 2011; Gustavsson *et al*., 2011; The Rockefeller Foundation, 2015). However, in developed countries, food waste and losses occurs significantly at the early stage of the FSC but more waste occurs at the consumption level. Contrarily, in developing countries such as SA, food is lost mostly during the early and middle stages of the food supply chain while less food is wasted at the consumer level (Gustavsson *et al*., 2011). Waste at the retail and consumption level in SA is estimated in thousand tonnes at 2008 and 501 per annum, respectively (Nahman & de Lange, 2013). Regardless of this volume, food waste needs to be reduced as numbers of South Africans are still experiencing hunger (Kirsten, 2012; Department of Social development & DAFF, 2013; Oelofse, 2013). At the consumer level, insufficient purchase planning and expiring 'best-before-dates' has been suggested to cause large amounts of waste, in combination with the careless attitude of those consumers who can afford to waste food (Gustavsson *et al*., 2011). Lack of understanding, combined with habitual behaviour however hamper the correct interpretation of the 'use-by' dates (Van Boxstael *et al*., 2014). However in SA, many consumers consider 'expiry date' as the most important information on a food label (Jacobs, de Beer & Larney, 2010). There is evidence that many consumer actively consider 'use-by' literarily, interpreting them as food must be discarded on that particular date (Lungqvist *et al*., 2008; Wansink & Wright, 2006). This perception may lead to the practice of discarding unspoiled and safe food or consuming products after the 'use-by' date which may pose

potential health risk to the consumer as the case may be. The causes of food losses and waste in developing countries are mainly connected to financial, managerial and technical limitations in harvesting techniques, storage and cooling facilities in difficult climatic conditions, infrastructure, packaging and marketing systems (Gustavsson *et al*., 2011). However, one of the technical challenges affecting SMEs in SA is the unscientific and conservative determination of 'sell-by and use-by' dates of food products which negatively affects consumer perception of these labelling leading to unnecessary substantial food wastes and losses (Mather, 2005; Newsome *et al*., 2014; Ceuppens *et al*., 2016). Although no research to the best of the author's knowledge has been presented to suggest that conservative shelf life estimation is a major contributor to food wastes and losses in SA. However, Lipinski *et al*. (2013) suggested that optimisation of the use of shelf life labels is one way to reduce food waste.

Consumer demand and sales of fresh-like RTE foods has risen during the last few years due to the trend towards convenience and healthy food. These products are gaining more market share on a global scale every year as processed food retail sales are growing due to advances in technology and global trade (Panagiotis & Nychas, 2011; Kotzekidou, 2013; Stratakos & Koidis, 2015). Few examples of these RTE food products include minimally processed fruits and vegetables such as pre-cut fruits and vegetables and their salads as well as RTE heat-and-eat meals such as lasagne, noodles, spaghetti Bolognese, and pasta. Whilst trying to cope with consumer demands for innovative, healthy and low priced products, food producers must balance this with the need to ensure food safety and an appropriate shelf life estimation for their products. Food manufacturers have to meet consumer demands for freshness and convenience without compromising the safety and shelf life of RTE foods (Stratakos & Koidis, 2015) in order to reduce food wastes and losses. Most perishable RTE foods are to be kept refrigerated. SA regulations (Foodstuffs, Cosmetics and Disinfectants Act No 54 of 1972; No. R. 429) states that FBOs are responsible for stating appropriate storage conditions relevant to food to ensure food quality and safety and no specific temperature need to be stated. According to Ceuppens *et al*. (2016), stating a specific storage temperature unanimously agreed between stakeholders can contribute to food waste reduction as it greatly influence the shelf life.

Hence, the objective of this study was to estimate and verify the shelf life of some selected RTE food products by means of evaluating microbial quality of these products during their shelf life (storage at ± 5ºC) with the aim of investigating the shelf life estimation of RTE foods by FBOs as a contributing factor to food wastes and losses in SA.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Survey on the RTE food products available on the shelf of supermarkets and their estimated shelf life ('Use-by and Sell-by' dates)

Three supermarkets from the Hatfield environs of Pretoria owned by three different major SA retailers was included in the survey. With permission, lists and photographs of the different types of RTE food products carrying a 'use-by and sell-by' date labelling that were available on their display shelf was taken. After which the food products were grouped based on different food categories such as pre-packed fruits and vegetables and their salads (pre-cut), RTE meat products and RTE meals and the gathered information was used to register the different types of RTE food products available on the shelf of each of the supermarkets, the estimated shelf life as well as storage instructions given for each RTE food product (Table 4).

3.2.2.2 Microbiological quality and storage test to verify the shelf life of selected RTE food products

Products sampling

The preliminary survey assisted in the selection of 4 different RTE food products based on 3 scenarios described below. This was conducted to undergo storage test for verification of the 'use-by' and 'sell-by' dates stated on these food products by food producers in order to estimate remaining shelf life of the products after purchase. The day of purchase represents day 0. Variation in the remaining shelf life estimates were considered as all the different batches of the food products were timed and purchased based on the same remaining shelf life. However, these products representing the scenarios were selected based on availability. The three scenarios adopted show how growth of microorganisms impact shelf life of RTE foods. They demonstrate the growth of spoilage microorganisms and pathogenic bacteria in food during chilled storage (New Zealand Guidance document, 2014). The scenarios are:

Scenario 1: Food supports the growth of both pathogenic and spoilage bacteria; the pathogenic bacteria reach unsafe levels before the food is visibly spoiled.

Scenario 2: Food supports the growth of both pathogenic and spoilage bacteria but the food is visibly spoiled before pathogenic bacteria have reached unsafe levels.

Scenario 3: The pathogenic bacteria may sometimes be present at very low (safe) levels but do not grow in the food. Spoilage bacteria can grow and the food becomes visibly spoilt.

Consumer units of the 4 selected RTE food products were used in this study. Samples were purchased at the point of sale (Day 0) and also obtained from different batches on the shelf from one of the three supermarkets used during the survey. Two sample units were purchased per batch and for each batch, 3 replicates were analyzed. Sampling was done in different stores of the supermarket in Hatfield environs in SA and food products were immediately transported to the laboratory and were stored in a cold room (± 5^{o}C). Microbiological analysis was carried out within 3 h of sample purchase, pre-cut mango and papaya was analysed every 72 h for a storage period of 12 days, while egg noodles and beef lasagne every 48 h over a storage period of 6 days. These intervals were used considering the perishability and microbial growth history of this category of products. On each day of analysis, pH and water activity (a_w) of the food samples were determined.

Table 4: Shelf life information and parameters of RTE food products selected for shelf life studies

RTE product category	Pre-cut mango	Pre-cut papaya	Beef lasagne	Egg noodles
Composition	Mango cubes (160g)	Papaya cubes (250g)	Layers of fresh durum wheat pasta, egg, beef Bolognese and creamy béchamel sauce, water, milk, cheddar cheese, dried chicken meat, pepper and turmeric (300g)	Fresh pasta containing flour, water, egg and canola oil (300g)
Scenario♯ category	3	2	1	2
Remaining shelf life*(days)	4	4	3	3
Storage information	Keep refrigerated	Keep refrigerated	Keep refrigerated. Freeze on day of purchase and use within one month of freezing. Defrost thoroughly before use. Microwave for 2 mins 30 sec (850-1100 watts) or in oven at 180^{o}C for 15 mins	Keep refrigerated. Heat for approx. 2 mins 30 sec or microwave for 2-3 mins on high heat or until heated thoroughly.
A_w	0.99	0.97	0.96	0.95
pH	3.55	5.21	5.60	6.60

* Remaining shelf life as estimated by food business operator (Calculated from day of purchase as difference between day 0 and use-by date labelled by the food producer)
♯ Predicted scenario category RTE falls into before microbiological study

Microbiological analysis

At each sampling time, packaging containers were opened aseptically 25 g of each sample was aseptically weighed into a stomacher bag with 225 mL of buffered peptone water (BPW) (Oxoid. Hampshire, UK) and stomached (Stomacher 400, ART MEDICAL EQUIPMENT PTY LTD. Johannesburg) at high speed for 3 mins. 10-fold serial dilutions were made from the homogenate in the stomacher bag with 0.1 % buffered peptone water (Oxoid) and the homogenate was tested for Total viable Count (TVC) on Nutrient Agar (LAB-M. Lancashire, UK) with incubation at 25 ^{o}C for 72 h. Lactic Acid Bacteria on de Man, Rogosa and Sharpe 1960 (MRS) Agar (Merck biolab. Darmstadt, Germany), incubated for 72 h at 30^{o}C. *Pseudomonas* spp. on *Pseudomonas* Agar Base with supplement (Oxoid) and incubated at 25^{o}C for 48 h. Yeasts and moulds on Potato Dextrose Agar (Merck) with the addition of chloramphenicol (Sigma-Aldrich. St. louis, MO. USA), incubated at 25°C for 3-5 days. Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA) (Oxoid) incubated for 24 h at 37^{o}C. *Escherichia coli* on Sorbitol Mac Conkey Agar (SMAC) (Oxoid) incubated for 24 h at 37^{o}C. *Staphylococcus aureus* was enumerated with 3M Petrifilm (3M, Saint Paul. Minnesota. USA). Typical colonies were counted and calculated as cfu/g and then converted into $\log_{10}$ value for statistical analysis. Samples were analysed in duplicate on each day of analysis.

Detection of *Listeria* spp. was done in accordance with ISO standard 11290-1:1996 by weighing 25 g of each sample into 225 mL of Half Fraser broth supplemented with half-Fraser supplement (Oxoid) and incubated for 24 h at 30^{o}C. From the first enrichment, 0.1 mL of homogenate was incubated in 10 mL of Fraser broth supplemented with Fraser supplement (Oxoid) for 48 h at 37^{o}C. After plating onto Palcam agar with supplement (Oxoid) and incubated for 48 h at 37^{o}C, plates were observed for typical black colonies. Presumptive *Listeria* spp. were confirmed by enriching individual isolates on Palcam agar in 9 mL Modified Listeria Recovery broth with supplement (Oxoid) and incubated for up to 48 h at 37^{o}C, aliquots were removed and analysed with 3M MDA Listeria model kit (3M).

Detection of *Salmonella* spp. was done in accordance with ISO standard 6579:2002 by weighing 25 g of each sample into 225 mL of full strength buffered peptone water (Oxoid) for pre-enrichment and incubated 18 h at 37^{o}C. From the first enrichment, 0.1 mL was incubated for 24 h at 41.5^{o}C in 10 mL of Rappaport-Vassiliadis soya (RVS) peptone broth (Oxoid). After plating onto xylose lysine desoxycholate (XLD) agar (Oxoid), plates were incubated for 24 h at 37^{o}C and were observed for typical black colonies.

Physico-chemical analysis during refrigerated storage

The pH (Instrulab, Johannesburg) and a_w (Pawkit water activity meter) was determined.

Statistical Analysis

The results were analysed statistically by the analysis of variance (ANOVA) using the software SPSS for Windows Version 11.5.0 (SPSS Inc., Chicago, IL USA). Statistical analyses to assess and compare the effect of the different storage days on the microbial growth of the different samples were computed by least square difference (LSD). Mean separation was determined using the Tukey test at $P < 0.05$. All experiment were repeated at least twice (n=2).

3.2.3 RESULTS

3.2.3.1 Types of RTE food products in supermarkets and their shelf life estimation

An indication of the different types and categories of RTE food products that can be found on the retail shelves in SA as well as their shelf life as estimated by the food producers are presented.

Table 5: Total number of different RTE food products sold by three retailers from their supermarket outlets in Hatfield, SA with the shelf life estimation as indicated on product packages.

RTE FOOD PRODUCT CATEGORY	SUPERMARKET A	SUPERMARKET B	SUPERMARKET C	TOTAL	RANGE OF SET SHELF LIFE OF RTE PRODUCTS (Days)
RTE meal	28	13	16	57	
Fruit and jelly based					1 – 2
Other RTE meals					4 – 9
Pre-cut fruit and salad	8	9	19	36	3 – 5
Pre-cut vegetable and salad	9	7	16	32	1 – 6
RTE meat products	15	38	17	70	
Cured					10 – 30
Uncured					3 – 10
Total RTE food products	60	67	68	195	

During the sample collection visit to three different supermarkets for a survey carried out to identify different RTE food products sold in retail stores in SA, a total of 195 RTE food products were studied based on their shelf life estimation, shelf life date marking (mainly food products with 'use-by and sell-by dates') and storage instructions written on the product packages. As shown in Table 4, supermarket A, B, and C had a total number of RTE food products of 60, 67 and 68 respectively with supermarket A having the highest number (38) of RTE meal products being sold while supermarket C had the highest number of pre-cut fruits and vegetables with their salad (19 and 16 respectively) been sold. On the other hand supermarket B sold the highest number (38) of RTE meat products.

According to the labelling on the RTE food products, 'sell-by' and 'use-by' dates were used for date marking of the food products used in this study. These dates according to the SA regulation (Foodstuffs, cosmetics and Disinfectants Act No 54 of 1972; No. R. 429) means the date which signifies the end of the estimated period if stored in accordance with any stated storage conditions after which the intact package of food should not be sold or consumed because of health or safety reasons. This law is however, similar to the Australian legislation on date marking (Food Standards Australia

New Zealand Act 1991. Standard 1.2.5-2) except it was stated that the food product is not meant to be consumed because of health or safety reasons. It was noted as expected that different RTE food products had different shelf life based on the food category and compositions and it was revealed that within a specific supermarket there was not much variation in the shelf life estimation (Table 5). Cured RTE meat products had the longest shelf life (10 – 30 days) while jelly and fruit based RTE meals had the shortest shelf life (1 – 2 days). Other RTE meals containing food products such as pasta, meat products, dairy and so on had a shelf life of between 4 – 9 days. Fruits and vegetables, and salads had shelf life of 1 – 6 days depending on their type. However, uncured RTE meat products had shelf life of between 3 – 10 days. Also worthy to mention is the fact that only a few portion of the food products had no storage condition recommendations specified on their packages while none of the RTE food products from the three supermarkets had a specific storage temperature recommendation (data not shown). Storage condition predominantly stated was to keep refrigerated.

The representative RTE food products used for this study was selected based on the stated 3 scenarios. On completion of the preliminary survey, it was suggested that pre-cut mango, pre-cut papaya, beef lasagne and egg noodles falls in to scenario 3, 2, 1 and 2 respectively (Table 5). However, microbiological analysis in this study suggest that these RTE food products falls into scenarios 3, 2, 2, and 1 respectively (Table 6).

Table 6: Shelf life estimation and how growth of micro-organisms impact shelf life (using scenarios from New Zealand Guidance document, 2014) of four selected RTE products purchased at supermarkets in Hatfield, SA.

RTE FOOD PRODUCTS	SET SHELF LIFE (Days)*	SHELF LIFE ATTAINED (Days)♯	SCENARIO CATEGORYβ	SCENARIO CATEGORY ATTAINED¥
Pre-cut mango	4 (day 3)	12 (day 12)	3	3
Pre-cut papaya	4 (day 3)	6 (day 6)	2	1
Beef lasagne	3 (day 2)	4 (day 4)	1	1
Egg noodles	3 (day 2)	-	2	1

* Shelf life set by FBO (indicates remaining shelf life after purchase)
♯ Shelf life attained during study
β Scenario category selected before microbiological study
¥ Scenario category attained during study

3.2.3.2 Microbiological quality and shelf life of selected RTE food products sold in supermarket in SA

In this study, the term initial microbial counts of the RTE products at the point of purchase (day 0) were interpreted as mean microbial count of food placed on the shelf of the retail store for sale to consumers. According to EC No 178/2002, food placed on the market means food or feed held for the purpose of sale. This includes foods offered for sale or any other form of transfer, whether free of charge or not, and the sale, distribution and other forms of transfer themselves. Such as sampled at the point of sale in the supermarket, food stall, convenience stores, distributors, wholesalers, catering establishments and point of import.

The cut-off point of microbial growth of the organisms in the RTE food products tested in this study was compared to the microbiological limits set by Gilbert *et al*., 2000, Health protection Agency, 2009 and Food Safety Authority of Ireland, 2014.

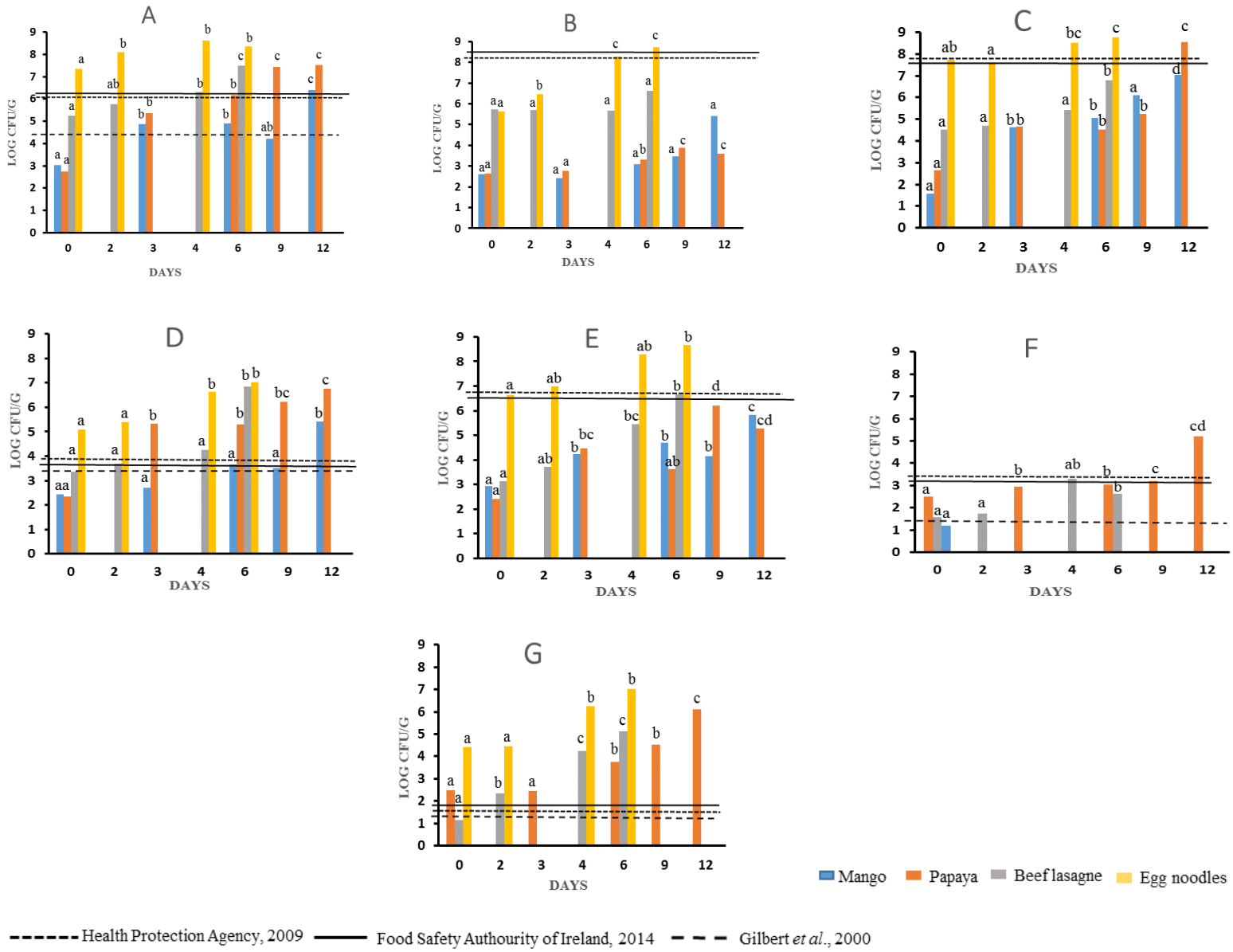


Figure 6: Microbial count and shelf life of pre-cut mango, pre-cut papaya, beef lasagne and egg noodles stored at 5ºC for 6 and 12 days. A. TVC B. LAB C. *Pseudomonas* spp. D. Enterobacteriaceae E. Yeasts and Moulds F. *Staphylococcus aureus* G. *E. coli*

Packaged pre-cut mango

Pre-cut mango which was selected as one of the relevant RTE products for the shelf life verification test had a remaining shelf life of 4 days after purchase as stated by the FBO (Table 6) which equates to day 3 of this study (Fig 6). Storage instruction indicates the product to be kept refrigerated.

Mean count of yeasts and moulds (2.93 $\log_{10}$ cfu/g at day 0, 4.25 $\log_{10}$ cfu/g at day 3, 4.70 $\log_{10}$ cfu/g at day 6, 4.16 $\log_{10}$ cfu/g at day 9 and 5.82 $\log_{10}$ cfu/g at day 12), TVC (3.04 $\log_{10}$ cfu/g at day 0, 4.88 $\log_{10}$ cfu/g at day 3, 4.89 $\log_{10}$ cfu/g at day 6, 4.21 $\log_{10}$ cfu/g at day 9 and 6.39 $\log_{10}$ cfu/g at day 12), and *Pseudomonas* spp. (1.57 $\log_{10}$ cfu/g at day 0, 4.63 $\log_{10}$ cfu/g at day 3, 5.07 $\log_{10}$ cfu/g at day 6, 6.09 $\log_{10}$ cfu/g at day 9 and 7.04 $\log_{10}$ cfu/g at day 12) increased significantly ($p<0.05$) throughout the storage period, that is after day 0 (Fig 6). However, for yeast and moulds and TVC, there was no significant difference between the mean counts of these organisms at day 3 compared to day 6 and day 9 while for *Pseudomonas* spp., there was no significant difference between the mean counts at day 3 compared to day 6 but increased significantly ($p<0.05$) after day 6 (day 9 and day 12). Mean counts of LAB (2.61 $\log_{10}$ cfu/g at day 0, 2.42 $\log_{10}$ cfu/g at day 3, 3.10 $\log_{10}$ cfu/g at day 6, 3.46 $\log_{10}$ cfu/g at day 9 and 5.41 $\log_{10}$ cfu/g at day 12) and Enterobacteriaceae (2.45 $\log_{10}$ cfu/g at day 0, 2.70 $\log_{10}$ cfu/g at day 3, 3.66 $\log_{10}$ cfu/g at day 6, 3.50 $\log_{10}$ cfu/g at day 9 and 5.41 $\log_{10}$ cfu/g at day 12) at day 0 on the other hand do not differ significantly with the counts at day 3 and 6, but increased significantly ($p<0.05$) after day 6. Also, counts of these organisms at day 3 do not differ significantly with counts at day 6 and 9. *S. aureus* was positive for 20 % of the samples which was present (1.20 $\log_{10}$ cfu/g) only at day 0 and was not detected on day 3 and for the rest of the storage period. No *E. coli*, *Listeria* and *Salmonella* spp. were detected in the pre-cut mangoes at the day 0 and also throughout the storage period.

Packaged pre-cut papaya

Pre-cut papaya had a remaining shelf life of 4 days after purchase as stated by the FBO (Table 6) which equates to day 3 of this study (Fig 6). Storage instruction indicates the product to be kept refrigerated. Microbial counts for all organisms increased significantly ($p<0.05$) throughout the storage period, that is after day 0 (Fig 6). Mean count of yeasts and moulds were 2.41 $\log_{10}$ cfu/g at day 0, 4.46 $\log_{10}$ cfu/g at day 3, 3.62 $\log_{10}$ cfu/g at day 6, 6.20 $\log_{10}$ cfu/g at day 9 and 5.28 $\log_{10}$ cfu/g at day 12. TVC were 2.74 $\log_{10}$ cfu/g at day 0, 5.36 $\log_{10}$ cfu/g at day 3, 6.16 $\log_{10}$ cfu/g at day 6, 7.42 $\log_{10}$ cfu/g at day 9 and 7.52 $\log_{10}$ cfu/g at day 12. Enterobacteriaceae were 2.36 $\log_{10}$ cfu/g at day 0, 5.34 $\log_{10}$ cfu/g at day 3, 5.31 $\log_{10}$ cfu/g at day 6, 6.21 $\log_{10}$ cfu/g at day 9 and 6.75 $\log_{10}$ cfu/g at day 12. *S. aureus* counts were 2.49 $\log_{10}$ cfu/g at day 0, 2.96 $\log_{10}$ cfu/g at day 3, 3.04 $\log_{10}$ cfu/g at day 6, 3.19 $\log_{10}$

cfu/g at day 9 and 5.22 $\log_{10}$ cfu/g at day 12 and *Pseudomonas* spp. (2.63 $\log_{10}$ cfu/g at day 0, 4.65 $\log_{10}$ cfu/g at day 3, 4.52 $\log_{10}$ cfu/g at day 6, 5.24 $\log_{10}$ cfu/g at day 9 and 8.54 $\log_{10}$ cfu/g at day 12. Counts of these organisms also increased significantly ($p<0.05$) from day 6 to 12 compared with count at day 3 with the exception of Enterobacteriaceae and *Pseudomonas* spp., where there was no significant difference between their mean counts at day 3 compared to day 6 while there was significant increase ($p<0.05$) in their mean counts at day 9 and 12. There was no significant difference between mean counts of LAB (2.63 $\log_{10}$ cfu/g at day 0, 2.78 $\log_{10}$ cfu/g at day 3, 3.32 $\log_{10}$ cfu/g at day 6, 3.83 $\log_{10}$ cfu/g at day 9 and 3.61 $\log_{10}$ cfu/g at day 12) at day 0 compared to counts at day 3, whereas, significant increase ($p<0.05$) occurred after day 0 (from day 3 to day 12). On the other hand, counts of *E. coli* (2.48 $\log_{10}$ cfu/g at day 0, 2.46 $\log_{10}$ cfu/g at day 3, 3.74 $\log_{10}$ cfu/g at day 6, 4.51 $\log_{10}$ cfu/g at day 9 and 6.11 $\log_{10}$ cfu/g at day 12) at day 0 were higher ($p<0.05$) compared with day 3, but do not differ from the counts at day 6. However, counts increased significantly ($p<0.05$) from day 6 compared with day 3. No *Listeria* and *Salmonella* spp. were detected in the pre-cut papaya at the day 0 and also throughout the storage period.

RTE beef lasagne

Beef lasagne which was selected as one of the relevant RTE meals for the shelf life verification test had a remaining shelf life of 3 days (Table 6) with the storage instruction indicating the product to be kept refrigerated. Instruction on the food package also stated that if the product was frozen on day of purchase then it can be used within 1 month (Table 4). However, the 3 days equates to day 2 of this study (Fig 6).

Mean count of yeasts and moulds (3.13 $\log_{10}$ cfu/g at day 0, 3.71 $\log_{10}$ cfu/g at day 2, 5.46 $\log_{10}$ cfu/g at day 4, and 6.66 $\log_{10}$ cfu/g at day 6) as well as *E. coli* (1.15 $\log_{10}$ cfu/g at day 0, 2.34 $\log_{10}$ cfu/g at day 2, 4.23 $\log_{10}$ cfu/g at day 4, and 5.13 $\log_{10}$ cfu/g at day 6) increased significantly ($p<0.05$) throughout the storage period, that is from day 2 to 6 (Fig 6). TVC counts were 5.24 $\log_{10}$ cfu/g at day 0, 5.76 $\log_{10}$ cfu/g at day 2, 6.32 $\log_{10}$ cfu/g at day 4, and 7.48 $\log_{10}$ cfu/g at day 6. However, there was no significant difference in the counts between day 0 and 2. *S. aureus* were 1.57 $\log_{10}$ cfu/g at day 0, 1.75 $\log_{10}$ cfu/g at day 2, 3.31 $\log_{10}$ cfu/g at day 4, and 2.62 $\log_{10}$ cfu/g at day 6 and *Pseudomonas* spp. counts were 4.53 $\log_{10}$ cfu/g at day 0, 4.69 $\log_{10}$ cfu/g at day 2, 5.43 $\log_{10}$ cfu/g at day 4, and 6.79 $\log_{10}$ cfu/g at day 6. Similar to the TVC counts, individual counts of this organisms did not differ between day 0 and 2. However, counts of these organisms increased significantly ($p<0.05$) from day 4 to day 6 compared with day 0. Compared with day 2, counts of *S. aureus* increased significantly ($p<0.05$) at day 4 and 6 while counts of *Pseudomonas* spp. did not differ from day 4 but increased significantly ($p<0.05$) by day 6. Furthermore, there was no significant difference in counts of Enterobacteriaceae

(3.37 $\log_{10}$ cfu/g at day 0, 3.69 $\log_{10}$ cfu/g at day 2, 4.25 $\log_{10}$ cfu/g at day 4, and 6.86 $\log_{10}$ cfu/g at day 6) and LAB (5.73 $\log_{10}$ cfu/g at day 0, 5.71 $\log_{10}$ cfu/g at day 2, 5.66 $\log_{10}$ cfu/g at day 4, and 6.62 $\log_{10}$ cfu/g at day 6) between day 0 compared with days 2 and 4. However, counts of *E. coli* at day 2 did not differ from counts at day 4 but increased significantly ($p<0.05$) by day 6 whereas counts of LAB increased significantly ($p<0.05$) for days 4 and 6 compared to day 2. No *Salmonella* spp. were detected in the beef lasagne at the day 0 and also throughout the storage period. However, after the two-step enrichment process, *L. monocytogenes* was detected in 100 % of the beef lasagne samples analysed.

RTE egg noodles

Egg noodles which was selected as one of the relevant RTE meals for the shelf life verification test had a remaining shelf life of 3 days after purchase as stated by the FBO (Table 6) which equates to day 2 of this study (Fig 6). Storage instruction indicates the product to be kept refrigerated.

Mean count of TVC (7.34 $\log_{10}$ cfu/g at day 0, 8.09 $\log_{10}$ cfu/g at day 2, 8.60 $\log_{10}$ cfu/g at day 4, and 8.35 $\log_{10}$ cfu/g at day 6), *E. coli* (4.42 $\log_{10}$ cfu/g at day 0, 4.46 $\log_{10}$ cfu/g at day 2, 6.26 $\log_{10}$ cfu/g at day 4, and 7.03 $\log_{10}$ cfu/g at day 6) and LAB (5.63 $\log_{10}$ cfu/g at day 0, 6.47 $\log_{10}$ cfu/g at day 2, 8.27 $\log_{10}$ cfu/g at day 4, and 8.71 $\log_{10}$ cfu/g at day 6) increased significantly ($p<0.05$) throughout the storage period, that is from day 2 to 6 (Fig 6) compared with day 0. However, there was no significant difference in count of TVC at day 6 compared with count at days 2 and 4.

Between days 0 and 2, there was no significant difference in the counts of Enterobacteriaceae (5.09 $\log_{10}$ cfu/g at day 0, 5.40 $\log_{10}$ cfu/g at day 2, 6.64 $\log_{10}$ cfu/g at day 4, and 7.01 $\log_{10}$ cfu/g at day 6). Similarly, yeasts and moulds counts (6.64 $\log_{10}$ cfu/g at day 0, 7.00 $\log_{10}$ cfu/g at day 2, 8.30 $\log_{10}$ cfu/g at day 4, and 8.66 $\log_{10}$ cfu/g at day 6) did not differ between days 0 and 2. *Pseudomonas* spp. counts (7.72 $\log_{10}$ cfu/g at day 0, 7.61 $\log_{10}$ cfu/g at day 2, 8.53 $\log_{10}$ cfu/g at day 4, and 8.75 $\log_{10}$ cfu/g at day 6) between day 0 and 2 did not also differ. Whereas, counts of these individual organisms increased significantly ($p<0.05$) from day 4 to day 6. No significant difference was observed between days 4 and 6.

No *S. aureus* (Detection limit 10 cfu /g) and *Salmonella* spp. were detected in the egg noodles at the point of purchase and also throughout the storage period. However, after the two-step enrichment process, *L. monocytogenes* was detected in 61 % (out of 24 samples) of the egg noodles samples analysed.

3.2.4 DISCUSSION

From the current survey, no specific storage temperature was stated, the general term 'keep refrigerated' was used which according to SA regulation means a temperature ranging between 0 -7^{o}C but specifically a core temperature of 4^{o}C. This law is also similar to the Australian legislation which is below 5^{o}C and above 0^{o}C. In Belgium however, RTE products having the 'use-by' date marking is essentially assigned to a specific storage temperature (Ceuppens, 2016).

TVC provide information about remaining shelf life of food products based on its quality and not safety as it cannot directly contribute towards a safety assessment but can be used as part of quality assessment (HPA, 2009; FSAI, 2014). However, high level of TVC indicates the level of microbial contamination which means there is a predominant organism present in the food which can translate to quality issues and possibly poor temperature control. Acceptability therefore depends on which organism predominates (HPA, 2009).

TVC count for fresh-cut fruits and vegetables such as pre-cut mango and papaya used in this study are not applicable (Gilbert *et al*., 2000; HPA, 2009; FSAI, 2014) as they tend to naturally have a higher level. Nevertheless, the initial TVC counts of the pre-cut mango and papaya were within the acceptable microbiological limit. For this reason, TVC count is not the shelf life predictor of this category of food product. The low TVC levels may in turn translate into the low levels of other microorganisms (Pseudomonas spp., Yeasts and moulds, and LAB) tested for in the pre-cut fruits. However, if the TVC count of these products were to be considered for shelf life estimation, pre-cut mango and papaya will have a shelf life of 12 and 6 days, indicating an underestimation of shelf life by about 9 and 3 days respectively.

Enterobacteriaceae and *E. coli* on the other hand are known to be hygiene indicator organisms and basically reflects the hygienic quality of the food. Enterobacteriaceae group includes pathogenic species e.g. *E.coli*, *Salmonella*, *Shigella*, and *Yersinia*. The initial Enterobacteriaceae count of the pre-cut mango and papaya were within acceptable microbiological limit of ≤ 4 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014) but the microbiological limit for Enterobacteriaceae do not also apply (Gilbert *et al*., 2000; HPA, 2009; FSAI, 2014) for these category of RTE food products because these foods can naturally contain high levels of Enterobacteriaceae as part of their microflora (De Roever, 1998; Corbo *et al*., 2004; HPA, 2009; FSAI, 2014). Enterobacteriaceae has been reported to be isolated in different types of pre-cut fruits (Poubol & Izumi, 2005). Thus Enterobacteriaceae count cannot be used to determine the shelf life of this category of food product while *E. coli* was not detected in the pre-cut mango, similar to the findings of Abadias *et al*., (2008) which can be attributed to the low pH of the

fruit as well as low storage temperature (Strawn & Danyluk, 2010). *E. coli* however was isolated at the point of purchase in the pre-cut papaya and throughout the storage period which is at an unsatisfactory level compared to the acceptable limit of between 20 and ≤ 2 $\log_{10}$ cfu/g (Gilbert *et al*., 2000; HPA, 2009). This pose a potential food safety risk to consumers. *E. coli* O157: H7 was found to grow in papaya during storage at low temperature (Strawn & Danyluk, 2010) similar to the storage condition of this study. However, based on the limit set by FSAI, (2014) for *E. coli* (2 and 3 $\log_{10}$ cfu/g) for fruits and vegetables, papaya at the point of purchase and at day 3 was within the acceptable limit but exceeded this limit from day 6 till end of the storage period. Presence of *E. coli* in this type of RTE food is an indication of post-process contamination due to faecal contamination of raw materials, poor handling during processing and inadequate cleaning and sanitisation as well as poor temperature and time control (HPA, 2009; Baylis *et al*., 2011; FSAI, 2014). Based on this level, shelf life of papaya is attributed to safety and this study agrees with the set shelf life (3 days) by the FBO with regards to *E. coli*.

S. aureus was also isolated at the point of purchase in both the pre-cut mango and papaya but the organism in these RTE food products are within the acceptable limit of between 20 to ≤ 4 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014) and the organism was observed to also be within the acceptable limit from day 3 and throughout the storage period. Hence, *S. aureus* is not the shelf life predictor of this category of food product and with respect to this organism, pre-cut mango and papaya can have their shelf life extended by about 9 and 6 days respectively.

LAB and yeasts and moulds count in pre-cut mango and papaya were within acceptable limit of between 8 to 9 and 6 and 7 $\log_{10}$ cfu/g respectively (HPA, 2009; FSAI, 2014) from point of purchase and throughout the storage period. Hence, pre-cut mango and papaya can have their shelf life extended by about 9 and 6 days respectively with respect to these counts and is not the shelf life predictor of this category of food product.

Acceptable limit for *Pseudomonas* spp. was set to be between 7 and 8 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014). Pre-cut mango had an acceptable level of *Pseudomonas* spp. from point of purchase till the end of the storage period whereas, papaya had an acceptable level of *Pseudomonas* spp. from point of purchase till day 9. Thus, shelf life of pre-cut mango and papaya can have their shelf life extended by about 9 and 6 days respectively with respect to these counts and is not the shelf life predictor of this category of food product.

In pre-cut mango *S. aureus* was the only pathogen detected, however, was detected at a low level and did not grow in the food product during the storage period (12 days) while *Pseudomonas* spp. which

reached an unacceptable level at this stage (12 days) is the shelf life predictor of this product and therefore falls within scenario 3 where pathogenic bacteria (*S. aureus*) may sometimes be present at very low (safe) levels but do not grow in the food. Spoilage bacteria (*Pseudomonas* spp.) can grow and the food becomes visibly spoilt. While pre-cut papaya on the other hand supported the growth of both pathogenic (*S. aureus* and *E. coli*) and spoilage (yeasts and moulds LAB and *Pseudomonas* spp.) organisms during storage, and the food product however, became unsafe due to the growth of *E. coli* before the food is visibly spoiled, hence belong to scenario 1.

It has been suggested that RTE meals generally have high TVC especially RTE meals containing dressings or fillings as it is with beef lasagne. It can therefore be suggested that beef lasagne falls within the category of RTE food products for food mixed with dressings, dips and pastes. Hence, the acceptable TVC limit for this product is between 6 and 7 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014).

According to this study, TVC count of beef lasagne falls within acceptable microbiological limit at the point of purchase as well as at day 2 and 6 of the storage period. Thus, TVC is not the shelf life predictor of this category of food product and the beef lasagne can however, have its shelf life extended by 4 days that is from about 3 to 6 days. Egg Noodles are also a RTE meal (Gilbert *et al*., 2000) that falls into the category of RTE food products that are cooked and chilled but with minimal handling prior to sale or consumption (HPA, 2009; FSAI, 2014). Guideline TVC limit for this products is between 4 and 7 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014) or between 4 and 5 $\log_{10}$ cfu/g (Gilbert *et al*., 2000).

Results from this study shows that TVC level of egg noodles does not fall within the acceptable limit at the point of purchase and throughout the storage period, due to the high TVC which is >7 $\log_{10}$ cfu/g. However, unsatisfactory results as observed in egg noodles do not also directly relate to the safety of a food and cannot directly contribute towards a safety assessment of RTE foods because TVC does not indicate the presence of pathogens (FSAI, 2014). Nevertheless, in the case of spoilage investigation, when TVC level is > 6 log $\log_{10}$ cfu/g as observed in the egg noodles, there is usually a predominant microorganism while TVC levels < 6 $\log_{10}$ cfu/g are usually associated with mixed microflora (HPA, 2009). This may be linked to the high level of *Pseudomonas* spp. in the product. Psychrotrophic bacteria have been reported to be able to multiply during retail (Abadias *et al*., 2008).

The Enterobacteriaceae counts of the RTE beef lasagne and egg noodles at the point of purchase indicates that the counts in beef lasagne is within the acceptable limit of between 2 and 4 $\log_{10}$ cfu/g (Gilbert *et al*., 2000; HPA, 2009; FSAI, 2014). Enterobacteriaceae in the beef lasagne was also observed to be within acceptable limit at day 2, however, the limit was exceeded at day 4 and 6 while

the limit was exceeded and unsatisfactory in egg noodles at the point of purchase and throughout the storage period. *E. coli* on the other hand is part of the Enterobacteriaceae family and can indicate faecal contamination of raw materials, poor handling during processing and post-processing contamination, inadequate cleaning and sanitisation as well as poor temperature and time control in this type of RTE food product (HPA, 2009; Baylis *et al*., 2011; FSAI, 2014).

Similar to the trend found in Enterobacteriaceae, *E. coli* in the beef lasagne was observed to be within acceptable limit at day 2, whereas, the limit was exceeded after day 2 while the limit was also exceeded and unsatisfactory in egg noodles at the point of purchase and throughout the storage period. Outbreaks of *E. coli* infection has been linked to foods such as noodles while egg products has been suggested to be of high risk (Michino & Otsuki, 2000). This can be linked to inadequate cooking of the egg noodles or cross contamination. The high count of *E. coli* in the product may be linked to the high count of Enterobacteriaceae and may suggest a food safety risk. However, according to Article 14 of EC 178/2002, the batch of the food product is not considered unsafe but FBO should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GMP to prevent the food products from reaching unsatisfactory levels (FSAI, 2014). *E. coli* however is the shelf life determining factor of these RTE meals. Hence, their shelf life cannot be extended.

S. aureus was not detected in egg noodles at the point of sale and throughout the storage period but an acceptable level was found in beef lasagne at the point of purchase but increased after purchase similar to previous findings in lasagne (Stratakos *et al*., (2015) but was found to remain at the acceptable level throughout the storage period. *S. aureus* does not compete well with other microorganisms, spoilage caused by non-pathogenic microbiota will precede the development of high population of this pathogen (Harris *et al*., 2003). Hence, with respect to this organism, beef lasagne can have its shelf life extended by 4 days.

LAB and yeasts and moulds count in beef lasagne was within acceptable limit of between 8 to 9 and 6 to 7 $\log_{10}$ cfu/g respectively (HPA, 2009; FSAI, 2014) at the point of purchase and throughout the storage period. Hence, beef lasagne can have its shelf life extended by about 4 to 10 days with respect to these counts. However, counts of LAB falls within the acceptable limit from point of purchase till the end of shelf life in egg noodles while for yeasts and moulds, counts exceeded the acceptable limit from point of purchase and throughout the storage period.

Acceptable limit for *Pseudomonas* spp. was set to be between 7 and 8 $\log_{10}$ cfu/g (HPA, 2009; FSAI, 2014). Beef lasagne had an acceptable level of *Pseudomonas* spp. from point of purchase till the end of the storage period whereas, egg noodles exceeded the limit for *Pseudomonas* spp. from point of

purchase till end of storage period. Thus, shelf life of beef lasagne can be extended by about 10 days with respect to this organism.

Beef lasagne supported the growth of both pathogenic (*S. aureus* and *E. coli*) and spoilage (yeasts and moulds LAB and *Pseudomonas* spp.) organisms during storage, the food product however, become unsafe before it is visibly spoilt by the spoilage organisms. *E. coli* growth after day 2 suggested a food safety risk for this product. Hence, *E. coli* is the shelf life predictor of this category of food product.

Salmonella and *Listeria* spp. were not detected in the pre-cut fruits used for this study from the point of sale till end of the storage period, while *Listeria* spp. was detected in 61% (out of 24 samples) of egg noodles and 100% (24 samples) of beef lasagne. The contamination level was < 1 $\log_{10}$ cfu/g, and falls within the food safety criteria for *Listeria* spp. defined in the Commission Regulation (EC) No 2073/2005 for RTE products which are considered as able to support the growth of *Listeria* spp. The presence of this pathogen will not pose a food safety risk to healthy consumers. However, this may not be the case in immunocompromised individuals. In 2017, about 7 cases of Listeriosis was reported in SA. This number increased to 190 in 2017. Also, over 365 cases of meningitis due to *L. monocytogenes* has been documented for this year (National Institute for Communicable Diseases (NICD), 2017) with newborn babies mostly implicated.

3.2.5 CONCLUSIONS

Conservative determination of shelf life by FBO is one of the major causes of food waste. Most of the RTE food products have a longer shelf life compared to the shelf life estimated by the FBOs. Those with compromised shelf life is mainly due to safety and not spoilage issues which relates to the food safety management system. Ultimately, food producers must be able to scientifically (using predictive modelling) determine shelf life of RTE food products to accurately estimate the shelf life of food products. This will minimise the risk of unwarranted disposal of wholesome food and also the risk of consumers buying spoilt or unsafe food.

3.3. EVALUATION OF SHELF LIFE AND FOOD SAFETY IMPLICATIONS OF EXTENDED SHELF LIFE RTE FOOD PRODUCTS WITH THE AIM OF REDUCING FOOD WASTE IN SOUTH AFRICA

ABSTRACT

Food spoilage is wasteful, costly and can adversely affect a country's trade and food security as well as consumer confidence. This problem can be reduced by implementing a process for perishable RTE food products to have an accurate shelf life. However, consumers have the right to safe and good quality food suitable for consumption. A great diversity of refrigerated RTE foods with a prolonged shelf life has an increased potential for pathogenic bacteria to grow if present. Hence, the ability of representative RTE food products (beef lasagne, egg noodles, and pre-cut mango) to support the growth of relevant food borne pathogens (*L. monocytogenes*, *Salmonella* Typhimurium, and *E. coli*) throughout storage period of 12 days at ± 5^{o}C was evaluated. This was carried out using challenge test. The growth potential (δ) of these pathogens in the RTE foods were calculated using the concept of EU-CRL technical guidance on shelf life for *L. monocytogenes* on RTE foods as δ values can be very useful in potential food safety risk evaluation. Challenge test results indicated that RTE egg noodles and beef lasagne may support the growth of *L. monocytogenes* ($\delta > 0.5$ $\log_{10}$ cfu/g) if present in the food while egg noodles may not support the growth of *S.* Typhimurium ($\delta \leq 0.5$ $\log_{10}$ cfu/g). Beef lasagne and pre-cut mango may also not support the growth of *E. coli* ($\delta \leq 0.5$ $\log_{10}$ cfu/g). However, shelf life of all the products evaluated can be extended (by 6 – 9 days) with no food safety risk associated with the extension thereby supporting the aim of food waste reduction.

3.3.1 INTRODUCTION

Ready to eat foods (RTE) which are highly perishable from a microbiological point of view are likely to constitute danger to human health. By the nature of these RTE foods, they present challenges to ensure microbiological quality and safety especially when their shelf life is prolonged. Certain refrigerated RTE foods with a prolonged shelf life has a high risk of pathogen growth (Huss, Jørgensen & Vogel, 2000; Uyttendaele *et al*., 2009; Lambertz *et al*., 2012; Ceuppens *et al*., 2016) hence, FBOs have to meet consumer demands for freshness and convenience without compromising the safety and shelf life of RTE foods (Stratakos & Koidis, 2015). The use of 'Use-by' and 'Sell-by' dates are hereby encouraged for this category of foods as they are an indicator of food safety (Food Standards Australia New Zealand Act 1991. Standard 1.2.5-2; EC, 2000; EC, 2011) while in SA, these dates are an indication of low quality attributes expected by consumers and after which the product is not marketable (Foodstuffs, cosmetics and Disinfectants Act No 54 of 1972; No. R. 429). According to this act, sell-by date is the last date of offer of a product to the consumers after which there remains a reasonable storage period at home. 'Use-by' date on the other hand is the date that signifies the estimated period under the stated storage conditions which a product will not have the quality attributes expected by the consumers and after which the product is not marketable. These food products however are not meant to be consumed after these dates (Foodstuffs, cosmetics and Disinfectants Act No 54 of 1972; No. R. 429).

Globally, about one-third of food produced for human consumption, is lost or wasted, which is about 1.3 billion ton per year (Gustavsson *et al*., 2011) while the estimated food waste in SA is approximately 9.04 million tonnes per year which tends to aggravate the problem of food insecurity, hence having a negative impact on food and nutritional security in SA (Oelofse & Nahman, 2013). The short shelf life of food products for instance, fresh noodles has been reported to result in high levels of wastage, and might also be a potential source of food poisonings (Xu *et al*., 2008; Ghaffar *et al*., 2009).

Microbiological risks attributed to foods depends on the hazards involved, pathogenicity, survival and growth of pathogens in the food and potential control measures applied during production, distribution and storage (Stella *et al*., 2013). Worldwide, about 600 million people are estimated fall ill and 420, 000 die every year after eating contaminated food (World Health Organization, 2015). Studies have shown that RTE food products have been implicated in foodborne disease outbreaks caused by a variety of pathogenic microorganisms (Sommers & Boyd, 2006; Gaul *et al*., 2013; Lardeux *et al*., 2015; CDC, Foodnet, 2017). As a result, numerous studies has been performed to determine the prevalence or growth potential of pathogens such as *Salmonella* spp. (Meldrum, Smith & Garside,

2006; Uyttendaele *et al*., 2009a; Sant'Ana *et al*., 2012a; Scolforo *et al*., 2017), *L. monocytogenes* (Meldrum, Smith & Garside, 2006; Uyttendaele *et al*., 2009b; Vermeulen *et al*., 2011; Sant'Ana *et al*., 2012a; Fang & Huang, 2014; Scolforo *et al*., 2017; Sahu *et al*., 2017) and *E. coli* (Meldrum, Smith & Garside, 2006) in different types of RTE food products which consists of minimally processed fruits and vegetables, sea foods, dairy, meat and poultry products (Uyttendaele *et al*., 2009b; Sant'Ana, Franco & Schaffner, 2012; Scolforo *et al*., 2017). Although the ability of these organisms to survive or grow on food will depend on product constituents, microbe-microbe interactions and response to unfavourable conditions of processing and storage (Brandl, 2006: Capozzi *et al*., 2009).

From the previous research chapter, it was established that shelf life of most of the selected RTE food products were underestimated thereby considered as a contributor to food waste. Hence, a scientific approach for accurate estimation of shelf life of these RTE food products is required to reduce this problem. In the overall risk assessment of RTE foods associated with *L. monocytogenes*, not only control measures to prevent *L. monocytogenes* contamination needs attention, but also the ability of the organism to survive if the pathogen is occasionally present in low levels (FAO/WHO, 2004; Vermeulen *et al*., 2011). Conversely, if the shelf life of these RTE food products are extended, what are the food safety implications? The new European regulation however is directing the food industry towards the adoption of quantitative microbiology approach to food safety assurance, such as the growth/no growth boundaries and kinetics models which are basically modelling approach called predictive microbiology (Koutsoumanis & Angelidis, 2007).

Microbiological challenge testing is a useful tool for determining the ability of a food to support the growth of spoilage organisms or pathogens (Vermeulen *et al*., 2011). The time to reach hazardous levels by pathogens should determine the shelf-life limit and in these cases, shelf-life is greatly influenced by the initial contamination level (Valero *et al*., 2012). Nonetheless, the quantitative output (behaviour of food-borne pathogens in RTE foods) generated from this study based on product characteristics (pH and water activity) as well as storage temperature, serves as data set for comparing predicted data from models in order to estimate 'use-by' dates of the selected RTE food products. However, challenge test has been used for this purpose in many studies (Koutsoumanis & Angelidis, 2007; Uyttendaele *et al*., 2009; Mejlholm *et al*., 2010; Vermeulen *et al*., 2011; Lee *et al*., 2014). But no study has compared stated shelf-life against challenge test data to extend shelf-life with the intention of reducing food waste.

Hence, the objective of this study is to evaluate, if the shelf life of the selected RTE food products can be extended beyond expiration date estimated by FBOs with the aim of waste reduction. Consequently,

the ability of the selected RTE food products (Beef lasagne, egg noodles, and pre-cut mango) to support the growth of relevant foodborne pathogens (*L. monocytogenes*, *S.* Typhimurium, and *E. coli)*. The data from this study will also serves to generate scientifically based microbial behaviour on the selected RTE food products that can be used by the industry to estimate use-by dates and also applied in the next research chapter (3.4).

3.3.2 MATERIALS AND METHODS

Product Sampling

Product sampling was done as described in the previous research chapter (3.2.2). However, microbiological analysis was carried out on the day of purchase (Day 0) for both Inoculated and uninoculated samples and subsequently every 72 h for all the inoculated samples for a period of 12 days.

Shelf life of the RTE products as estimated by the FBO is the remaining shelf life of the products after purchase. That is, it was determined by estimating the remaining shelf life based on the use-by date on the product label. For instance, if the product was purchased on 8th November, 2017 and the use-by date on the products was 11th November, 2017. The remaining shelf life of the product will be 7 days which equates to day 4 of the storage period.

Table 7: Shelf life information, physico-chemical characteristics, relevant pathogens in relation to selected RTE food products used for challenge test studies at ± 5^{o}C for 12 days storage period

RTE food products	Pre-cut mango (160g)	Beef Lasagne (300g)	Egg Noodles (300g)
pH	3.55	5.60	6.60
a_w	0.99	0.96	0.95
Composition	Pre-cut mango cubes	Layers of fresh durum wheat pasta, beef Bolognese and a creamy béchamel sauce, containing egg, water, milk, cheddar cheese, dried chicken meat, pepper and turmeric.	Fresh pasta (contains flour, water, egg, canola oil and gluten).
End of shelf life specified by FBO	Day 3	Day 4	Day 4
Pathogen tested	*E. coli*	*L. monocytogenes* *E. coli*	*L. monocytogenes* *S.* Typhimurium

Bacterial strain and culture

Bacteria used in this study were, *L. monocytogenes* ATCC 19115 which is a reference strain, *S.* Typhimurium isolated from egg and *E. coli* isolated from irrigation water and lettuce. These bacterial cultures were maintained at -75^{o}C on cryobeads. The bacteria cells were revived by incubating a cryobead at 37^{o}C for 24 h in Brain Heart Infusion broth (BHI), this culture was sub-cultured twice in BHI and then streaked on BHI agar. The stock culture from the agar was kept frozen (-75^{o}C) for further use in BHI supplemented with 25 % glycerol. When needed for further experiment the stock culture was streaked on BHI agar and incubated at 37^{o}C for 24 h. Bacterial suspension was prepared with individual colonies in 0.1 % buffered peptone water (Oxoid) to obtain bacterial suspension with same density as compared with Mc Farland standard using a densitometer DEN-1/28712/2.01 (Grant Instruments, UK). An initial bacterial suspension of 3 and 6 $\log_{10}$ cfu/g of food samples was achieved

representing low and high inoculum sizes respectively. This was done by diluting bacterial suspension serially in 0.1 % buffered peptone water (Oxoid). The suspension was in turn measured in the densitometer to achieve the required comparable Mc Farland unit.

Microbiological Analysis

At each sampling time, packaging containers were opened aseptically 25 g of each sample was aseptically weighed into a stomacher bag with 225 mL of buffered peptone water (Oxoid) and stomached (Stomacher 400, ART MEDICAL EQUIPMENT PTY LTD. Johannesburg) for 3 mins. 10-fold serial dilutions were made from the homogenate in the stomacher bag with 0.1 % buffered peptone water (Oxoid) and the homogenate was tested for *E. coli* on SMAC (Oxoid) incubated for 24 h at 37ºC, *L. monocytogenes* on Palcam agar with supplement (Oxoid) and incubated for 48 h at 37ºC, and *Salmonella* spp. on XLD (Oxoid), incubated for 24 h at 37ºC. With the presence of *L. monocytogenes* and *E.coli* in uninoculated foods samples, the challenge test is still valid as it provides additional information that naturally occurring *L. monocytogenes* and *E.coli* strains at realistic levels were present in addition to the added mixture of strain (Beaufort, 2011).

Detection of *Listeria* spp. and *Salmonella* spp. was done in accordance to the procedure described in the previous research chapter (3.2.2).

Physico-chemical analysis during refrigerated storage

pH (Instrulab, Johannesburg) and a_w (water activity meter - Pawkit) of the food products were measured at day 0 for the uninoculated samples.

Food samples inoculation

The challenge test was done in triplicate as three different batches of the RTE food products were used. A whole pack of each food product were homogenously contaminated with the bacteria based of the size of the product in order to obtain an initial concentration of 3 and 6 $\log_{10}$ cfu/g. The high inoculum level of 6 $\log_{10}$ cfu/g was used to demonstrate the extent of reduction in challenge organisms. The inoculated food samples were allowed to interact with the food matrix for one hour before proceeding to storage at ± 5ºC. Enumeration of *E. coli, L. monocytogenes* and *S.* Typhimurium were carried out at interval by homogenising 25 g of food samples in 225 mL of 0.1% BPW, followed by serial dilution and inoculation in duplicate plates of SMAC (Oxoid), PALCAM with supplement (Oxoid) and Brilliance Salmonella agar with supplement (Oxoid) and plates were incubated at 37ºC for 24 h, 24 – 48h and 24 h respectively.

Determination of growth potential (δ) of the pathogens

The growth potential (δ) of the pathogens relevant to each type of RTE food products was determined by calculating the difference between the mean counts in log cfu/g of each microorganism at the beginning (day 0) and at the end (day 3 and 12) of storage period. Growth potential (δ) of the pathogens was also determined for in between storage days. The pathogens with δ values higher than 0.5 $\log_{10}$ cfu/g was considered as able to grow in the RTE food products while the pathogens which had δ values of negative or lower than 0.5 $\log_{10}$ cfu/g were considered not able to grow in the RTE food products (EU-CRL, 2008; Beaufort, 2011).

Statistical Analysis

The results were analysed statistically by the analysis of variance (ANOVA) using the software SPSS for Windows Version 11.5.0 (SPSS Inc., Chicago, IL USA). Statistical analyses to assess the effect of storage time (over each storage day) on the growth of pathogens in the different food samples were computed by least square difference (LSD). Mean separation was determined using the Tukey test at $P < 0.05$. All experiment were repeated at least three times (n=3).

3.3.3 RESULTS

3.3.3.1 Challenge tests performed to study the behaviour of foodborne pathogens at low and high initial inoculum levels in selected RTE food products as observed during storage for 12 days at ± 5°C.

The inoculum levels (3 and 6 $\log_{10}$ cfu/g) was used in this microbiological challenge study. This is to determine the stability and shelf life of the selected RTE products during storage (Figure 7). The ability of the food products to support the growth of *L. monocytogenes*, *S.* Typhimurium and *E. coli* as the case may be was also measured. Applying the EU-CRL, (2008) technical guidance on shelf-life for *L. monocytogenes* on RTE foods, growth potential (δ) of the pathogens was used to classify the RTE foods, as to when $\delta > 0.5$ $\log_{10}$ cfu/g, the food is classified as "RTE foods able to support the growth of the pathogens and when $\delta \leq 0.5$ $\log_{10}$ cfu/g, the food is classified as "RTE foods unable to support the growth of the pathogens. This calculation for δ was also applied to other pathogens (*S.* Typhimurium and *E. coli*) used for the challenge test in this study (Table 8).

As stated on the label by the food producer, the 'use – by' date of egg noodles and beef lasagne selected as part of the relevant RTE food products for the challenge test study was day 4 with pH of 6.60, a_w of 0.95 and pH of 5.60, a_w of 0.96 respectively. The pre-cut mango however had a pH of 3.55 and a_w of 0.99 with 'use – by' date at day 3. Storage instruction on the RTE food products indicate the product

to be kept refrigerated (Table 7). Selection of these RTE food products was based on the three scenarios described above and also based on availability of the products during the period of the study.

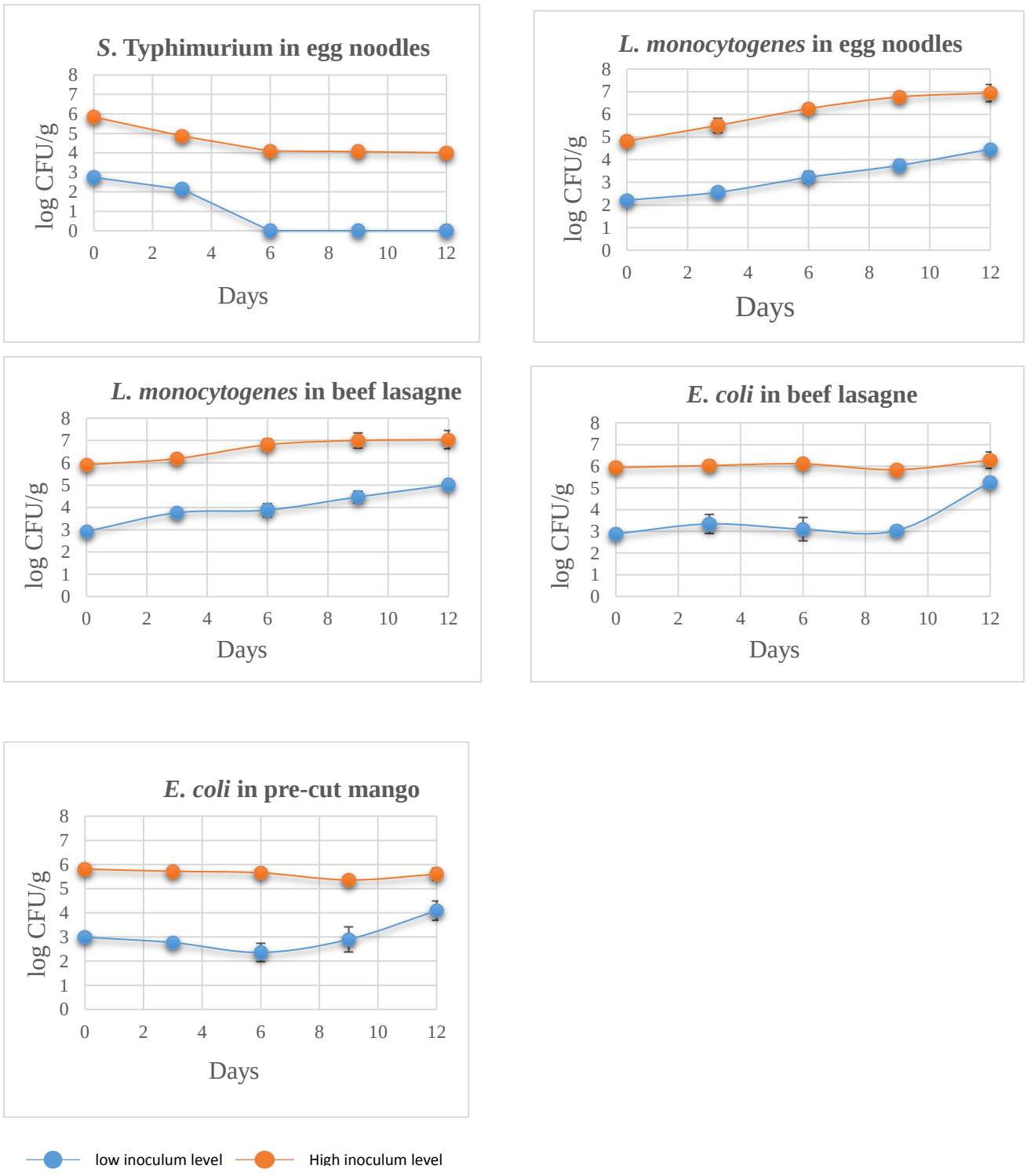


Figure 7: Challenge test to observe the behaviour of relevant foodborne pathogens at low inoculum level of 3 log $_{10}$ cfu/g and high inoculum level of 6 log $_{10}$ cfu/g in selected RTE food products as observed during storage for 12 days at ± 5^{o}C

Behaviour of S. Typhimurium *in egg noodles during storage at ± 5°C for 12 days*

S. Typhimurium was not detected in the egg noodles samples before inoculation. However, after inoculation of the egg noodles with 3 and 6 $\log_{10}$ cfu/g, counts obtained after the inoculation were 2.74 ± 0.17 and 5.84 ± 0.1 $\log_{10}$ cfu/g respectively with both inoculum levels having a < 1 $\log_{10}$ cfu/g decrease in counts of the pathogen immediately after inoculation. As seen in Fig 7, significant decrease ($p<0.05$) in the concentration of *S.* Typhimurium for the low inoculum level, at day 0 was observed during storage to 2.13 ± 0.06 $\log_{10}$ cfu/g compared to day 3 of storage which indicates a day before the end of shelf life stated by the FBO and the pathogen was not detected afterwards and till the end of storage period of 12 days. Same trend with the low inoculum level was observed for the high inoculum level of *S.* Typhimurium with a significant decrease ($p<0.05$) in the concentration of the pathogen to 4.87 ± 0.07 $\log_{10}$ cfu/g at day 3 and also during the rest of the storage period (day 6 to 12). Nevertheless, *S.* typhimurium showed a growth potential for both inoculum levels to be < 0.5 $\log_{10}$ cfu/g after day 3 of storage and throughout the storage period (Table 8).

Behaviour of L. monocytogenes in beef lasagne and egg noodles during storage at ± 5°C for 12 days

L. monocytogenes was detected (detection limit < 1 $\log_{10}$ cfu/g) in both beef lasagne and egg noodles. However, the initial counts before inoculation could not be estimated because they were not quantifiable with normal enumeration method. Growth of the organisms were not observed in the plates. As seen in Fig 7, the concentration of *L. monocytogenes* after inoculation of the beef lasagne with 3 and 6 $\log_{10}$ cfu/g was 2.92 ± 0.02 and 5.91 ± 0.09 $\log_{10}$ cfu/g respectively. The pathogen declined by < 1 $\log_{10}$ cfu/g immediately after inoculation. In egg noodles, the concentration of *L. monocytogenes* was 2.21 ± 0.11 and 4.82 ± 0.16 $\log_{10}$ cfu/g respectively. The pathogen also declined by < 1 $\log_{10}$ cfu/g immediately after inoculation.

At about the end of shelf life of beef lasagne stated by the FBO (day 3), there was a significant increase ($p<0.05$) in the count of *L. monocytogenes* at low inoculum level throughout the storage period compared to day 0. That is, increase was observed from 2.92 ± 0.02 $\log_{10}$ cfu/g to 3.76 ± 0.15 $\log_{10}$ cfu/g at day 3, to 3.88 ± 0.29 $\log_{10}$ cfu/g at day 6, 4.47 ± 0.26 $\log_{10}$ cfu/g at day 9 and to 5.01 ± 0.06 $\log_{10}$ cfu/g at day 12. For the high inoculum level on the other hand, there was no significant increase from the initial count at day 0 (5.91 ± 0.09 $\log_{10}$ cfu/g) compared to counts at day 3 (6.19 ± 0.11 $\log_{10}$ cfu/g). However, a significant increase ($p<0.05$) was observed at day 6 (6.81 ± 0.25 $\log_{10}$ cfu/g) and 9 (7.00 ± 0.34 $\log_{10}$ cfu/g). At the end of storage period (day 12) for both the low and high inoculum levels (5.01 ± 0.06 $\log_{10}$ cfu/g and 7.04 ± 0.41 $\log_{10}$ cfu/g respectively) in beef lasagne, a significant increase ($p<0.05$) was also observed.

In egg noodles, there was a significant increase ($p<0.05$) of *L. monocytogenes* at low inoculum *level*. The pathogen increased from the initial count at day 0 (2.21 ± 0.11 $\log_{10}$ cfu/g) to 2.56 ± 0.11 $\log_{10}$ cfu/g for day 3, 3.22 ± 0.26 $\log_{10}$ cfu/g for day 6, 3.75 ± 0.23 $\log_{10}$ cfu/g for day 9 and 4.46 ± 0.08 $\log_{10}$ cfu/g for day 12. On the other hand, there was no significant increase in the growth of *L. monocytogenes* for high inoculum level between day 0 and 3. But a significant increase ($p<0.05$) in concentration of the pathogen was observed from days 6 to 12. The pathogen increased from the initial count at day 0 (4.82 ± 0.16 $\log_{10}$ cfu/g) to 5.51 ± 0.32 $\log_{10}$ cfu/g for day 3, 6.25 $\log_{10}$ cfu/g ± 0.19 for day 6, 6.77 ± 0.11 $\log_{10}$ cfu/g for day 9 and 6.94 ± 0.38 $\log_{10}$ cfu/g for day 12.

The δ values were calculated from the difference of initial population after inoculation at day 0 and the population at subsequent storage days. With day 3 representing that end of shelf life stated by FBO and days 6 to 12 representing the extended shelf life period.

Furthermore, δ values of *L. monocytogenes* in beef lasagne was > 0.5 $\log_{10}$ cfu/g throughout the storage period for the low inoculum level. Outgrowth of the pathogen at days 3 to 12 was 0.84, 0.96, 1.55 and 2.09 $\log_{10}$cfu/g respectively. For high inoculum level, the δ value of *L. monocytogenes* was < 0.5 $\log_{10}$ cfu/g between day 0 and 3 (0.28 $\log_{10}$cfu/g outgrowth). The δ values however was > 0.5 $\log_{10}$cfu/g between day 0 and throughout the remaining storage period (day 6 -12), with an outgrowth of the pathogen for days 6 to 12 at 0.90, 1.09 and 1.13 $\log_{10}$cfu/g respectively.

Whereas, for the low inoculum level in egg noodles, *L. monocytogenes* had a δ value < 0.5 $\log_{10}$ cfu/g between day 0 and 3 (0.35 $\log_{10}$cfu/g outgrowth) while the δ values were > 0.5 $\log_{10}$ cfu/g between day 0 and throughout the storage period (day 6 -12) (Table 7), with outgrowth of 1.01, 1.54 and 2.25 $\log_{10}$cfu/g respectively. For the high inoculum level, the δ values of *L. monocytogenes* was > 0.5 $\log_{10}$ cfu/g between day 0 and throughout the storage period, with outgrowth of 0.69, 0.96, 1.43, 1.95 and 2.12 $\log_{10}$cfu/g respectively

Behaviour of E. coli in packaged pre-cut mango and beef lasagne during storage at ± 5°C for 12 days

The concentration of *E. coli* after inoculation of the pre-cut mango with 3 and 6 $\log_{10}$ cfu/g was 2.99 ± 0.03 and 5.81 ± 0.06 $\log_{10}$ cfu/g respectively. In beef lasagne, the concentration of *E. coli* after inoculation was 2.88 ± 0.1 and 5.94 ± 0.05 $\log_{10}$ cfu/g respectively. The pathogen declined by < 1 $\log_{10}$ cfu/g in both RTE food products immediately after inoculation. *E. coli* was not detected in pre-cut mango before inoculation. Whereas, the organism was detected in beef lasagne (data not shown).

For counts at day 0 (2.99 ± 0.03 $\log_{10}$ cfu/g) compared to days 3 to 9 (2.77 ± 0.18 $\log_{10}$ cfu/g, 2.36 ± 0.38 $\log_{10}$ cfu/g and 2.90 ± 0.52 $\log_{10}$ cfu/g) respectively, no significant decrease was observed for the

low inoculum level of *E. coli* in pre-cut mango. The pathogen had a significant increase ($p<0.05$) > 1 $\log_{10}$ cfu/g to 4.09 ± 0.40 $\log_{10}$ cfu/g at day 12 compared to day 0. For the high inoculum level, no significant decrease was observed at day 3 to 6 compared to day 0 (Figure 7). However, a significant decrease ($p<0.05$) was observed at storage day 9 (5.36 ± 0.13 $\log_{10}$ cfu/g) followed by a non-significant increase at day 12 (5.60 ± 0.25 $\log_{10}$ cfu/g).

Nonetheless, there was no significant increase in the concentration of *E. coli* for both low and high inoculum levels in beef lasagne between days 0 to 12. With the exception of storage day 12 for low inoculum level, where a significant increase ($p<0.05$) was observed (5.26 ± 0.24 $\log_{10}$ cfu/g). Counts of low inoculum level *E.coli* at day 3 was 3.34 ± 0.44 $\log_{10}$ cfu/g, 3.10 ± 0.54 $\log_{10}$ cfu/g at day 6 and 3.04 ± 0.20 $\log_{10}$ cfu/g at day 9. Counts of *E. coli* for days 3 to 12 at high inoculum level was 6.03 ± 0.23 $\log_{10}$ cfu/g, 6.11 ± 0.06 $\log_{10}$ cfu/g, 5.84 ± 0.16 $\log_{10}$ cfu/g and 6.28 ± 0.38 $\log_{10}$ cfu/g respectively compared to day 0.

The δ values of *E. coli* in the pre-cut mango (-0.22, -0.63, -0.09 $\log_{10}$ cfu/g) were < 0.5 $\log_{10}$ cfu/g from days 3 to 9 respectively compared to day 0 for the low inoculum level. However, it was > 0.5 $\log_{10}$ cfu/g at day 12. At high inoculum level, the δ values of the pathogen in the pre-cut mango (-0.09, -0.15, -0.45 and -0.21 $\log_{10}$ cfu/g) were < 0.5 $\log_{10}$ cfu/g from day 3 to 12 respectively compared to day 0.

In beef lasagne on the other hand, the δ values (0.46, 0.22, 0.16 $\log_{10}$ cfu/g) of the low inoculum level of *E.coli* between day 0 and 3 to 9 were also < 0.5 $\log_{10}$ cfu/g while the value was > 0.5 $\log_{10}$ cfu/g between day 0 and 12 (2.38 $\log_{10}$ cfu/g). However for the high inoculum level, δ values (0.09, 0.17, 0.1 and 0.34 $\log_{10}$ cfu/g) were < 0.5 $\log_{10}$ cfu/g for days 3 to 12 respectively compared to day 0 (Table 8).

Table 8: Growth potential (δ) result for the different relevant pathogens at low and high inoculum levels inoculated in selected RTE food products stored at ± 5°C for 12 days.

Food Products & Pathogen		Storage period (Day)	Growth Potential (δ)*
Egg noodles			
S. Typhimurium -	3 $\log_{10}$ cfu/g	Day 3	-0.61
		Day 6	-2.74
		Day 9	-2.74
		Day 12	-2.74
	6 $\log_{10}$ cfu/g	Day 3	-0.97
		Day 6	-1.75
		Day 9	-1.78
		Day 12	-1.84
L. monocytogenes -	3 $\log_{10}$ cfu/g	Day 3	0.35
		Day 6	1.01
		Day 9	1.54
		Day 12	2.25
	6 $\log_{10}$ cfu/g	Day 3	0.69
		Day 6	1.43
		Day 9	1.95
		Day 12	2.12
Beef lasagne			
L. monocytogenes -	3 $\log_{10}$ cfu/g	Day 3	0.84
		Day 6	0.96
		Day 9	1.55
		Day 12	2.09
	6 $\log_{10}$ cfu/g	Day 3	0.28
		Day 6	0.90
		Day 9	1.09
		Day 12	1.13

Table 8 continues on next page

E. coli -	3 $\log_{10}$ cfu/g	Day 3	0.46
		Day 6	0.22
		Day 9	0.16
		Day 12	2.38
	6 $\log_{10}$ cfu/g	Day 3	0.09
		Day 6	0.17
		Day 9	-0.1
		Day 12	0.34
Pre-cut mango			
E. coli -	3 $\log_{10}$ cfu/g	Day 3	-0.22
		Day 6	-0.63
		Day 9	-0.09
		Day 12	1.10
	6 $\log_{10}$ cfu/g	Day 3	-0.09
		Day 6	-0.15
		Day 9	-0.45
		Day 12	-0.21

* Growth potential calculated by difference of counts between day 0 and remaining storage period (days 3 to 12)
Day 0 represents the day of sample purchase (after pathogen inoculation)
Day 3 represents the end of shelf life as indicated by FBO
Day 12 represents end of storage period in this study

3.3.4 DISCUSSION

Estimation of shelf life should essentially be based on scientific principles that can take into account relevant intrinsic and extrinsic factors and should be done to reduce food waste generated due to conservative shelf life estimation. However, to adopt a scientific method for accurate shelf life prediction, quantitative methods have been recommended which is based on models generated to predict microbial behaviour in food products (Koutsoumanis & Angelidis, 2007). The growth data predicted from these models are reliant on the data generated from challenge studies.

Nevertheless, when shelf life of refrigerated RTE food products are extended with the aim to reduce food waste for instance, there is a high risk of pathogen growth (Ceuppens *et al*., 2016). Evaluation of these categories of RTE foods with regards to the relative potential risk they pose to public health should be based upon prevalence and the ability of each RTE food product to support the growth of their relevant foodborne pathogen to numbers exceeding their safety limits under foreseen refrigeration storage condition. Hence, the results of this study gives an indication of the ability or inability of the selected RTE food products to support the growth of their respective relevant foodborne pathogen if the shelf life of these RTE food products are prolonged under refrigeration. From the challenge test

studies, the behaviour of each pathogen in the different RTE food products are also observed. This behavioural data of relevant foodborne pathogens on selected RTE foods will be used as a comparison data for modeling purposes.

From the storage test study (3.2), *S.* Typhimurium was not detected in all samples of egg noodles tested. It was also observed that this organisms did not survive when inoculated into the egg noodles according to this challenge test study, with the organism gradually declining till the end of the storage period at both low and high inoculation levels. If the organism is present in the RTE food product, it shows that there will not be growth and the organism will eventually be inactivated during the shelf life. The local microenvironment in multi-ingredient foods (egg, flour and oil) critically affects *Salmonella* survival (Li *et al*., 2014; Zhou *et al*., 2014). Essentials oils and certain edible oils such as olive and canola oil and products containing them are suggested to have antimicrobial substances (Medina *et al*., 2006, 2007; Karaosmanoglu *et al*., 2010; Palumbo & Harris, 2011; Keerthirathne *et al*., 2016). According to Medina *et al*. (2006), greater than 4 log reduction was observed with 1 hour exposure of *Salmonella enterica* to virgin olive oils. Oils are reported to interact with the cell membranes of bacteria, causing the cell components to leach out from the cell (Lambert *et al*., 2001). Growth potential however, indicates that egg noodles may not support the growth of *S.* Typhimurium. Li *et al*. (2014) and Zhou *et al*. (2014) reported a similar pattern of reduction of *Salmonella* spp. in non-fat dry milk, peanut butter and chicken meat. Consequently, *S.* Typhimurium will not pose food safety risk with regards to its presence in egg noodles if the shelf life of the product is extended. Albeit, the pathogen must be absent in 25g of the food product (Food Standards Australia New Zealand Act 1991. Standard 1.2.5-2; FSAI, 2014; HPA, 2009).

L. monocytogenes was found to be present in both egg noodles and beef lasagne during the storage test study and it was observed to grow ($\delta > 0.5$ $\log_{10}$ cfu/g) in both products during this challenge test study though a reduction in the bacterial count was observed in the food products immediately after inoculation. *L. monocytogenes* has been reported to be psychrophilic in nature (Gandhi & Chikindas, 2007; Scolforo *et al*., 2017). pH and a_w has been reported to also have an influence in the ability of RTE food product to support the growth of *L. monocytogenes* (Hwang and Tamplin, 2005; Uyttendaele *et al*., 2009; Skalina & Nikolajeva, 2010; Sahu *et al*., 2017). Despite the growth of *L. monocytogenes* in beef lasagne and egg noodles, the shelf life of these products can be extended beyond that set by the FBO. The growth observed was quite slow (< 2 $\log_{10}$ cfu/g increase) in both products between days 0 and days 3 to 9. Fang & Huang, (2014) and (Claire *et al*., 2004) reported a similar slow growth (< 2 log increase) of *L. monocytogenes* in boiled eggs. Hence, the shelf life of these products can be extended by 6 days because the organisms remain at acceptable limit till this date. For RTE foods that

are able to support the growth of *L. monocytogenes*, EC Regulation 2073 specifies that the 2 $\log_{10}$ cfu/g criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, and the product will not exceed the limit of 2 $\log_{10}$ cfu/g throughout the shelf life. Whether a food supports the growth of *L. monocytogenes* or not is mainly determined by the physico-chemical factors such as pH, a_w, packaging atmosphere of the food matrix rather than being defined as such by the food type (Uyttendaele *et al*., 2009). There will be no food safety risk associated with the extension of shelf life of these RTE food products (Beef lasagne and Egg noodles) despite the retarded growth of the pathogen. The uninoculated product had low level of *L. monocytogenes* which will not grow to an unacceptable limit before the end of shelf life (Day 9). Although, a study showed that chicken salad caused listeriosis even with the low level of *L. monocytogenes* in the product (Gaul *et al*., 2013) and according to NICD, (2017), in the year 2017, there was an increased number of infection cases linked to *Listeria* spp. in SA. With new born babies implicated mostly in this study. Caution needs to be taken with regards to shelf life extension of these products. For RTE food products such as beef lasagne and egg noodles, *L. monocytogenes* should not be detected in 25 g of the food products. However, EC 2073/2005 stated that if food producers can prove that the RTE food products will not support the growth of the pathogen, then the 100 cfu/g limit apply. Growth similar to that observed in beef lasagne was also reported in smoked ham salad and chicken salad (Skalina & Nikolajeva, 2010; Sahu *et al*., 2017), with similar properties.

E. coli was isolated in beef lasagne while the organism was not detected in pre-cut mango during the storage test study. However, no growth was observed in pre-cut mango and the beef lasagne sample stored at ± 5^{o}C used in this challenge test study during the storage period. Growth ($\delta > 0.5$ $\log_{10}$ cfu/g) at low inoculum level was observed in both food products at day 12 of the storage period though with < 1 log cfu/g increase in pre-cut mango. Hence, the shelf life of pre-cut mango and beef lasagne can be extended by 6 days. Considering the rate of increase at day 12 for pre-cut mango, the shelf life of the product will be 12 days with an extension by 9 days beyond date estimated by the FBO. Presence of *E. coli* in food is an indication of post-process contamination and insufficient heat treatment (Baylis *et al*., 2011). *E. coli* was also detected in RTE meat products (Ciekure *et al*., 2016). Inability of this pathogen to grow in pre-cut mango can be attributed to the low pH of the fruit as well as low storage temperature (Strawn & Danyluk, 2010). However, fresh-cut mango has not been associated with disease outbreaks, but a challenge test study on cut mango found *E. coli* O157:H7 to remain relatively constant during storage at 4^{o}C before decline in the pathogen (Strawn & Danyluk, 2010). This result is evident of the food waste associated with conservative shelf life estimation. The acceptable limit of

between 20 and $\leq 2 \log_{10}$ cfu/g is however stated for *E. coli* in RTE food products (Gilbert *et al*., 2000; HPA, 2009).

3.3.5 CONCLUSIONS

To reduce food waste, the shelf life of RTE food products used in this study can be extended beyond that stated by the FBO with regards to the behaviour of the relevant pathogens as supported by the previous storage test study. In other words, *Salmonella* Typhimurium if present will not survive and will be inactivated in egg noodles, hence extending the shelf life of this RTE product by 9 days pose a very low to no food safety risk. On the other hand, shelf life of beef lasagne can be extended by 6 to 9 days with regards to the growth behaviour of *L. monocytogenes* and *E. coli* in the product while shelf life of egg noodles can be extended by 6 days with respect to the presence of *L. monocytogenes*. The shelf life of pre-cut mango nonetheless, can also be extended by 9 days with regards to the presence of *E. coli.* Though the RTE food products support the growth of the relevant pathogens, the shelf life extension of these RTE food products pose no food safety risk as the growth of these pathogens are quite slow (< 2 log increase). It is however important to highlight the risks involved in the consumption of RTE food products for consumer health, to raise consumer awareness and to remind manufacturers to monitor hygiene during food production and storage. The behaviour of the relevant pathogens in the selected RTE food products also generated a growth data for *L. monocytogenes* and *E. coli* while non-thermal inactivation was generated for *Salmonella* Typhimurium. This data will be used in comparing the predicted data generated from the next research chapter (3.4).

3.4. PERFORMANCE EVALUATION OF TERTIARY PREDICTIVE MODELS FOR SHELF LIFE ESTIMATION OF RTE FOOD PRODUCTS WITH THE AIM OF FOOD WASTE REDUCTION IN SOUTH AFRICA

ABSTRACT

With the aim of reducing food waste, application of a scientific approach known as predictive modeling for shelf life estimation of RTE food products was explored. Performance of 4 software (ComBase, PMP, MicroHibro and FSSP) was evaluated. These software were selected based on different criteria (User-friendly, accessibility and availability and types of pathogens for its application). The predicted growth data from these software were compared to observed growth data. These observed growth data was generated from experimental data got from challenge test carried out on *L. monocytogenes* in beef lasagne and egg noodles. Coefficient of determination (R^2), root mean square error (*RMSE*), bias factor (B_f) and accuracy factor (A_f) were used as indices to evaluate the performance of these software. All the software evaluated in this study was in agreement with the observed data from the challenge test with a fail-safe prediction. However, ComBase predictor had the best performance for prediction of *L. monocytogenes* growth in beef lasagne and egg noodles compared to the other software. This is because the software had the closest prediction to the observed data. Albeit, all the software evaluated in this study can be explored for use in shelf life prediction of RTE food products by SMEs in South Africa.

3.4.1 INTRODUCTION

Unscientific determination of 'sell-by and use-by' dates especially of packaged products may contributed to the problem of food loss and waste (Lyndhutst, 2011) as FBOs are conservative in determining shelf life of food products. SMEs in SA are faced with technical challenges (Mather, 2005) which include scientifically predicting shelf life of food products. However, accurate determination of shelf life can readily extend shelf life of food products thereby reducing food waste. That is, scientifically determined shelf life are more accurate and less conservative.

A research area of food microbiology known as "predictive microbiology" which emerged few decades ago has been applied as a tool to support decisions concerning food safety and quality (Pérez-Rodríguez & Valero, 2013). Predictive food microbiology however involves the quantification of microbial ecology in foods by means of mathematical models (Ross *et al*, 2000) and are able to predict microbial behaviour in food environments. This is done by integrating traditional microbiology knowledge with those found in the disciplines of mathematics, statistics and information systems and technology (Ross *et al*., 2000; McMeekin & Ross, 1996; Fakruddin *et al*., 2011; Pérez-Rodríguez & Valero, 2013). Most of these models have been evaluated and successfully validated for specific types of food to provide a more accurate, fast and cost-effective alternative to traditional challenge test method for calculation of growth parameters of the pathogens present in foods (Mellefont *et al*., 2003; Lardeux *et al*., 2015; Vermeulen *et al*., 2011; Mejlholm *et al*., 2010). They can then be used to predict food safety and shelf life and have also been incorporated as helpful elements into the self-control systems such as HACCP programs and food safety risk-based metrics (Ross & McMeekin, 2003; Pérez-Rodríguez & Valero, 2013).

Predictive modeling however can accurately determine shelf life of food using predictive models and its application can readily extend shelf life of food products which is of great use to the food industry thereby reducing food loss and waste (Mejlholm *et al*., 2010). Time taken to determine shelf life using challenge testing, which tends to be the most common technique in the food industry may also be significantly reduced as well (Bernaerts *et al*., 2004). This area of food microbiology has been explored in developed countries and can be a useful tool in SA to support SMEs to accurately and scientifically determine shelf life of food products. The use of predictive modelling has been recommended for the determination of shelf life of RTE food products (EC, 2005; FAO/WHO, 2009; New Zealand Guidance document, 2014) and according to testimonies on ComBase (https://www.combase.cc/index.php/en/testimonials) use, Unilever and Heinz makes use of these types of software to support better and faster product and process designs as well as to assess and manage

risks to consumer health. These 'tertiary models' have become widely available in the form of user-friendly software (Ross & Dalgaard, 2004; McMeekin *et al*., 2006; Purac, 2007; Tamplin, 2009) developed for different types of food products. The model allows users without detailed knowledge of programing to easily apply as they are the interface between the scientist and the end-user and consist of simple input screens where the user can enter a set of product formulation conditions and receive a prediction of growth parameters aiding shelf life prediction, quality control or risk assessment (Steele, 2004; Huang, 2014). The different software differ in their structure (e.g. Ratkowsky, square root or cardinal parameter type models), module (e.g. growth or growth boundary), type of microorganisms (pathogen or spoilage), the number of intrinsic and extrinsic parameters that they take into account for prediction, what data (either with data from liquid laboratory media or food) was used for the software development. These difference probably makes performance of the different types of software differ. Therefore, a comparison of the performance of the different types of software for bacterial growth, kinetics in different foods is of great importance in its application by SMEs in order to understand the range of applicability of the models and limit of performance of the models (Ross, 1996). However, Mejlholm *et al*., (2015) suggested the range of applicability of predictive models with respect to other food types and preserving parameters can be possible. Hence, performance of these software needs to be evaluated to enable the assessment of the reliability of the models when compared to observations not used to generate the model. This particularly is important with regards to its applicability on other food types for which the software was not developed for, and hence to evaluate their utility to assist in food safety and quality decisions (Ross, 1999; Ross *et al*., 2000; Mellefont *et al*., 2003; Mejlholm *et al*., 2010; Mejlholm *et al*., 2015).

A number of research has been conducted to compare predictive models mainly in the form of primary and secondary models (Koutsoumanis & Nychas, 2000; Mejlholm *et al*., 2005; Hwang & Marmer, 2007; Uyttendaele *et al*., 2009; Mejlholm *et al*., 2010; Vermeulen *et al*., 2011; Bruckner *et al*., 2013; Lee *et al*., 2014; Lardeux, *et al*., 2015; Mejlholm & Dalgaard, 2015). Nonetheless, significant number of tertiary models (Software) are available in scientific literature and internet for shelf life estimation purposes. Quite a few have been involved in predictive model comparison studies and the commonly used software are ComBase, FSSP, PMP, PURAC and DMRI (Mellefont *et al*., 2003; Uyttendaele *et al*., 2009; Mejlholm *et al*., 2010; Vermeulen *et al*., 2011). However, many other user-friendly, effective and readily available software recently developed are yet to be explored by comparing their performance for shelf life prediction. On the other hand, the performance of the software application on food products not used to generate the model is also yet to be evaluated.

For model performance evaluation, coefficient of determination (R^2), mean square error (*MSE*), root mean square error (*RMSE*), bias factor (B_f) and accuracy factor (A_f) has frequently been applied (Ross, 1996; Lee *at al*., 2014; Drosinos *et al*., 2006) involving comparison of predicted growth responses with those observed in the food products. Challenge test carried out by inoculating food products with test organisms is a basis for growth or survival data generation used for the evaluation of the performance of predictive models, but data from naturally contaminated food are important and should be used when they can be obtained (Mejlholm *et al*., 2010). It is however imperative to present a scientific basis for the model comparison, hence, from the previous research chapter (3.3), a realistic microbial behaviour data set in some selected RTE foods was generated based on the variability of factors affecting microbial growth (a_w, pH, temperature).

Hence, the objective of this study was to evaluate the performance of selected predictive tertiary models (software) to accurately predict the shelf life of RTE food products. The best performed software may be adopted by SMEs in predicting shelf life of RTE food products with the aim of reducing food waste arising as a result of the conservative method of shelf life estimation by FBOs.

3.4.2 MATERIALS AND METHODS

0bial responses to different food environment, with data obtained from the literature or provided by supporting institutions. Food model is included in the software for perfringens predictor and *Salmonella* in egg. The software also contain a shelf life predictor which was developed at the Institute of Food Research (Nowrich, UK). This application is available online for free after registration at the ComBase website (www.combase.cc). ComBase predictor is a collection of predictive models based on generated data to predict the responses of microorganisms as a function of temperature, pH and a_w/salt concentration, including in some cases the effect of a fourth environmental factor, such as the concentration of carbon dioxide or organic acids. Input data also include initial bacteria level and physiological state of the microorganism. These models were developed on the basis of kinetic data obtained in broth and mostly for pathogen (about 12 in number) and some spoilage microorganisms such *Brochothrix thermosphacta* and *Pseudomonas* spp. The primary model used by ComBase predictor is the Baranyi's model (Baranyi & Roberts 1994), and the secondary models are polynomial equations relating environmental factors and kinetic parameters. The ComBase predictor allows predictions under dynamic temperature, permitting introducing time–temperature profiles for all microorganisms considered in the application. Therefore, users are able to introduce data recorded by temperature loggers obtaining growth or inactivation predictions for the introduced profile and users are provided with estimates of maximum growth rate, lag time, doubling time, kinetic curve graphs

and data points. Furthermore, ComBase predictor permits simultaneous predictions for more than one microorganisms. This software can be applied in academia, food industry and research and is expected to be used by a large range of people including quality assurance, product development and legal professionals, legislators, retailers, trainers and students. The website is maintained by the IFR in collaboration with the Food Safety Center, University of Tasmania, and the Eastern Regional Research Center of the USDA Agricultural Research Service. For the predictions in this study, the growth model for *L. monocytogenes/innocua* was used with a physiological state of 1 which indicates that the culture is adapted and that no lag phase but growth occurrence (Vermeulen *et al*., 2011).

PMP is a software package of different models (growth, inactivation and cooling) that can be used to predict the growth and inactivation of selected foodborne pathogens under specific environmental conditions. The software was developed by the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) and particularly at the USDA-ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania. The PMP 7. 0 software (Buchanan, 1993) is available online to be downloaded for free at the PMP website http://www.ars.usda.gov/Services/docs.htm?docid=11550. It is probably the most widely used predictive microbiology application software and it has more users within the food processing industry (Baranyi & Tamplin, 2002). Majority of the PMP models are growth models and predictions are made as a function of atmosphere (aerobic & anaerobic), temperature, pH, water activity, and in some cases nitrite and other additives. The survival also known as the thermal and non-thermal inactivation model predicts the inactivation of selected pathogens as a function of temperature, NaCl, pH, nitrite, lactic acid or sodium pyrophosphate. The cooling models predict the effect of cooling temperature profiles on growth of *Cl. botulinum* and *Cl. perfringens* after cooking. Irradiation, time to turbidity, time to toxin in fish and heat inactivation models for about 11 different pathogenic bacteria are also available and are based on extensive experimental data of microbial behaviour in liquid microbiological media and food.

The primary models in PMP are based on the Gompertz equation (Zwietering *et al*. 1990). Users are provided with estimates of generation time, lag time, kinetic curve graphs, and their confidence intervals for the selected values of environmental factors. Input data include initial bacteria level, a_w, pH, temperature, sodium nitrite content and organism's level of concern, that is, the arbitrary level of the pathogen to pose food safety risk. This software was designed for use in research and for estimating the effects of multiple variables on the growth, survival and inactivation of foodborne pathogens. For the predictions in this study, the aerobic growth model for *L. monocytogenes* in broth culture (a_w) was

used (Buchanan, Stahl & Whiting, 1989; Buchanan & Philips, 1990). Predictions can be exported and the software contains references to studies from which the models were developed.

MicroHibro version 1.7.7 is an on-line quantitative risk assessment tool that can be freely accessed after user registration at http://www.microhibro.com. The software was developed by the Predictive Microbiology Group (Optimum quality & Grupo Hibro) at University of Córdoba. The application incorporates growth and inactivation models for a variety of microorganisms mainly pathogens with LAB and different types of RTE foods. MicroHibro allows including any type of mathematical function enabling its easy update and making the tool dynamic and renewable (Pérez-Rodríguez & Valero, 2013). Because MicroHibro is an on-line tool, users can save its own predictive models and predictions in a virtual account, which can be accessed anytime and anywhere to retrieve saved data. The models are based on primary and secondary functions of Te Giffel & Zwietering, 1999 and Gompertz equation (Zwietering *et al*. 1990). Users are provided with estimates of maximum growth rate, lag time, kinetic curve graphs and input data include initial bacteria level, a_w, pH, temperature and organism's level of concern depending on the model used. MicroHibro also incorporate a validation module to allow users to assess available models using their own data. Finally, the application applies a stochastic approach intended for risk assessors to carry out probabilistic risk models based on an object-oriented system and allowing defining environmental factors as probability distributions (Pérez-Rodríguez & Valero, 2013). Results are displayed using a suitable graphic interface to improve interpretability and data analysis. For the predictions in this study, the growth model for *L. monocytogenes* in multifoods was used. Predictions can be exported and the software contains references to studies or functions from which the models were developed.

The Food Spoilage and Safety Predictor (FSSP) software is available to be downloaded free of charge from http://fssp.food.dtu.dk. The software was developed by the Predictive Microbiology research group (Paw Dalgaard) at the National Food Institute (DTU Food) within the Technical University of Denmark (DTU) in collaboration with Anchor Lab K/S (Brian J. Cowan). FSSP predicts growth of spoilage and pathogenic microorganisms in different food products, predicting the effect of constant or fluctuating temperature storage conditions on product shelf-life. The software includes: Four product-specific relative rate of spoilage (RRS) models, three generic RRS models, four product-specific microbial spoilage models, a generic model to predict microbial growth and shelf-life, modules to compare predictions from FSSP with users own data of shelf-life or growth of bacteria, model to predict growth of psychrotolerant Lactobacillis spp. in chilled seafood and meat products, models to predict growth and histamine formation by *Morganella psychrotolerans* and *Morganella*

morganii., model to predict growth and growth boundary model for *L. monocytogenes*, models to predict the simultaneous growth of *L. monocytogenes* and LAB in chilled seafood, meat products and cottage cheese, an extensive and generic model to predict growth in various foods for different microorganisms on the basis of their cardinal parameter values. In the FSSP software, the models to predict growth of *L. monocytogenes* in chilled seafood and meat products was used in this study and growth of *L. monocytogenes* was predicted with the effect of temperature, pH and a_w. The software uses WPS rather than a_w to predict growth responses of Lm and a_w which was converted to WPS using water activity calculation for square root type models, under the microbial spoilage model with user-defined parameter values. A_w for beef lasagne and egg noodles were converted to 6.56 and 8 WPS, respectively. Users are able to introduce data recorded by temperature loggers and are provided with estimates of maximum growth rate, lag time, kinetic curve graphs and time for 100 cfu/g increase and input data include initial bacteria level, a_w, pH, temperature, smoke components, % CO_2 in headspace gas at equilibrium, nitrite and different types of organic acids. The primary model used for the growth of *L. monocytogenes* in chilled seafood and meat products is the Logistic model with delay while the secondary model used is the Simplified cardinal parameter type model and the model has been extensively validated using data from challenge studies on RTE products (Mejlholm & Dalgaard 2007a, b; Mejlholm & Dalgaard, 2009, 2015; Mejlholm *et al*. 2010).

Indices of performance

Bias factor (B_f), accuracy factor (A_f), coefficient of determination (R^2) and root mean square error (*RMSE*) were used to evaluate the performance of the selected software by comparing observed growth of the microorganisms with that predicted by the software (Ross, 1996; Drosinos *et al*., 2006; Lee *et al*., 2014).

B_f measured the relative average deviation of the predicted and observed growth of *L. monocytogenes* and it estimated by how much the observed values lie above or below the line of equivalence (Ross, 1996). B_f of 1 indicates perfect agreement between the observed and predicted growth data, meaning there is no under or over-prediction. However, a $B_f > 1$ indicates the predicted growth data by the software is longer than the observed growth data while a $B_f < 1$ indicates the predicted growth data is shorter than the observed growth data. It is calculated by the equation:

$$B_f = 10^{\left\{\frac{\sum \log\left(\frac{predicted}{observed}\right)}{n}\right\}} \qquad \text{Equation 1}$$

Where predicted = predicted growth data by the software, observed = observed growth data from challenge test and n is the number of observations used in the calculation.

A_f is a measure of the spread of the results about the predicted values. That is, a measure of the relative average of the minimum 'distance' between each point and the line of equivalence as a measure of how close, on average, predictions are to observations. The A_f, however, reflect the extent of the bias of the software (Ross, 1996). It is calculated by the equation:

$$A_f = 10^{\left\{\frac{\sum \left|\log\left(\frac{predicted}{observed}\right)\right|}{n}\right\}} \qquad \text{Equation 2}$$

Where predicted = predicted growth data by the software, observed = observed growth data from challenge test and n is the number of observations used in the calculation.

R^2 is the fraction of the square of the deviations of the observed values about their mean as explained by the equation fitted to the experimental data (Drosinos *et al*., 2006; Lee *et al*., 2014) and is often used as an overall measure of the prediction attained (Te Giffel & Zwietering, 1999). It is calculated by the equation:

$$R^2 = 1 - \left(\frac{\sum e_i^2}{\sum (y_i - \bar{y})^2}\right) \qquad \text{Equation 3}$$

Where e_i is the error of predicted data (observed-predicted), y_i is the predicted data and $\bar{y}$ is the average of the predicted data.

RMSE is a widely used measure of 'goodness-of-fit', and can be used to derive a measure analogous to the A_f and is the standard deviation of the residuals of the software used for prediction (Ross, 1996; Drosinos *et al*., 2006). It is calculated by the equation:

$$\left[RMSE = \sqrt{\frac{\sum (observed - predicted)^2}{n}} \right]$$ Equation 4

Where predicted = predicted growth data by the software, observed = observed growth data from challenge test and n is the number of observations used in the calculation.

3.4.3 RESULTS

The data generated from challenge test studies to observe the behaviour of L. monocytogenes in RTE beef lasagne and egg noodles was compared with the data generated from software predictions. Software used include PMP, ComBase, MicroHibro and FSSP. The comparison are presented in the graphs below.

Performance of software

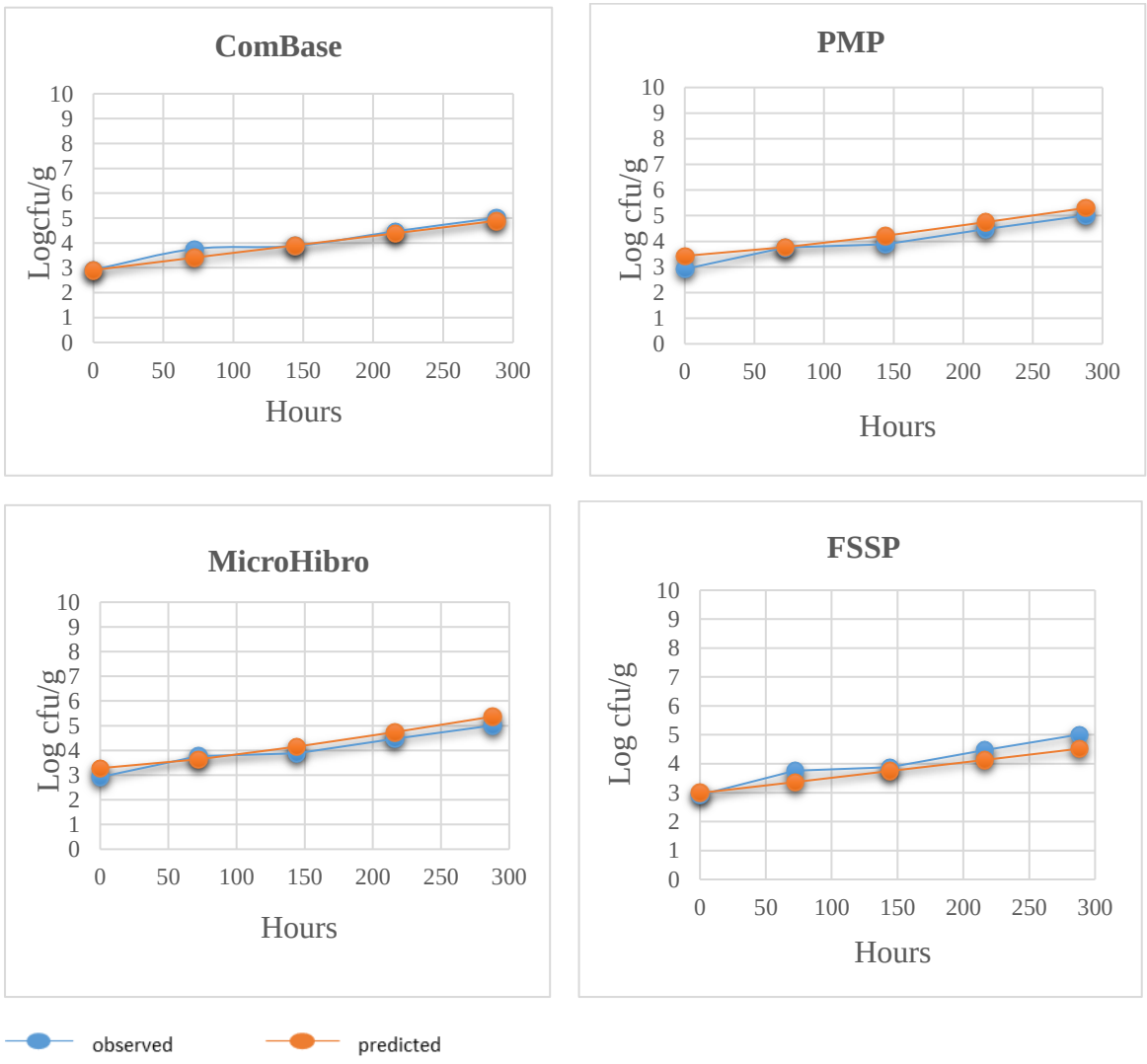


Figure 8: Growth curve of predicted versus observed data for the different types of software used for prediction of *L. monocytogenes* growth at low (3 log $_{10}$ cfu/g) inoculum level in beef lasagne.

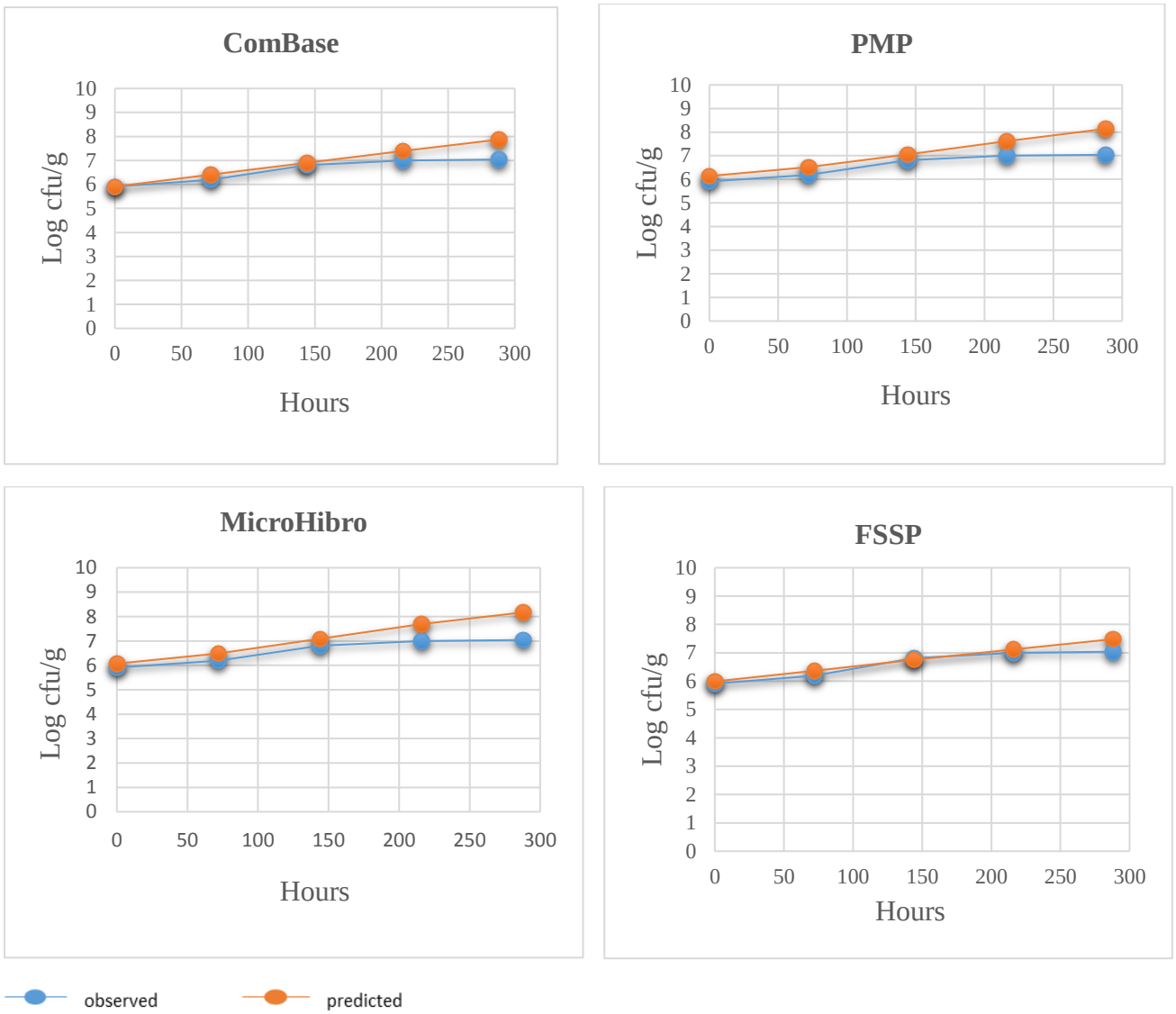


Figure 9: Growth curve of predicted versus observed data for the different types of software used for prediction of *L. monocytogenes* growth at high (6 log $_{10}$ cfu/g) inoculum level in beef lasagne.

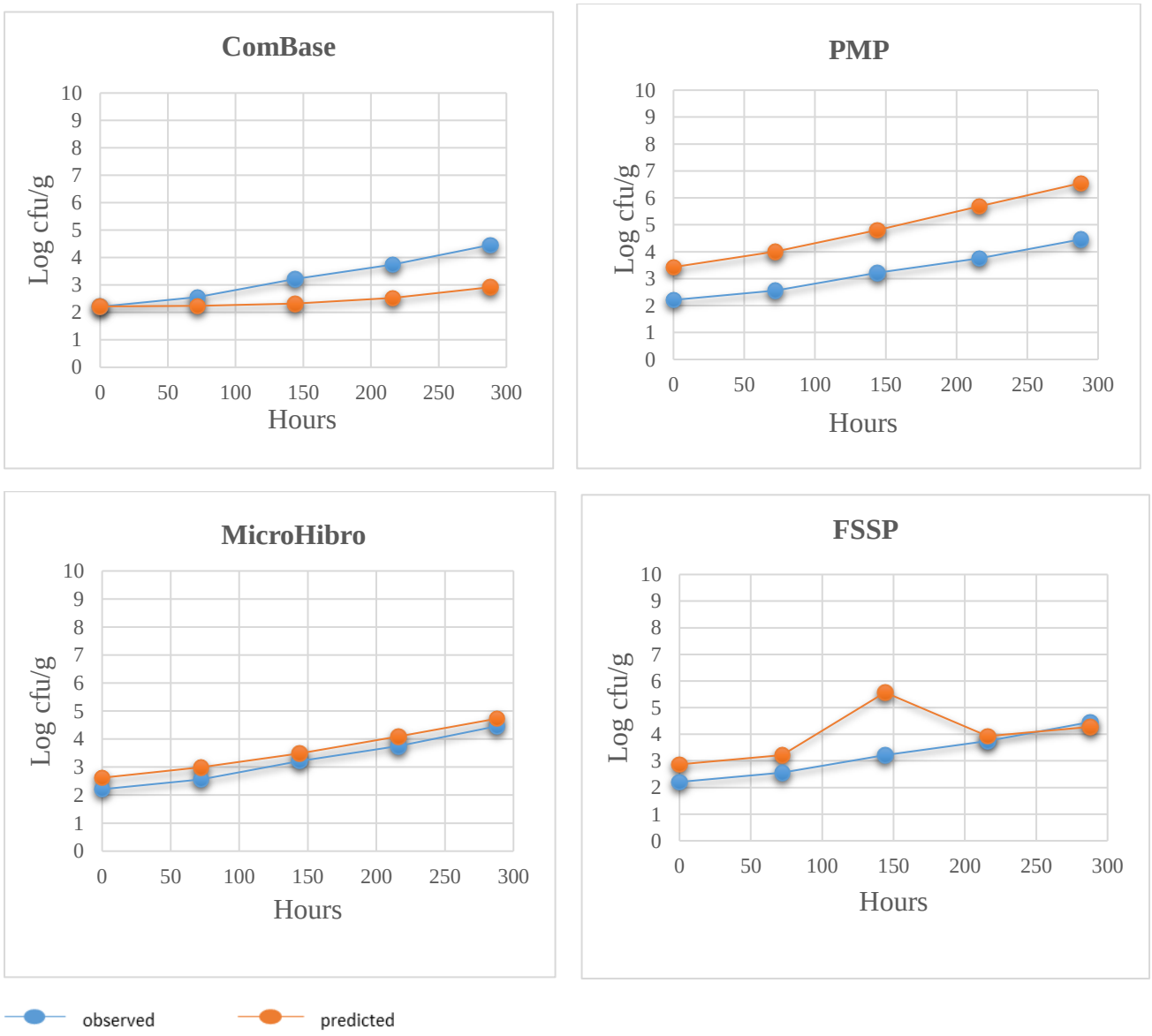


Figure 10: Growth curve of predicted versus observed data for the different types of software used for prediction of *L. monocytogenes* growth at low (3 log $_{10}$ cfu/g) inoculum level in egg noodles.

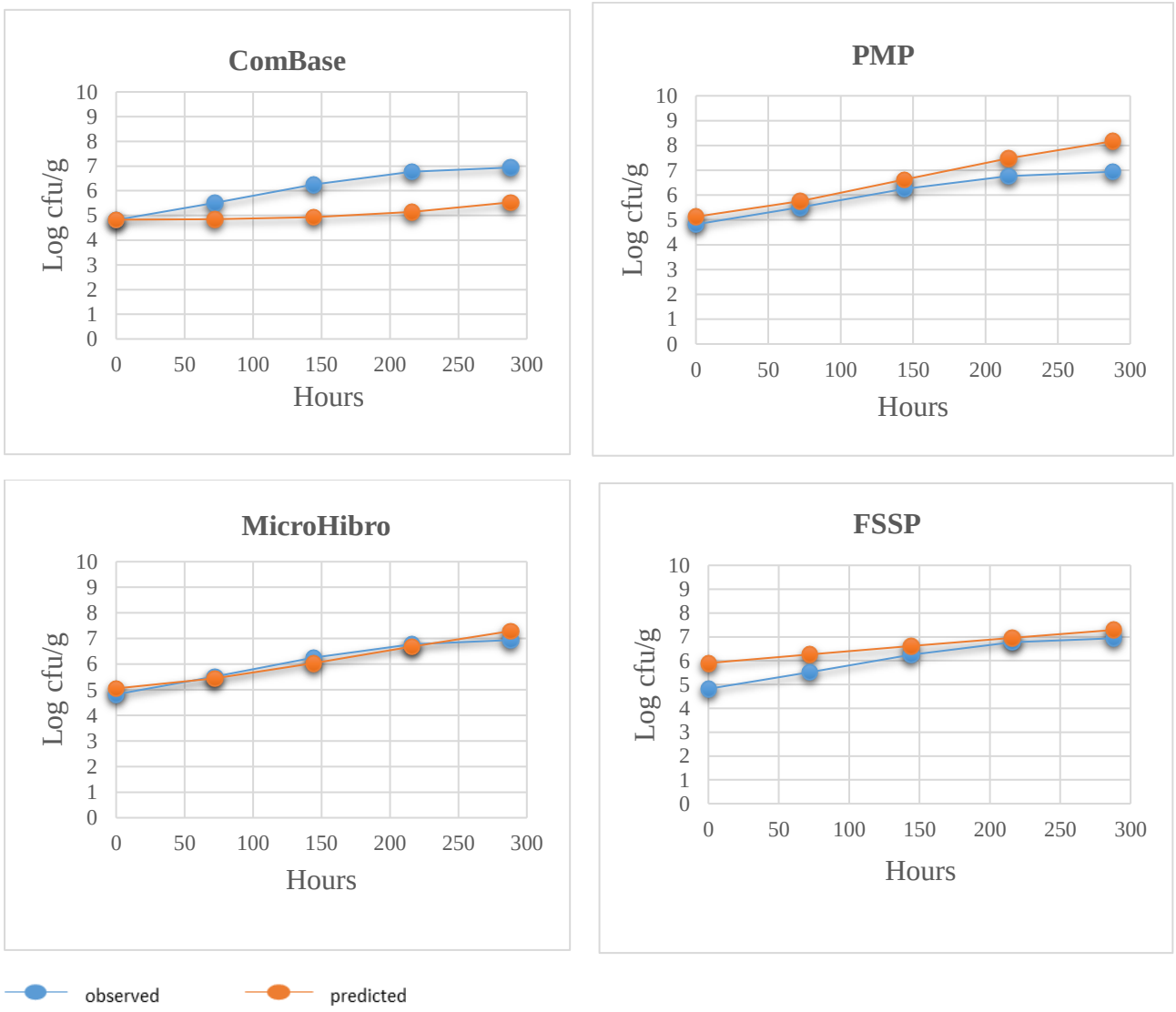


Figure 11: Growth curve of predicted versus observed data for the different types of software used for prediction of *L. monocytogenes* growth at high (6 log $_{10}$ cfu/g) inoculum level in egg noodles.

Table 9: Performance evaluation of selected software predicting the growth of *L. monocytogenes* on beef lasagne and egg noodles under the same environmental conditions.

Food product	Inoculation level	Indices of performance	Software			
			ComBase	***PMP***	***MicroHibro***	***FSSP***
Beef lasagne	3 log $_{10}$ cfu/g	y_o	2.91	3.43	3.28	2.99
		y_f	4.89	5.30	5.37	4.52
		μ_{max}	0.007	0.23	0.009	0.0122
		B_f	0.97	1.08	1.06	0.94
		A_f	1.03	1.08	1.07	1.07
		RMSE	0.17	0.33	0.29	0.33
		R^2	1.0	0.99	0.99	0.99
Beef lasagne	6 log $_{10}$ cfu/g	y_o	5.91	6.14	6.07	5.99
		y_f	7.87	8.14	8.17	7.48
		μ_{max}	0.007	0.23	0.009	0.0122
		B_f	1.04	1.07	1.07	1.02
		A_f	1.04	1.07	1.07	1.03
		RMSE	0.42	0.60	0.63	0.22
		R^2	0.99	0.99	0.99	1.0
Egg noodles	3 log $_{10}$ cfu/g	y_o	2.22	3.43	2.62	2.87
		y_f	4.52	6.55	4.73	4.28
		μ_{max}	0.008	0.29	0.009	0.113
		B_f	1.04	1.50	1.12	1.23
		A_f	1.04	1.52	1.12	1.25
		RMSE	0.15	1.69	0.35	1.14
		R^2	1.0	0.82	0.99	0.88
Egg noodles	6 log $_{10}$ cfu/g	y_o	4.83	5.13	5.05	5.90
		y_f	7.12	8.17	7.29	7.29
		μ_{max}	0.008	0.29	0.009	0.113
		B_f	0.99	1.09	1.01	1.10
		A_f	1.02	1.09	1.03	1.10
		RMSE	0.18	0.68	0.21	0.63
		R^2	1.0	0.98	1.0	0.99

y_o – Initial cell count at day 0 predicted in $\log_{10}$ cfu/g
y_f – Final cell count at day 12 predicted in $\log_{10}$ cfu/g
μ_{max} – Maximum growth rate predicted in $\log_{10}$ cfu/h

Model performance for high and low inoculum levels of *L. monocytogenes* in beef lasagne and egg noodles was evaluated. From the challenge test studies, the initial cell count of *L. monocytogenes* at low and high inoculum levels in beef lasagne was 2.92 and 5.91 log $_{10}$ cfu/g respectively, while it was 2.21 and 4.82 log $_{10}$ cfu/g respectively for egg noodles (Table 9). However, final cell count of *L. monocytogenes* at low and high inoculum levels at the end of storage period in beef lasagne was 5.01 and 7.04 log $_{10}$ cfu/g respectively, while it was 4.46 and 6.94 log $_{10}$ cfu/g respectively for egg noodles (Table 9).

At low inoculum level, ComBase prediction with an initial and final cell count of 2.91 and 4.89 log $_{10}$ cfu/g respectively for *L. monocytogenes* in beef lasagne had a maximum growth rate (μ_{max}) of 0.007 log $_{10}$ cfu/h. When compared to the experimental data (Fig 8), the ComBase underestimated growth of the pathogen by 3% with an average B_f of 0.97 and the prediction was quite close to the observed data with A_f 1.03 (Table 9). The *RMSE* and R^2 for the software prediction was 0.17 and 1.0 respectively. However, at high inoculum level (Fig 9), initial and final cell count predicted by ComBase was 5.91 and 7.87 log $_{10}$ cfu/g respectively with a maximum growth rate (μ_{max}) of 0.007 log $_{10}$ cfu/h. At an average B_f and A_f of 1.04 and 1.04 respectively, the ComBase software overestimated the growth of *L. monocytogenes* by 4% at this high inoculum level and the A_f value showed there was a relatively close prediction to the observed data and *RMSE* and R^2 was 0.42 and 0.99 respectively (Table 9).

At low inoculum level, ComBase prediction with an initial and final cell count of 2.22 and 4.52 log $_{10}$ cfu/g respectively for *L. monocytogenes* in egg noodles had a maximum growth rate (μ_{max}) of 0.008 log $_{10}$ cfu/h, with 4 % overestimation corresponding to an average B_f of 1.04 when compared to the observed data (Fig 10) and A_f of 1.04 showed a relatively close prediction to the observed data (Table 9). *RMSE* and R^2 for the software prediction was 0.15 and 1.0 respectively. At high inoculum level, initial and final cell count predicted by ComBase was 4.83 and 7.12 log $_{10}$ cfu/g respectively with a maximum growth rate (μ_{max}) of 0.008 log $_{10}$ cfu/h. B_f and A_f for the software prediction at this high inoculum level was 0.99 and 1.02 corresponding to 1% underestimation and a very close prediction to the observed data respectively (Fig 11). *RMSE* and R^2 was 0.18 and 1.0 respectively (Table 9). However, for prediction in beef lasagne, PMP had an initial and final cell count prediction for low inoculum level of *L. monocytogenes* as 3.43 and 5.30 $\log_{10}$ cfu/g respectively at μ_{max} of 0.23 $\log_{10}$ cfu/g with 8% overestimation of the growth of *L. monocytogenes*, having an average B_f of 1.08. A_f value at 1.08 indicated a close prediction to the observed data (Fig 8) while *RMSE* and R^2 was 0.33 and 0.99 respectively. When compared to the observed data (Fig 9), at high inoculum level, initial and final cell count of *L. monocytogenes* was 6.14 and 8.14 $\log_{10}$ cfu/g respectively at μ_{max} of 0.23 $\log_{10}$ cfu/g with

an overestimation by 7% corresponding to B_f of 1.07. A_f value of 1.07 indicated a close prediction to the observed data while *RMSE* and R^2 was 0.60 and 0.99 respectively (Table 9).

For prediction of *L. monocytogenes* growth in egg noodles, PMP had an initial and final cell count for low inoculum level at 3.43 and 6.55 $\log_{10}$ cfu/g respectively at μ_{max} of 0.29 $\log_{10}$ cfu/g with the prediction having B_f and A_f of 1.50 and 1.52 overestimating the growth of *L. monocytogenes* by 50% while *RMSE* and R^2 was 1.69 and 0.82 respectively when compared to the observed data (Fig 10). Comparing the prediction at high inoculum level (Fig 11), initial and final cell count was 5.13 and 8.17 $\log_{10}$ cfu/g respectively at μ_{max} of 0.29 $\log_{10}$ cfu/g with corresponding B_f and A_f of 1.09 and 1.09 overestimating growth of *L. monocytogenes* by 9% and the prediction was relatively close. *RMSE* and R^2 was 0.68 and 0.98 respectively (Table 9).

On the other hand MicroHibro software had an initial and final cell count at low inoculum level in beef lasagne as 3.28 and 5.37 $\log_{10}$ cfu/g respectively at μ_{max} of 0.009 $\log_{10}$ cfu/g with B_f and A_f of 1.06 and 1.07 respectively with an overestimation of the growth of *L. monocytogenes* by 6% (Fig 8) while *RMSE* and R^2 for the software prediction was 0.29 and 0.99 respectively. At high inoculum level, initial and final cell count was 6.07 and 8.17 $\log_{10}$ cfu/g respectively at μ_{max} of 0.009 $\log_{10}$ cfu/g. B_f and A_f was 1.07 and 1.07 respectively with *L. monocytogenes* growth overestimation by 7% and *RMSE* and R^2 for the software prediction was 0.63 and 0.99 respectively (Table 9) A_f values showed a relatively close prediction to the observed data (Fig 9).

For prediction of *L. monocytogenes* growth in egg noodles, MicroHibro software had an initial and final cell count at low inoculum level as 2.62 and 4.73 $\log_{10}$ cfu/g respectively at μ_{max} of 0.009 $\log_{10}$ cfu/g with B_f and A_f of 1.12 and 1.12 corresponding to 12 % overestimation (Fig 10). *RMSE* and R^2 for the software prediction was 0.35 and 0.99 respectively (Table 9). At high inoculum level, initial and final cell count was 5.05 and 7.29 $\log_{10}$ cfu/g respectively at μ_{max} of 0.009 $\log_{10}$ cfu/g. B_f and A_f was 1.01 and 1.03 with an overestimation of *L. monocytogenes* growth by 1% and a very close prediction compared to the observed data (Fig 11). *RMSE* and R^2 for the software prediction was 0.21 and 1.0 respectively (Table 9).

Furthermore, FSSP software had an initial and final cell count of *L. monocytogenes* in beef lasagne at low inoculum level as 2.99 and 4.52 $\log_{10}$ cfu/g respectively at μ_{max} of 0.0122 $\log_{10}$ cfu/g with B_f and A_f of 0.94 and 1.07 respectively corresponding to 6% underestimation (Fig 8). *RMSE* and R^2 for the software prediction was 0.33 and 0.99 respectively (Table 9). At high inoculum level, initial and final cell count was 5.99 and 7.48 $\log_{10}$ cfu/g respectively at μ_{max} of 0.0122 $\log_{10}$ cfu/g. B_f and A_f was 1.02 and 1.03, corresponding to 2% overestimation and A_f values showed the predicted data were quite

close to the observed data (Fig 9). *RMSE* and R^2 for the software prediction was 0.22 and 1.0 respectively (Table 9).

Comparing predictions (Fig 10) of *L. monocytogenes* growth in egg noodles, FSSP software had an initial and final cell count at low inoculum level as 2.87 and 4.28 $\log_{10}$ cfu/g respectively at μ_{max} of 0.113 $\log_{10}$ cfu/g with B_f and A_f of 1.23 and 1.25 and overestimation of *L. monocytogenes* growth was by 23%. *RMSE* and R^2 for the software prediction was 1.14 and 0.88 respectively. At high inoculum level, initial and final cell count was 5.90 and 7.29 $\log_{10}$ cfu/g respectively at μ_{max} of 0.113 $\log_{10}$ cfu/g. B_f and A_f was 1.10 and 1.10 respectively with growth estimation of 10% (Fig 11) and *RMSE* and R^2 for the software prediction was 0.63 and 0.99 respectively (Table 9).

3.4.4 DISCUSSION

To select the best performed software, performance indices (B_f, A_f, R^2 and *RMSE*) was used to evaluate the performance of the four different software calculated based on equations 1-4.

B_f of 1 indicates perfect agreement between the observed and predicted growth data, meaning there is no under or over-prediction. However, a $B_f > 1$ indicates the predicted growth of the pathogen generated by the software is longer than the observed growth during challenge test while a $B_f < 1$ indicates the predicted growth is shorter than the observed growth. Hence, over-prediction ($B_f > 1$) may be termed 'fail-safe' and under-prediction ($B_f <$) may be termed 'fail-dangerous' (Ross, 1996). Fail-dangerous predictions mean no-growth of the pathogen was predicted by the software when growth was actually observed during challenge test. For the fail-safe prediction, growth was predicted when no-growth was actually observed. Predicted growth values over- or under-predicted by more than 43% and 13%, respectively, corresponding to a B_f of between 0.87 and 1.43 are considered not good or unacceptable (Ross, 1999).

A_f of 1 represents a perfect agreement between the observed and predicted growth. $A_f > 1$ implies a less accurate average estimate of the observed and predicted growth.

The larger the value of *RMSE* the greater the influence of predictions which deviate widely from the observed result, resulting in larger values of the 'error' estimate (Ross, 1996). The lower the *RMSE*, the better the software is at describing the observed growth data (Drosinis *et al*., 2006).

R^2 shows the level of statistical fit of predicted growth data of *L. monocytogenes* to the observed growth. Predicted values close to 1 indicate a good statistical fit to the observed growth data for *L. monocytogenes*.

The ComBase software had a better performance compared to the other types of software for prediction of growth of *L. monocytogenes* at both low and high inoculum levels in beef lasagne. The average B_f and A_f values were quite close to 1 and shows the software slightly over- or underestimate the growth values (Table 9). Software used in shelf life prediction of food should predict as closely as possible the observed behaviour to avoid wastage of product (Ross, 1996). The *RMSE* and R^2values for the software indicated low level of error and close fit to the observed growth of *L. monocytogenes* in beef lasagne.

The ComBase software also had a good performance in predicting growth of *L. monocytogenes* in egg noodles (Table 9). The average B_f and A_f values were also close to 1, showing the software slightly over- or underestimate the growth values and the *RMSE* and R^2values for the software indicated low level of error and close statistical fit to the observed growth of *L. monocytogenes* in egg noodles.

The software prediction were fail safe for beef lasagne and egg noodles at 10^6 and 10^3 cfu/g inoculum level of *L. monocytogenes* respectively, while it was fail dangerous for beef lasagne and egg noodles at 10^3 and 10^6 cfu/g inoculum level of *L. monocytogenes* respectively which indicates that prediction depends on the level of *L. monocytogenes* contamination. de Araújo *et al*., (2017) reported that ComBase growth predictions under the temperature, pH, and a_w conditions in commercial coalho cheese samples were generally fail-safe for predicting the growth of *L. monocytogenes*. The ComBase software has physiological state of microorganism as an input data. This may influence the better performance of the software as Buchanan, Whiting & Damert. (1997) suggested that the basic physiological state of individual cells should form the basis of any growth model.

The PMP software had a good performance for prediction of growth of *L. monocytogenes* at both low and high inoculum levels in beef lasagne with the average B_f and A_f values close to 1 and a good R^2 statistical fit also close to 1 (Table 9). *RMSE* values on the other hand were high at both low and high inoculum levels with the high inoculum level having the higher error (0.60).

This software also performed well for the prediction of *L. monocytogenes* growth at high inoculum level in egg noodles (Table 9) with the average B_f and A_f values close to 1 while the prediction for low inoculum level was not acceptable at over 50 % overestimation of the growth of *L. monocytogenes* with an average B_f and A_f values of 1.50 and 1.52 respectively. Consequently, *RMSE* and R^2values were such that the prediction had the highest error margin compared to other software. The software prediction was generally fail safe.

MicroHibro software (Table 9) also had a good performance (third best) for the prediction of *L. monocytogenes* growth at both low and high inoculum levels in beef lasagne with the average B_f and

A_f values close to 1 and a good R^2 statistical fit also close to 1. However, the *RMSE* values were high at both low and high inoculum levels with the high inoculum level having the higher error (0.63).

This software on the other hand performed well (second best) for the prediction of *L. monocytogenes* growth at both high and low inoculum levels in egg noodles (Table 9) with the average B_f and A_f values close to 1 and a good R^2 statistical fit of almost 1 while the *RMSE* values were a bit high at both low and high inoculum levels with the low inoculum level having the higher error (0.35). The software prediction was generally fail safe. The good performance of MicroHibro software could be due to the allowance for modelling multifood in the software which was used for the prediction of the food product in this study. Predictive models should match the complex nature of foods of concern including low pathogen contamination levels in order to provide more realistic outputs (Mejlholm *et al*., 2010).

The FSSP software also performed better after the ComBase predictor for the prediction of *L. monocytogenes* growth at both high and low inoculum levels in beef lasagne (Table 9) with the average B_f and A_f values close to 1 and a good R^2 statistical fit of almost 1. *RMSE* values were a relatively low at both low and high inoculum levels with the low inoculum level having an error of 0.33. Mejlholm *et al*. (2010) reported that the cardinal parameter model which the FSSP software is built upon performed better compared to other models for the prediction of meat products similar to beef lasagne.

This software on the other hand had an acceptable performance for the prediction of *L. monocytogenes* growth at both high and low inoculum levels in egg noodles. The software was the third best performed compared to other types of software for prediction of *L. monocytogenes* growth in egg noodles. The average B_f values showed an overestimation of the growth of *L. monocytogenes* for high and low inoculum levels as overestimated by 10 and 23% respectively. A_f values were not as close to 1 as well with an overestimation of 10 and 25%, respectively. Mejlholm *et al*. (2010) also reported *L. monocytogenes* overestimation by 50% for poultry products using the cardinal parameter model although the model generally performed better compared to other models for the prediction of *L. monocytogenes* growth rate in meat products, non-fermented dairy products, seafood and dairy products. R^2 statistical fit for the FSSP prediction was close to 1 for the high inoculum level while R^2 for low inoculum level was not indicating a poor statistical fit. The *RMSE* values were relatively high at both low and high inoculum levels with the low inoculum level having the higher error (1.14). Predictions of this software was generally fail safe.

Fail safe prediction pose no public health or food safety risk to consumers as growth was predicted when growth was not observed. On the other hand, fail dangerous prediction can pose health risk to consumers due to growth of the pathogen not predicted when growth was actually observed. On the

other hand, overly fail safe predictions could lead to food waste as shelf life predictions are boosted beyond the observed growth of the pathogen. Also, software with fail safe prediction should be used with caution for instance in product development or risk assessment, as this might result in an excessive use of preservatives or an overestimation of the risk associated with *L. monocytogenes*, while fail dangerous prediction might result in underestimation of *L. monocytogenes* growth thereby increasing potential health risk (Mejlholm *et al*., 2010).

3.4.5 CONCLUSION

For the prediction of *L. monocytogenes* growth in beef lasagne and egg noodles, all the software evaluated in this study performed well with a fail-safe prediction. This translates to the products not posing food safety risk to the consumers as the growth of the pathogens are predicted to be faster. This can be applied for shelf life prediction of RTE food products by the South African food industry. However, ComBase software had the best performance compared to other software for the prediction of *L. monocytogenes* growth in beef lasagne and egg noodles. The prediction of the software was quite close to the observed. Hence, its application will aid in the alleviation of food waste problem. The FSSP and MicroHibro software can also be used to predict the growth of *L. monocytogenes* in beef lasagne and egg noodles. In other words, to reduce food waste due to the conservative shelf life prediction, SMEs can make use of predictive microbiology models (Software) for prediction of shelf life of food products. Furthermore, these software are applicable with respect to other food types and preserving parameters which are not used to develop the software.

CHAPTER 4: GENERAL DISCUSSION

4.1 Critical review of methodology

To explore the use of predictive modelling for accurate shelf life estimation of RTE foods. Performance of tertiary models (software) in the shelf life prediction of RTE food products were evaluated using challenge test data also gathered in this present study. Conservative shelf life estimation of food products set by FBOs was verified by carrying out a storage test for selected RTE food products.

These RTE products (Beef lasagne, egg noodles, papaya, and mango) were selected based on the assumption that these products' shelf life is a representative of the 3 scenarios (New Zealand guidance document, 2014) described earlier in this study. Studies shows that some specific foodborne pathogens has been linked to certain RTE food products such as *L. monocytogenes* in RTE meat products, (Uyttendaele *et al*., 2009; Vermeulen *et al*., 2011), *L. monocytogenes* in mayonnaise-based deli-salads (Hwang & Tamplin, 2005; Uyttendaele *et al*., 2009), minimally processed fruits such as mango, melon & papaya, has been linked with Salmonella (CDC, 2012a,b; 2017). In SA, *S. aureus* and *E. coli* was found in RTE chicken, meat products, cheese, fruits and vegetables (Christison *et al*., 2008; Oguttu *et al*., 2014), *E. coli* was also isolated in biltong (Naidoo & Lindsay, 2010). However, some of these products would have been selected as a representative for this study with specific pathogens but were not available during the challenge test study. Microbiological challenge testing is very useful for food products that may sustain the growth of pathogenic organisms and vulnerable to the growth of these foodborne pathogens as knowledge of formulation and history of the food for instance foods associated with illness outbreak and/or evidence of potential growth is essential (FDA, 2001).

Shelf life estimation of the RTE food products was carried out using microbiological basis due to the fact that the highest risk of RTE foods is microbiological contamination (Ciekure, 2016). This may give an underestimation of shelf life of the selected RTE products and the study was carried out with the notion that before the quality of a food product can be compromised or rejected due to sensory reasons, the microbial load will attain 7 $\log_{10}$ cfu/g and for certain toxin producing organisms for instance *S. aureus*, the microbial load will be 5 $\log_{10}$ cfu/g (Koutsoumanis & Nychas, 2000; Corbo *et al.*, 2006; Barth *et al*., 2009; Valero *et al*., 2012). In other words, shelf life of food products is an indication of both quality and safety of the products, hence, sensory and chemical changes in the food products needs to be considered to get the overall shelf life of food products (Koutsoumanis & Nychas, 2000; Barth *et al*., 2009; Valero *et al*., 2012).

To assess the growth potential of microorganisms especially foodborne pathogens in RTE foods, challenge tests are often performed. During the shelf life estimation of the selected RTE food products,

it was observed that *E. coli* was the shelf life determination factor in pre-cut papaya, beef lasagne and egg noodles. Challenge test studies carried out in this study should have included the shelf life estimation to determine the behaviour of *E. coli* in egg noodles. This would determine if the organism will grow to an unacceptable limit during the shelf life of the product and the ability to compare shelf life estimation during storage and challenge test. Eggs and food products prepared using eggs seem to be a major concern as such foods are involved with Salmonellosis (Elias *et al*., 2015).

RTE foods subjected to challenge testing should be characterized carefully with respect to intrinsic and extrinsic factors such as pH, salt, preservatives, packaging conditions and the background microbiota making sure that variability of factors is considered (Mejlholm *et al*., 2010; Vermeulen *et al*., 2011). In this study, selected physico-chemical factors (pH, storage temperature and a_w) were factored into the shelf life determination factors of the selected RTE food products. Vermeulen *et al*. (2011) addressed this point as particularly important for challenge testing in order not to underestimate the growth potential of *L. monocytogenes* and it was reported in this study that models, taking into account the interaction effects with background flora, performed the best. LAB which has been reported to be the relevant competing microflora of *L. monocytogenes* should be considered (Mellefont, Mcmeekin & Ross, 2008; Mejlholm & Dalgaard, 2013; Vermeulen *et al*., 2011). *L. monocytogenes* was reported to be suppressed by all other strains present in the tested samples (Mellefont *et al*., 2008). The FSSP software developed an extensive model for interaction between *L. monocytogenes* and LAB to be able to model the importance of microbial interaction based on the Jameson effect between the two types of organisms (Ross *et al*., 2000). Without the Jameson effect, it was reported that the maximum cell concentration of *L. monocytogenes* in cold-smoked salmon was overestimated by as much as 5-6 $\log_{10}$ cfu/g (Mejlholm & Dalgaard, 2013).

4.2 Main research scientific findings

Based on the outcome of the storage test, it was observed that beef lasagne, egg noodles and pre-cut mango fall under scenarios 1, 1 and 3 respectively. However, outcome of the challenge test for the selected pathogens in these RTE products suggested that these products fall under scenarios 2, 2 and 3, respectively. Beef lasagne and egg noodles according to the challenge test study might be visibly spoilt before the pathogens reach unsafe levels (New Zealand guidance document, 2014). However, the shelf life of these products (beef lasagne, egg noodles and pre-cut mango) was estimate to be 7, 6 and 12 days respectively. Outcome of the challenge test for the selected pathogens in the RTE products suggested that these products have shelf life of 9, 9 and 12 days respectively. A study have shown that shelf life of mayonnaise-based salad with similar characteristics with beef lasagne can be extended

(Uyttendaele *et al*., 2009). The SA law however states that 'use-by' date used as date marking on these selected RTE food products is an indication of quality acceptance after which the product will not be marketable rather than safety. This is contrary to the outcome of this study which shows that safety is of paramount concern in these product, and is in agreement with the Australian and European laws that the use of 'use-by' date marking for this category of foods as an indicator of food safety (Food Standards Australia New Zealand Act 1991. Standard 1.2.5-2; EC, 2000; EC, 2011).

After inoculation of the pathogens into the different RTE food products, there was a reduction in the count of the pathogens. *L. monocytogenes* declined from 3 $\log_{10}$ cfu/g to about 2 $\log_{10}$ cfu/g in egg noodles and just a little below 3 $\log_{10}$ cfu/g for beef lasagne and from 6 $\log_{10}$ cfu/g to about 5 $\log_{10}$ cfu/g in egg noodles and just a little below 6 $\log_{10}$ cfu/g for beef lasagne. For *E. coli*, reduction occurred to just below 3 and 6 $\log_{10}$ cfu/g for beef lasagne and for pre-cut mango, about 0.01 and 0.19 cfu/g reduction occurred for 3 and 6 $\log_{10}$ cfu/g respectively. Reduction in the concentration of the different pathogens in egg noodles and beef lasagne immediately after inoculation can be attributed to the fact that pathogen survival is influenced by the immediate spatial location of the organism cells within the local microenvironment of the food product as these category of RTE food products are multi-ingredient foods (Li *et al*., 2014).

S. Typhimurium did not survive in egg noodles while *E.coli* had a slow growth in the product. However, the rate of decline of the pathogen at low level inoculation was > 2 log cfu/g and < 2 log cfu/g for high inoculum level. According to Hwang & Marmer, (2007), growth of *L. monocytogenes* was reported to be more rapid in egg salad compared to pasta salad. This behaviour can be attributed to the microstructure and microscopic water distribution of egg noodles (Hills *et al*., 2001). Similar decline of *S.* Typhimurium and *E.coli* in packed silica bed having comparable intrinsic property with egg noodles was reported by Hills *et al*., (2001). *Salmonella* spp. however requires higher temperature (> 7^{o}C) for growth to occur (Sant'Ana *et al*., 2012b). Also, a critical factor affecting the capacity for growth of various initial pathogen levels on a food surface is the interaction with the epiphytic flora (Manios *et al*., 2013).

L. monocytogenes was observed to be present in uninoculated samples of egg noodles (50%) and beef lasagne (100 %) at low level (as organism was not detected with direct enumeration but detected with a 2-step enrichment) while *E.coli* was also enumerated at day 0 (data not shown). Despite this, and the increase in the population of this organisms in the inoculated samples, the growth was quite slow (< 1 log increase) throughout the storage period but slow between day 3 and 6 compared to day 0 in the beef lasagne at both low and high inoculation levels which signifies the effect of low temperature

refrigeration storage such as 5^{o}C on *L. monocytogenes* (Hwang & Marmer, 2007; Sant'Ana *et al*., 2012a). *L. monocytogenes* particularly is of paramount concern in these RTE products as absence or slow growth of this pathogen can still be of food safety risk due to presence in the environment (Pouillot *et al*., 2015) especially if present in the FBO's facility unsuspected. It was reported by Centre for disease control and prevention (CDC, 2015) that in four states in the United State there was an outbreak of illness due to listeriosis between 2010 and 2015, also in 2016, there was listeriosis outbreak linked a packaged salad (CDC, 2016b). This outbreaks was due to the consumption of the products contaminated with *L. monocytogenes* that was however isolated from the facilities of the companies such as non-food contact areas within the processing room and kitchen and from non-food contact surfaces of production equipment (CDC, 2015) despite the cleaning and sanitizing programmes that has been put in place by the companies.

All the software evaluated in this study was observed to perform well for the shelf life estimation of the selected RTE food product. Generally, the ComBase software performed best for the prediction in egg noodles, followed by MicroHibro, then FSSP and PMP. For beef lasagne on the other hand, the performance is in the order ComBase, followed by FSSP, then MicroHibro and PMP. However, depending on the inoculation level, some of the predictions by the software were faster than the observed data while some were slower. FSSP performed better compared to ComBase as observed by the study carried out on cold smoked salmon and fast software predictions were observed in this study. This is probably due to the extra stress factors (for instance packaging) which are present in the RTE food products that are not incorporated into the models (Uyttendaele *et al*., 2009; Vermeulen *et al*., 2011). Nevertheless, PMP software performed well for growth prediction in melon (Scolforo *et al*., 2016).

The varying performance of some software, that is, those that predicted faster or slower growth compared to the others can be attributed to the fact that the tertiary models may not include all the growth limiting factors which is taken into account during the challenge test studies (Uyttendaele *et al*., 2009). Hence, the software applicability and grading were rated based on the closest predictions to the observed pathogen growth. Using available tertiary models has the major advantage that the most important intrinsic and extrinsic factors can be included for the prediction of the growth parameter. FSSP which is one of the evaluated software in this present study has been approved by the Danish Veterinary and Food Administration as a means to predict growth of *L. monocytogenes* and to document compliance of RTE foods with regulation EC 2073/2005 (Mejlholm *et al*., 2015).

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The result of this study demonstrated that field of predictive food microbiology can be explored for shelf life prediction in SA. Successfully validated models are powerful tools for evaluating shelf life and improving food safety, particularly when models are included in user-friendly application software. This can pave way to reducing the problem of food waste due to conservative shelf life estimation of RTE food products by FBOs in SA. Majority of these software are available for free online, meanwhile these selected user friendly software can be explored by SA food industry especially the SMEs for shelf life estimation of RTE food products without compromising the quality and safety of these products. Shelf life predictions can be obtained within a short period (including time to carefully determine product characteristics) and it is relatively easy to extrapolate, that is, change one or more of the environmental parameters in order to obtain combinations of product characteristics and storage conditions that prevent or limit the growth of foodborne pathogens to an acceptable level. It is however recommended that the performance of these software be validated by extrapolation and changing parameters. Observed predictions should be carried using the growth parameters of the organisms in products with challenge test for proper validation.

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