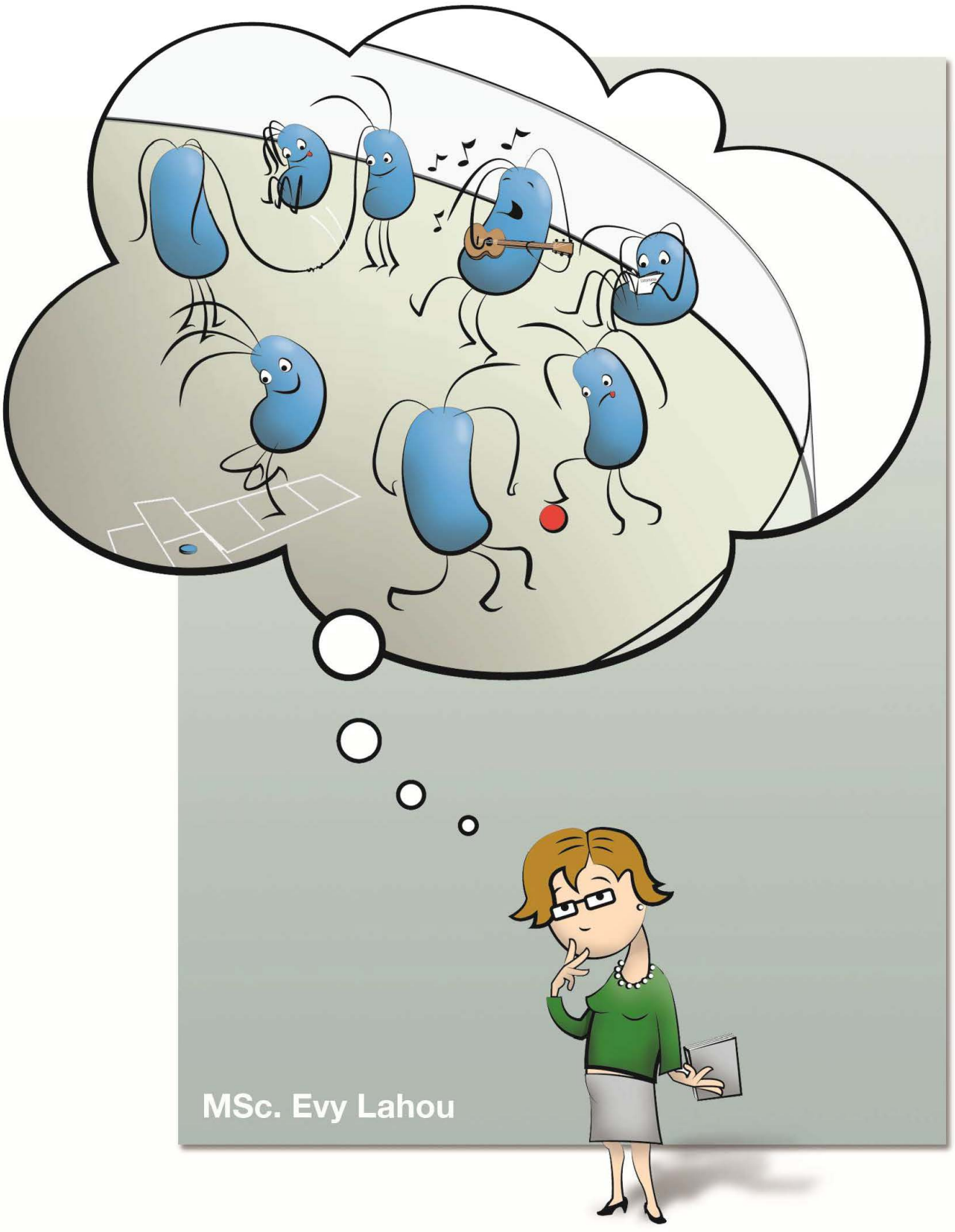
Microbiological Analysis for Validation and Verification of Food Safety Management



MSc. Evy Lahou

Promotors:

Prof. dr. ir. Mieke Uyttendaele

Prof. dr. ir. Liesbeth Jacxsens

Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University

Dean: Prof. dr. ir. Marc Van Meirvenne

Rector: Prof. dr. Anne De Paepe

Examination Committee Chairman: Prof. dr. ir. Nico Boon

Examination Committee Secretary: Prof. dr. ir. Monica Höfte

Examination Committee: Prof. dr. ir. Mieke Uyttendaele (UGent), Prof. dr. ir. Liesbeth Jacxsens (UGent), Prof. dr. med. vet. Martin Wagner (Vetmeduni Vienna, Austria), Dr. Annemarie Pielaat (RIVM, The Netherlands), Prof. dr. Lieven De Zutter (UGent)

MSc. Evy Lahou

Microbiological Analysis for Validation and Verification of Food Safety Management

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Dutch translation of the title:

Microbiologische analyse om de beheersing van voedselveiligheid te valideren en te verifiëren.

ISBN: 978-90-5989-907-0

To refer to this thesis:

Lahou, E. (2016). Microbiological analysis for validation and verification of food safety management. Thesis submitted in fulfillment of the requirements for the degree of doctor (PhD) in Applied Biological Sciences. Faculty of Bioscience Engineering, Ghent University, Belgium.

Dankwoord

Het lijkt al een eeuwigheid geleden dat ik dit avontuur begon. Maar nu, na zeven plezante jaren is het dan eindelijk zover… het afronden van mijn doctoraat. Het was een periode waarin ik veel heb geleerd op wetenschappelijk gebied, maar ook op persoonlijk vlak. Aangezien ik er nooit alleen voor heb gestaan en dus hulp, aanmoediging en inspiratie van een groot aantal mensen heb gekregen, wil ik dit avontuur afronden met een dankwoordje aan hen.

Mijn oprechte dank gaat uit naar mijn promotoren Prof. Mieke Uyttendaele en Prof. Liesbeth Jacxsens voor de mogelijkheid om dit doctoraat te starten. Prof. Uyttendaele, Mieke, bedankt voor het vertrouwen dat jij in mij stelde, niet alleen als doctorandus, maar ook als assistente! Zonder jouw vertrouwen, steun, enthousiasme en gedrevenheid zou ik dit werk niet hebben kunnen voltooien met de nodige motivatie en inspiratie. Prof. Jacxsens, Liesbeth, bedankt voor je steun en begeleiding van dit doctoraat! Ook jij wist me steeds te motiveren en niet alleen op wetenschappelijk vlak… zo stimuleerde jij me ook om o.a. links te rijden in Chipping Campden en mijn loopschoenen aan te trekken om 6u ’s morgens ☺. Ervaringen die ik nooit zal vergeten.

Daarnaast wil ik ook mijn (ex-)collega’s bedanken. Gedurende mijn periode aan het LFMFP heb ik vele mensen zien vertrekken, maar ook vele mensen zien starten en het is onbegonnen werk om jullie allen afzonderlijk te bedanken voor de leuke samenwerking, medewerking in de practicums, vlotte babbels, gezellige en ontspannende lunchpauzes, het aangename reisgezelschap, bureaufeestjes, world food party’s, logeerpartijtjes, … Toch zou ik graag een speciaal woordje van dank richten aan An De Coen voor de ondersteuning en handige tips tijdens mijn eerste ervaringen in de wereld van de microbiologie. Ook Ann Dirckx, Elien Verguldt en Xiang Wang verdienen een speciaal dankwoordje om mijn labowerk te helpen verlichten. Bedankt aan jullie allen om mijn tijd op het LFMFP tot een onvergetelijke tijd te maken!

Ook mijn thesisstudenten mag ik niet vergeten te bedanken voor hun inzet en bijdrage aan dit doctoraatproefschrift. In het bijzonder, Elien De Boeck, voor haar harde en nauwkeurige werk. Ik ben blij dat jij daarna ons LFMFP-team kwam verrijken!

Tevens werd deze bundeling van onderzoeksresultaten nauwgezet gelezen door de leden van de examencommissie. De geleverde commentaren en suggesties hebben een aanzienlijke bijdrage geleverd tot dit doctoraal proefschrift, waarvoor dank!

Graag wil ik ook mijn familie bedanken en in het bijzonder mijn ouders en schoonouders. Bedankt voor de ondersteuning op de moeilijke momenten, maar vooral voor de opvang van de kids wanneer het ons niet goed uitkwam!

Als laatste, maar daarom niet de minst belangrijke... Bert, mijn beertje, bedankt dat jij er voor mij bent! Tijdens dit doctoraat heeft ons leven ook niet stil gestaan en ik besef maar al te goed hoe zwaar dit alles weegt op jou als partner. We verbouwden een huis, zijn getrouwd en kregen 2 prachtige zonen Jesper en Lars. Daarnaast had ik ook nog een uit de hand gelopen (smakelijke) hobby, waardoor jij niet altijd de aandacht kreeg die je verdiende. Ik beloof je dat daar snel verandering in zal komen! Samen met jou en de kids ben ik nu klaar voor een nieuw avontuur in Zweden! Een droom van ons samen die we nu gaan waarmaken…

Vi ses!

Evy *Geraardsbergen, juni 2016*

TABLE OF CONTENTS

Table of Contents

List of abbreviations xi
Objectives and outline xv
Summary xxiii
Samenvatting xxix
CHAPTER 1 1
1.1 Management of (microbiological) food safety 3
1.1.1 Food safety management at the level of competent authorities 4
1.1.2 Food safety management at the level of industry associations 6
1.1.3 Food safety management at the level of individual food business operators 7
1.2 Microbiological analysis 9
1.2.1 Sampling: an essential tool in microbiological analysis 10
1.2.2 The selection of microbiological parameters 17
1.2.3 Interpretation of microbiological analysis: the use of microbiological criteria 23
1.3 Microbiological Analysis for ensuring safety in the food supply chain 27
1.3.1 Sampling and testing for ensuring safety in food service operations 27
1.3.2 Challenge testing for assessing the growth potential of *L. monocytogenes* 29
1.3.3 Inactivation studies to validate the efficacy of heat treatment of foods 31
1.4 Conclusion 33
CHAPTER 2 35
2.1 Introduction 38
2.2 Materials and Methods 40
2.2.1 Characterization of the food service operation 40
2.2.2 Microbiological Assessment Scheme 40
2.2.3 Data analysis and interpretation: microbiological safety level profiles. 45
2.2.4 Hand hygiene 47
2.3 Results and Discussion 47
2.4 Conclusion 56
CHAPTER 3 57
3.1 Introduction 60
3.2 Materials and methods 62
3.2.1 Definition of the case study 62
3.2.2 Food categorization and risk attribution 62
3.2.3 Elaboration of the sampling plan 68
3.2.4 Implementation of the sampling plan at the selected food service operation 69
3.2.5 Interpretation of results and feedback on elaborated sampling plan 72
3.3 Results 73
3.3.1 Food categorization and risk attribution 73

3.3.2 Implementation of the elaborated sampling plan for verification of supplier selection and microbial quality and safety of end products 74
3.4 Discussion 80
3.5 Conclusion 87
CHAPTER 4 89
4.1 Introduction 92
4.2 Materials and methods 93
4.2.1 Characterization of the hospital food service operation 93
4.2.2 (Self-)assessment questionnaire 94
4.2.3 Risk-based sampling plan 97
4.3 Results 100
4.3.1 (Self-)assessment questionnaire 100
4.3.2 Risk based sampling plan 104
4.4 Discussion 105
4.5 Conclusion 111
CHAPTER 5 113
5.1 Introduction 116
5.2 Materials and methods 118
5.2.1 Selection of bacterial strains and culture conditions 118
5.2.2 Determination of D-values of bacterial strains 119
5.2.3 Simulation of home pan frying of meat and meat preparations 121
5.3 Results 126
5.3.1 Determination of D-values of bacterial strains 126
5.3.2 Assessment of initial quality and safety of the meat and meat preparations 128
5.3.3 Temperature profile during pan-frying of meat and meat preparations 129
5.3.4 Detection and enumeration of surviving bacteria 133
5.4 Discussion 137
5.5 Conclusion 142
CHAPTER 6 143
6.1 Introduction 146
6.2 Materials and methods 148
6.2.1 Survey on *L. monocytogenes* in artisanal cheeses from delicatessen shops 148
6.2.2 Challenge testing 149
6.2.3 Data analysis 150
6.3 Results 150
6.3.1 Survey on *L. monocytogenes* in artisanal (semi-)soft and (semi-)hard cheeses from cheese shops 150
6.3.2 Challenge testing 152
6.4 Discussion 155
6.5 Conclusion 163
CHAPTER 7 167

7.1 Management of microbiological food safety 169
7.1.1 Microbiological analysis to verify food safety management in FSO 169
7.1.2 Microbiological analysis to validate food safety management systems 175
7.2 Conclusion 181
7.3 Recommendations and future perspectives 182
Reference list 185
Curriculum vitae 223

List of Figures

Figure 1.1 Overview of EU legislation on food safety 3

Figure 1.2 A schematic overview of a Food Safety Management System 8

Figure 1.3 Overview of the relevant phenomena in food safety control, indicating the position of validation and verification 14

Figure 1.4 Example of a microbial safety profile 16

Figure 2.1. Schematic overview of the main production processes in a FSO 42

Figure 2.2. Microbial safety level profile for three production processes in the catering establishments 49

Figure 2.3. Distribution of bacterial contamination on hands/or gloves of all food handlers (A) and of food handlers performing a specific food activity (B). 52

Figure 3.1. Overview of concentrations (in log CFU/g) and variability of spoilage indicators of high risk raw materials used in food service operation 75

Figure 3.2. Overview of concentrations (in log CFU/g) and variability of spoilage indicators of high risk meals produced in food service operation 79

Figure 4.1. Structure and relationship between the groups of the (self-)assessment questionnaire 95

Figure 4.2. Example of an indicator for the context (A), an activity (B) and system performance (C) as used in the (self-) assessment questionnaire 97

Figure 5.1 Thermal inactivation curves of foodborne pathogens at 60 °C in adjusted broth (pH 5.6 and 1.5% NaCl) 127

Figure 6.1. Growth potential of *L. monocytogenes* on soft, semi-soft and semi-hard cheeses regardless to storage temperature and contamination profile 152

Figure 6.2. Influence of storage temperature (A), inoculation spot (B), cheese type (C), and milk treatment (D) on the growth potential of *L. monocytogenes*……..156

List of Tables

Table 1.1 Examples of sampling plans for batch control as adopted in Regulation EC No. 2073/2005. 11

Table 1.2 The most important food pathogens and their taxonomic situation, morphology, growth characteristics, infective dose and (known) virulence genes. 20

Table 1.3 Reported cases and notification rates per 100,000 of human food infections in the EU and Belgium during the period 2009–2013. 21

Table 1.4 Reported collective foodborne outbreaks (FTI) in Belgium during the period 2009-2014. 22

Table 1.5 Microbiological criteria established by processors, trade associations or governments. 26

Table 1.6 Definitions of related heat processing variables. 31

Table 2.1 Overview of critical sampling locations (CSLs) encompassing food products and production environment with the corresponding analyzed microbiological parameters and legal requirements or microbiological guidelines 43

Table 2.2 Score attribution system. 46

Table 2.3 Table 2.3. Detailed results of the microbiological assessment scheme. 48

Table 3.1 Categorization and risk attribution of raw materials in the inventory list of the food service operation. 64

Table 3.2 Example of the motivation of the risk characterization for food category meat and meat products. 66

Table 3.3 Number of samples required for a certain confidence level in order to detect a maximum fraction of contamination given all samples are negative.. 69

Table 3.4 Overview of high risk categories of raw materials with their analysed parameters and microbiological criteria and guidelines 71

Table 3.5 Overview of high risk meal servings with their analysed parameters and microbiological criteria and guidelines. 72

Table 3.6 Detection and unsatisfactory results of hygiene indicators and pathogens. 77

Table 4.1 Results of the (self-)assessment questionnaire. 101

Table 4.2 Overview of analysed samples and number of positive *L. monocytogenes* samples. 105

Table 5.1 Bacterial strains used for thermal inactivation experiments. 119

Table 5.2 Characteristics of the meat and meat preparations subjected to the treatments. 123

Table 5.3 Standardized commonly used cooking practices for intact meat and meat preparations. 125

Table 5.4 Estimated D-values at 60°C in normal and adjusted broth in this study compared with D-values reported in previous studies. 128

Table 5.5 Microbial quality (in log CFU/g) of the meat and meat preparations prior to heat treatments. 129

Table 5.6 Process lethality *P* calculated with a z-value of 7°C (Table A) or a z-value of 5°C (Table B) and core temperatures (≥ 55°C) measured during the heat treatment of the different meat and meat preparations. 132

Table 5.7 Residual recovery of pathogenic bacteria on inoculated meat and meat preparations after simulated home-cooking practices. 135

Table 5.8 Residual recovery of pathogenic bacteria on inoculated steaks (beef, horse, kangaroo, crocodile and turkey) after simulated home-cooking practices with different gradients of frying (i.e. rare, medium, well done). 136

Table 6.1 Summary of the results from the survey on *L. monocytogenes* in (semi-)soft and (semi-) hard cheeses. 151

Table 6.2 Intrinsic characteristics of the different cheeses involved in challenge testing. 153

Table 6.3 Growth potential of *L. monocytogenes* as obtained during challenge testing. 154

LIST OF ABBREVIATIONS AND TERMS

AFNOR	Association Française de Normalisation
APC	Aerobic Psychotrophic Count
BP	Baird-Parker
B2C	Business-to-Consumer
c	The maximum number of units that may be accepted with counts above the microbiological limit m, provided none of these units exceeds M
CCP	Critical Control Point
CFU	Colony Forming Unit
CSL	Critical Sampling Location
EC	European Commission
ECDC	European Center for Disease Prevention and Control
ELFA	Enzyme-Linked Fluorescent Assay
EFSA	European Food Safety Authority
EU	European Union
FASFC	Belgian Federal Agency for the Safety of the Food Chain
FAVV	Federaal Agentschap voor de Veiligheid van de Voedselketen
FBO	Food Business Operator
FSMS	Food Safety Management System
FSO	Food Service Operation
FTI	Collective food-borne outbreak
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
ICMSF	International Commission on Microbiological Specification for Foods
ISO	International Organization for Standardization
kve	kolonie vormende eenheden
LAB	Lactic acid bacteria

LFMFP	Laboratory of Food Microbiology and Food Preservation
m	Microbiological limit which separates good quality from marginally or defective quality
M	Microbiological limit which separates marginally quality from defective quality in a three-class attributes sampling plan
MANCP	Multi-Annual National Control Plan
MAS	Microbiological Assessment Scheme
MRS	Man Rogosa Sharpe
MSLP	Microbiological Safety Level Profiles
MYP	Mannitol egg Yolk Polymyxin
n	Number of sample units that need to be randomly taken from one and the same lot and which are to be examined individually
PCR	Polymerase Chain Reaction
PRP	Pre-Requisite Programs
RASFF	Rapid Alert System for Food and Feed
RODAC	Replicate Organism Detection and Counting
RTE	Ready-To-Eat
STEC	Shiga toxin-producing *E. coli*
TVC	Total Viable Count
VRBG	Violet Red Bile Glucose
VTEC	Verotoxin producing *E. coli*
YOPI	Young, Old, Pregnant, Immunodeficient

OBJECTIVES AND OUTLINE

European food hygiene regulations (EC No. 852/2004 and EC No. 853/2004) put pressure on food business operators to develop and implement a food safety management system (FSMS) to govern food safety and to prevent foodborne outbreaks. Such a FSMS consists of generic Pre-Requisite Programs (PRP) and specific structured procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP). One of the seven principles of the HACCP system is verifying whether the FSMS is functioning properly. One of the tools which can be applied by food business operators for verification is establishing a sampling and testing scheme. The food hygiene legislation is complemented by EC Regulation No. 2073/2005 on microbiological criteria for foods (as amended by EC Regulation No. 1441/2007) which gives guidance to all food businesses involved in the production and handling of food on the acceptability of foodstuffs and their manufacturing, handling and distribution processes. This guidance is provided by setting microbiological food safety and process hygiene criteria that need to be met in putting food stuffs on the market or in operating activities according to good hygienic practices.

In addition, EC Regulation No. 882/2004 demands from the competent authorities to set up a Multi-Annual National Control Plan (MANCP) and an approach to be adopted to conduct official controls and to perform monitoring to enforce businesses' compliance with food and feed law. Therefore, the overall objective of this PhD study was to gain insight in how microbiological analysis of foods or during food processing can be used as a performance indicator of adequate food safety management and compliance to relevant European regulations. A microbiological analysis not only deals with selection of the method of analysis and its performance characteristics, but also concerns the elaboration of a sampling plan, selection of microbial parameters and the interpretation of test results and subsequent related actions. As such a comprehensive approach of all relevant factors surrounding microbiological analysis to ensure food safety was the focus of the current PhD thesis.

Overall, the presented research is divided into two objectives and seven chapters as shown in the overview below and Figure 0.1. A general introduction on the different aspects of food safety management and microbiological analysis in foods is given in **CHAPTER 1.**

The first objective is to develop a systematic approach in setting up a sampling plan and microbiological analysis to be fit for purpose for validation (**CHAPTER 2**) or verification (**CHAPTER 3**) of a FSMS. In particular, if multiple food types are handled and various food production processes are part of the food business' activities. Therefore, it was decided to focus on food service operations (FSO) as an example. As FSO are business-to-consumer (B2C) operations and although being prone to official controls, they have less pressure to demonstrate the performance of their FSMS in place compared to the food production companies. The developed approach was also applied on site as a case study in a specific food service operation. In **CHAPTER 2**, a vertical microbiological assessment scheme which focuses on sampling throughout the processing line from raw material to finished product in one of the institutional kitchens of Ghent University is presented. It was the objective to demonstrate how microbiological analysis can be used as a tool for identification of bottlenecks within the operation of this food business and as a validation of the food service operations' FSMS. In addition, as food service operations are confronted with a diverse range of raw materials and served meals, a microbiological sampling plan directed at i) incoming products in the framework of verification of supplier selection and ii) finished products (meals being served) to verify the functionality of their PRP and HACCP program in place is presented in **CHAPTER 3**. In **CHAPTER 4**, the FSMS of a hospital food service operation has been evaluated in its operation with particular focus on *L. monocytogenes.* The unique aspects of a hospital environment, such as the multitude of dietary needs and thus the variety of meals to be served and incoming (raw) materials to be used, challenge the development and implementation of appropriate control and assurance measures to ensure food safety. Besides, *L. monocytogenes* is considered a relevant microbial hazard for most food service operations producing and serving ready-to-eat foods to vulnerable people.

The second objective is to use microbiological analysis to evaluate processing and storage conditions at the level of a sector. This type of information will serve both industry associations, individual food businesses and competent authorities to define new or reinforce current guidelines or control measures to be taken to ensure food safety. In particular for micro and small-sized enterprises there might be a lack of resources and knowledge to validate their operations and to comply to the complexity of food safety

regulations. As such it was decided to focus on the following two examples which are also defined as being of priority for food protection and public health by the Belgian Food Safety Agency and the Federal Public Service Health, Food Chain Safety and Environment who also partially supported and funded this research.

In **CHAPTER 5**, the effectiveness of inactivation of foodborne pathogens during simulated pan-frying of meat and meat preparations was evaluated. The heat treatment of raw meat in the sector of food service operations (hotels, restaurants, catering) - as well as consumer domestic kitchens - is a major critical control point enabling to significant reduce numbers of pathogenic bacteria possibly being present in the meat. It is generally accepted that when meat and meat preparations (including hamburgers or any other comminuted meat) is subjected to a core temperature of 70 °C for 2 min or was subjected to a heat treatment equivalent to 2 min at 70 °C, it will accomplish a substantial inactivation (6 log reduction) of zoonotic pathogens and therefore renders the meat to be safe. However, it is not always clear which temperatures are actually reached during pan-frying of meat as applied in Belgian food service operations (and at home) where doneness of meat is usually defined by culinary preferences and visually judged. Therefore, it was decided to perform microbiological analysis to actually validate the current procedures in place in terms of pathogen reduction.

Microbiological analysis, and in particular challenge testing, can also be used as a tool to establish the growth potential of *L. monocytogenes*. Listeriosis infections tends to increase again in the European Union (EU), especially in the elderly population. Soft and semi-soft cheeses are acknowledged at risk products for listeriosis. These types of cheeses are often produced as artisanal products in the short supply chain or delicatessen shops. Not only during production of cheeses (on the farm) but also during storage conditions of cheese opportunities for post-contamination with *L. monocytogenes* may occur. The aim of the case study in **CHAPTER 6** was to use challenge testing to evaluate the behavior of *L. monocytogenes* on soft, semi-soft and semi-hard artisanal cheeses as a result of post-contamination during distribution, (short) further ripening, cold display and slicing at delicatessen shops. This case study illustrates also the quite complexity of microbiological analysis to comply to the microbiological criterion defined for *Listeria monocytogenes* stated in EC Regulation No. 2073/2005. This criterion defines as end product limit "<100 CFU *L.*

monocytogenes cells per ml or per g" only when the producer can proof that *L. monocytogenes* is not able to grow further to elevated numbers in the product throughout the shelf life.

In **CHAPTER 7** a general discussion of the research is presented. As such the research aims to cover the approach to be taken for the various stakeholders in the food chain, covering various food supply chains and their various purposes to perform microbiological analysis.

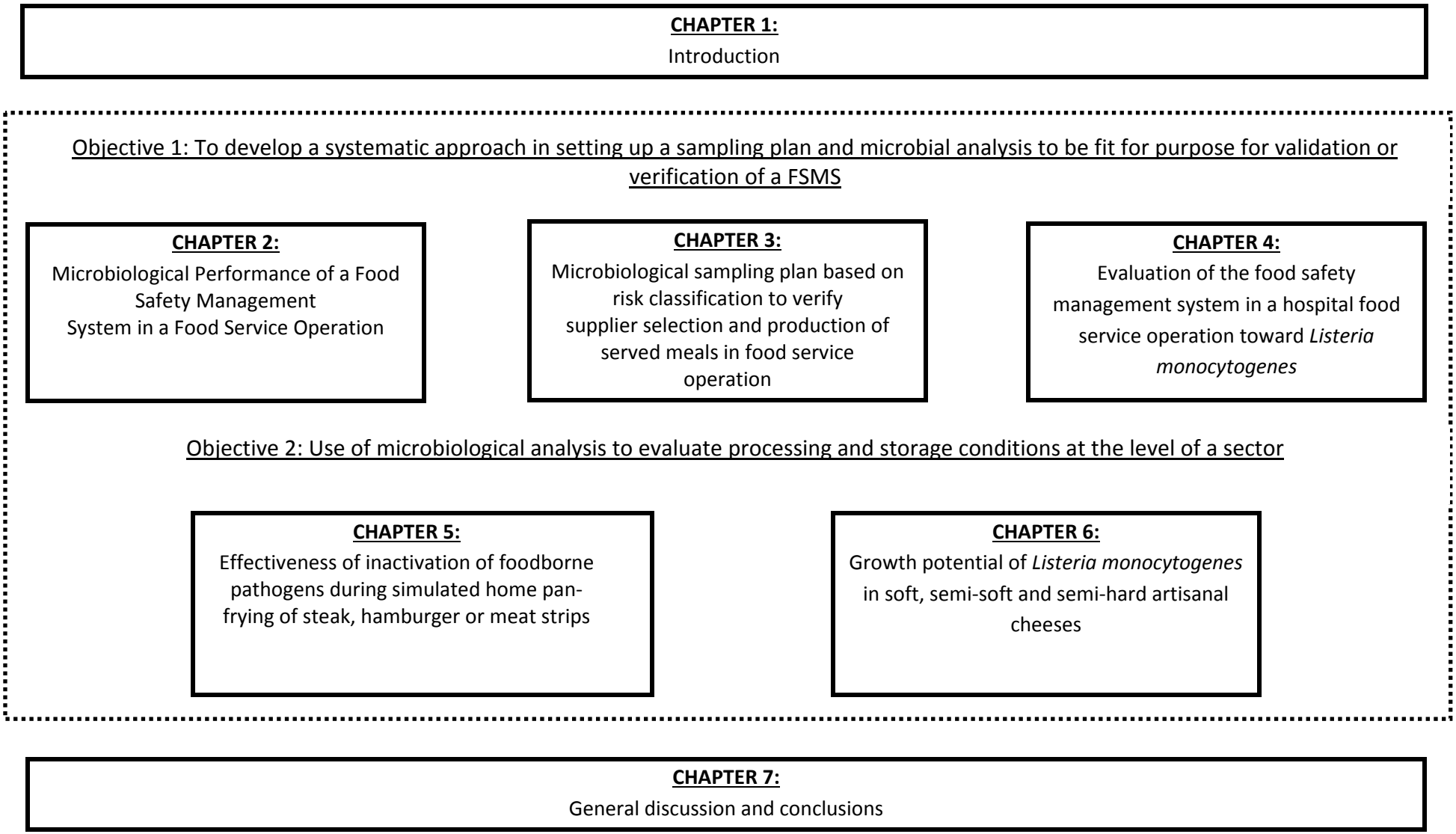


Figure 0.1.Overview of the different chapters of the present research.

SUMMARY

Microbiological analysis of foods or food production environment plays an important role in the management of microbiological hygiene and safety in the food chain. Microbiological analysis not only deals with the selection of the method of analysis and its performance characteristics, but also needs to take care of the objective and the set-up of a sampling plan, selection of microbiological parameters and interpretation of test results. These aspects are reviewed in **CHAPTER 1**. As such a comprehensive overview of all relevant factors surrounding microbiological analysis to ensure food safety and hygiene is presented.

Food service operations serve food directly to the consumer (i.e. perform business-to-consumer (B2C) activities) and are frequently involved in reported foodborne outbreaks. Therefore, control of food safety and hygiene with adequate food safety management is especially important for these sectors. However, expertise on performing microbiological analysis and resources to perform those are often lacking in this part of the food chain. As such, **CHAPTER 2, 3 and 4** focus on sampling and testing for validation and verification of core control measures within a Food Safety Management System (FSMS) in food service operations (FSO).

CHAPTER 2 concentrated on the setting up and implementation of a vertical microbiological assessment scheme in one of the institutional kitchens of Ghent University to validate the FSMS in place. The sampling plan focuses on sampling throughout the processing line from raw materials to final product of 1) a high-risk sandwich production process (involving raw meat preparation), 2) a medium-risk hot meal production process (starting from undercooked raw materials), and 3) a low-risk hot meal production process (reheating in a bag). When three times performed, insight into the microbial contamination and the variability of a production process is obtained. Besides, the sampling plan was also able to pinpoint the bottlenecks in the FSMS. As high levels of total aerobic bacteria (> 3.9 log CFU/50 cm^2) were noted occasionally on gloves of food handlers and on food contact surfaces, especially in highly contaminated areas (e.g. during handling of raw material, preparation room), core control activities such as hand hygiene of personnel and cleaning and disinfection were considered points of attention.

CHAPTER 3 reveals a more horizontal approach in sampling and testing to set up an annual risk based sampling plan for 1) year-around monitoring and surveillance of raw materials as

a verification of supplier selection and 2) sampling of selected end products as an overall verification of the well-functioning of Pre-Requisite Programs (PRP) and Hazard Analysis and Critical Control Points (HACCP) implemented to serve high quality and safe foods to the customers at the FSO. This type of sampling plan can deduce major non-compliances and systematic failures in "best practices" of both suppliers and the food service operation itself. As FSO are confronted with a diverse range of raw materials and served meals, a microbiological risk categorization of food products was needed to enable a focused sampling plan. The implementation of the sampling plan in the institutional kitchens of Ghent University resulted in 123 samples of raw materials and 87 samples of meal servings (focused on high risk categorized food products) which were analyzed for spoilage bacteria, hygiene indicators and foodborne pathogens. Although sampling plans are intrinsically limited in assessing the quality and safety of sampled foods, it was shown to be useful to reveal major non-compliances and opportunities to improve the FSMS in place. Points of attention deduced in the case study were control of *L. monocytogenes* in raw meat spread and raw fish as well as overall microbial quality of served sandwiches and salads.

In **CHAPTER 4**, it was demonstrated that the systematic risk based approach can also be used to evaluate the implemented control activities towards a specific pathogen, e.g. *L. monocytogenes*. This can be of interest for FSO producing and serving ready-to-eat foods for vulnerable people (e.g. a hospital institutional kitchen). In this case study the sampling plan implemented in a hospital food service operation, resulted in 49 food and 145 environmental samples, and was accompanied with a self-assessment questionnaire. As such, it was demonstrated that the use of a (self-)assessment questionnaire to evaluate the current FSMS performance and the implementation of a risk based sampling plan to verify the implemented control measures toward the presence of *L. monocytogenes* in a hospital service setting, are useful tools for a food service operation to gain more insight into their own food management system and to adjust it.

In addition to use microbiological analysis to verify the FSMS in place, microbiological analysis and more in particular challenge testing using artificially inoculated samples, were performed to provide an insight on the concern of microbiological safety in two sectors involving many small businesses operators in a B2C operation. As such, in **CHAPTER 5**, the effectiveness of heat inactivation of *Campylobacter jejuni*, *E. coli* O157:H7, *Salmonella* spp.

and *L. monocytogenes* was assessed using raw meat from various animal species (including pork, beef, lamb, chicken, turkey, horse, kangaroo and crocodile) and different types of meat and meat preparations (i.e. steak, hamburger and pitta meat strips) that were pan fried (or stir fried for the pitta meat) according to a standardized procedure as applicable in the sector of hotels, restaurants and catering in Belgium. It is generally accepted that when meat and meat preparations (including hamburgers or any other comminute meat) is subjected to a core temperature of 70 °C for 2 min or was subjected to a heat treatment equivalent to 2 min at 70 °C, it will accomplish a substantial inactivation (6 log reduction) of zoonotic pathogens and therefore renders the meat to be safe. However, on several occasions, residual survivors of the initial inoculated foodborne pathogens (4 log CFU/g) could be recovered either by enumeration (detection limit of 1 log CFU/g) or by the presence/absence testing per 25 g. Therefore, good hygiene practices at farms and slaughterhouses is needed to minimize pathogen contamination on the meat and the cold chain during further distribution and storage should be respected to prevent further multiplication. However, eating insufficient heat-treated meat will always pose a certain risk to consumers.

CHAPTER 6 focused on microbiological analysis and challenge testing to evaluate the presence and growth potential of *L. monocytogenes* in soft, semi-soft and semi-hard artisanal cheeses being sold in small delicatessen shops in the region of Ghent. Small scale on-farm cheese producers are increasingly aware on hygiene requirements during production to prevent *L. monocytogenes* contamination, but opportunities for post-contamination may occur also in delicatessen shops, during the sometimes short further ripening of cheese in the shop (at temperatures up to maximum 14°C), during storage and display in the refrigeration cabinet (at temperatures up to maximum 7°C), or during slicing when selling a piece of cheese to customers. Therefore, the growth potential of *L. monocytogenes* in three soft cheeses (one white-molded raw cow's milk cheese, one pasteurized cow's milk cheese with spicy herbs, one washed rind pasteurized cow and sheep's milk cheese) and two semi-hard artisanal cheese (one smear-ripened raw cow's milk cheese and one natural-ripened raw cow's milk cheese) was evaluated by challenge testing. The results demonstrated that if occasional post-contamination takes place during storage or handling of the cheese, *L. monocytogenes* has the potential to grow to elevated numbers

throughout a reasonably expected storage period of up to 14 days notwithstanding the presence of high numbers of indigenous lactic acid bacteria being present in these cheese samples. Therefore, current storage conditions are not able to control this hazard. Besides, due to the obtained high variability in growth potential, the food business operator may not be able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout the shelf life. To ensure for the batch of these types of cheeses to comply to EU legislation 2073/2005, it is needed for *L. monocytogenes* to be absent in 25g of cheese using a multiple sample subunit approach (n=5) at the time of production.

The knowledge acquired in the above-mentioned chapters have led to the formulation of a general discussion and final conclusions in **CHAPTER 7**, including the discussion on the systematic approach in elaboration of microbiological analysis for validation and verification of the FSMS in institutional food service operations and the use of challenge testing to evaluate handling conditions of at risk food products at small businesses selling to consumers.

SAMENVATTING

De beheersing van de microbiologische kwaliteit en veiligheid van de voedselketen berust, gedeeltelijk, op de uitvoering van microbiologische analyse van levensmiddelen (en hun productieomgeving) tijdens of na het productieproces. Een microbiologische analyse heeft echter niet enkel betrekking op de selectie van de juiste analysemethode met bijhorende technische prestatiekenmerken, maar dient ook rekening te houden met het beoogde doel dat men voor ogen heeft, de geschikte uitwerking van een bemonsteringsplan, de selectie van aangepaste microbiologische parameters en de correcte interpretatie van de testresultaten in hun context. Daarom werden deze aspecten bestudeerd in **HOOFDSTUK 1**. Op deze manier wordt een overzicht bekomen van alle relevante factoren die invloed hebben op een microbiologische analyse om de voedselveiligheid te garanderen.

Diensten die betrokken zijn bij maaltijdvoorzieningen, serveren maaltijden direct aan de consument (m.a.w. zijn betrokken in *business-to-consumer* (B2C) activiteiten). Omdat deze diensten vaak betrokken zijn in voedselgebonden uitbraken, is het belangrijk dat ze een goede beheersing van de voedselveiligheid op basis van de *Hazard Analysis and Critical Control Points* (HACCP) principes nastreven. Ze hebben echter vaak niet de nodige kennis omtrent het opzetten van bemonsteringsplannen en microbiologische analyses noch de (financiële) middelen om deze uit te voeren. Daarom werd er in **HOOFDSTUK 2, 3 en 4** geopteerd om een aanpak uit te werken voor microbiologische analyse ter validatie en verificatie van de kernactiviteiten in de beheersing van voedselveiligheid voor deze maaltijdvoorzieningen.

HOOFDSTUK 2 concentreert zich daarbij op de uitwerking en toepassing van een verticaal microbiologisch bemonsteringsplan in één van de restaurants van de dienst maaltijdvoorzieningen van de Universiteit Gent. Op deze manier werd getracht hun beheersing van de voedselveiligheid te valideren. Het bemonsteringsplan focust op monsternames doorheen het productieproces van grondstoffen tot eindproduct en werd driemaal uitgevoerd voor een productieproces met 1) een hoog risico waarbij een belegd broodje met préparé werd bereid, 2) een middelmatig risico waarbij een maaltijd werd bereid vanuit rauwe grondstoffen en 3) een laag risico waarbij een maaltijd werd geregenereerd vanuit voorgegaarde grondstoffen die enkel dienden te worden opgewarmd in hun originele verpakking (*reheating-in-bag*). Op deze manier werd een inzicht verkregen in de microbiologische contaminatie en de variabiliteit van een productieproces. Daarnaast

was het ook mogelijk om de knelpunten in de beheersing van de voedselveiligheid aan te duiden. Zo werden bijvoorbeeld regelmatig hoge aerobe kiemgetallen (> 3.9 log kve/50 cm^2) aangetroffen op de handschoenen van de werknemers en de voedselcontactoppervlakten en dit voornamelijk in de risicozones (vb. tijdens het omgaan met grondstoffen, voorbereidingsstappen). Daarom verdienen de kernactiviteiten zoals handhygiëne en reiniging en desinfectie voldoende aandacht te krijgen.

HOOFDSTUK 3 onthult een meer horizontale benadering voor microbiologische analyse met als doel een jaarlijks risico-gebaseerd bemonsteringsplan uit te werken voor 1) continue controle en toezicht van de microbiologische kwaliteit van de grondstoffen in het kader van leverancierscontrole en 2) microbiologische analyse van eindproducten ter verificatie van het functioneren van het geïmplementeerde basisvoorwaardenprogramma en de HACCP principes om zo goede kwaliteit en veiligheid van eindproducten (de geserveerde maaltijden) te garanderen naar de klanten toe. Er werd aangetoond dat dit type bemonsteringsplan in staat is om grote tekortkomingen en systematische fouten in “goede werkpraktijken” van zowel leveranciers als zichzelf als dienst maaltijdvoorziening op te sporen. Omdat maaltijdvoorzieningen gepaard gaat met een zeer gevarieerd aanbod aan grondstoffen en eindproducten, diende het bemonsteringsplan een risicokarakterisatie van de levensmiddelen op te nemen om zo een gerichte bemonstering mogelijk te maken. De uitvoering van dit bemonsteringsplan op één van de sites van de dienst maaltijdvoorzieningen van de Universiteit Gent resulteerde in 123 monsters van grondstoffen en 87 monsters van eindproducten of deelproducten hiervan waarbij geconcentreerd werd op de ‘hoog risico’ producten. Deze monsters werden vervolgens geanalyseerd op verschillende bederfflora, hygiëne-indicatoren en voedselgebonden pathogenen. Hoewel het bemonsteringsplan beperkt is in het beoordelen van de kwaliteit en veiligheid van voedingsmiddelen, was het toch in staat om grote tekortkomingen aan het licht te brengen en dus aan te duiden welke verbeteringen in de beheersing van de voedselveiligheid kunnen worden aangebracht. Zo werd bijvoorbeeld de aandacht gevestigd op het toezicht van *L. monocytogenes* in aangeleverde grondstoffen, maar ook op de algemene kwaliteit van de eigen belegde broodjes en salades.

In **HOOFDSTUK 4** werd aangetoond dat de risico gebaseerde systematische benadering ook gebruikt kan worden om de geïmplementeerde kern- en borgingsactiviteiten naar een

specifiek micro-organisme, bv. *L. monocytogenes*, te evalueren. Dit kan interessant zijn voor institutionele restaurants die maaltijden voorzien voor gevoelige bevolkingsgroepen zoals bijvoorbeeld een ziekenhuiskeuken. In deze gevalsstudie werd het bemonsteringsplan uitgevoerd in een ziekenhuis wat resulteerde in 49 voedselmonsters en 145 omgevingsmonsters. Het bemonsteringsplan werd daarbij vergezeld van een vragenlijst voor zelfbeoordeling van het voedselveiligheidsbeheerssysteem. Het werd aangetoond dat op deze manier de vragenlijst kan dienen om het huidige beheer te evalueren en het bemonsteringsplan gebruikt kan worden om de kernactiviteiten te verifiëren. Zo krijgt de institutionele keuken inzicht of de uitvoering van kernactiviteiten met betrekking tot beheersing van een bepaald microbiologische risico voldoende kan geacht worden.

Naast het uitvoeren van microbiologische analyses ter verificatie van kernactiviteiten in institutionele keukens, werden microbiologische analyses ook uitgevoerd om een inzicht te krijgen in de beheersing van de voedselveiligheid op het niveau van sectoren die vele kleine operatoren met B2C-activiteiten omvatten. Hiervoor werden voornamelijk challengetesten uitgevoerd waarbij levensmiddelen artificieel geïnoculeerd worden met het relevante micro-organisme. Zo werd in **HOOFDSTUK 5**, de efficiëntie van inactivatie van *Campylobacter jejuni, E. coli* O157:H7*, Salmonella spp.* en *L. monocytogenes* nagegaan door rauw vlees van verschillende dierlijke oorsprong (varken, rund, lam, kip, kalkoen, paard, kangoeroe en krokodil) en verschillende vleessoorten en vleesbereidingen (vb. steak, hamburger, pittareepjes) te onderwerpen aan een bakproces (of roerbakproces in geval van vleesreepjes) volgens een gestandaardiseerde procedure zoals deze wordt toegepast in hotels, restaurants en catering in België. Het is namelijk ingeburgerd dat wanneer bij bereiding van vlees in de kern een temperatuur van 70°C gedurende 2 minuten wordt aangehouden (of een equivalent hiervoor), een 6 log reductie van pathogene cellen verkregen wordt waardoor het vlees veilig is voor consumptie. Er werden echter sporadisch pathogene bacteriën zoals *L. monocytogenes* gevonden via tellingen (detectielimiet van 1 log kve/g) of detectie na aanrijking van 25g vlees, wat er op wijst dat lage aantallen overblijven van de initiële hoge aantallen (4 log kve/g). Daarom zijn goede hygiënepraktijken op de boerderij en in het slachthuis nodig om de initiële contaminatie zo laag mogelijk te houden. Daarnaast is het behoud van de koude keten tijdens verdere distributie en opslag

belangrijk om verdere uitgroei te voorkomen. Echter, het consumeren van onvoldoende doorbakken vlees zal altijd het nodige risico voor de consument met zich meebrengen.

HOOFDSTUK 6 concentreert zich op microbiologische analyse en challengetesten om de aanwezigheid en het groeipotentieel van *L. monocytogenes* te evalueren in producten zoals zachte, halfzachte en halfharde kazen die te koop worden aangeboden in de detailhandel. De ambachtelijke producenten zijn zich er zeker van bewust dat strikte hygiënemaatregelen nodig zijn tijdens de productie van hun kazen om zo besmetting met *L. monocytogenes* te voorkomen, maar de mogelijkheid bestaat ook dat nabesmetting gebeurt in de detailhandel, tijdens het verdere rijpen van de kazen in de winkel (bij een maximum temperatuur van 14°C), of tijdens de opslag en uitstalling in de toonbank (bij een maximum temperatuur van 7°C), of tijdens het versnijden van de kaas op vraag van de consument. Daarom werd met behulp van challengetesten nagegaan in hoeverre *L. monocytogenes* kan uitgroeien door nabesmetting in drie ambachtelijke zachte kazen (zoals een rauwmelkse witschimmel kaas, een gepasteuriseerde kaas met kruidige korst en een rauwmelkse roodbacterie kaas) en twee ambachtelijke halfharde kazen (zoals een rauwmelkse roodbacterie kaas en een rauwmelkse natuurlijk gerijpte kaas). De resultaten tonen aan dat als er zich een nabesmetting voordoet tijdens opslag en omgang met de kaas, *L. monocytogenes* de kans heeft om uit te groeien tot hogere aantallen tijdens een aanvaardbare bewaarperiode van 14 dagen desondanks de aanwezigheid van hoge aantallen nevenflora zoals melkzuurbacteriën. De huidige bewaaromstandigheden zijn dus niet (altijd) in staat om de groei van *L. monocytogenes* te beperken. Bovendien werd opgemerkt dat er een grote variatie is in de groeipotentieel tussen de verschillende herhalingen maar ook tussen verschillende productie-eenheden. Hierdoor kan een producent niet aantonen aan de bevoegde overheid dat het micro-organisme niet zal uitgroeien tot aantallen hoger dan 100 kve/g tijdens zijn houdbaarheidsdatum. Dus om aan te tonen dat een batch voldoet aan microbiologische criteria zoals opgelegd in de verordening (EG) Nr. 2073/2005 van de Europese Unie, moet *L. monocytogenes* na productie afwezig zijn in 25g en dit in alle monsters zoals voorgeschreven in het bemonsteringsplan.

De kennis die werd opgedaan in de bovengenoemde hoofdstukken heeft geleid tot het formuleren van enkele algemene conclusies in **HOOFDSTUK 7.** Daarnaast werd in dit hoofdstuk ook de nodige bedenkingen geformuleerd bij de verschillende stappen die nodig

zijn voor de uitwerking van een systematische aanpak voor het uitvoeren van microbiologische analyse als indicator voor de beheersing van voedselveiligheid. Tevens werden een aantal reflecties gemaakt bij de opzet en het gebruik van challengetesten voor het beoordelen van de “goede werkpraktijken” bij de bereiding en opslag van risicoproducten door kleine operatoren met B2C-activiteiten in de voedselketen.

CHAPTER 1

INTRODUCTION

1.1 Management of (microbiological) food safety

Risk management of food and feed safety lies with the European Union (EU) institutions (i.e. European Commission, European Parliament and the Council) and is covered by food and feed law at Union level (Figure 1.1).

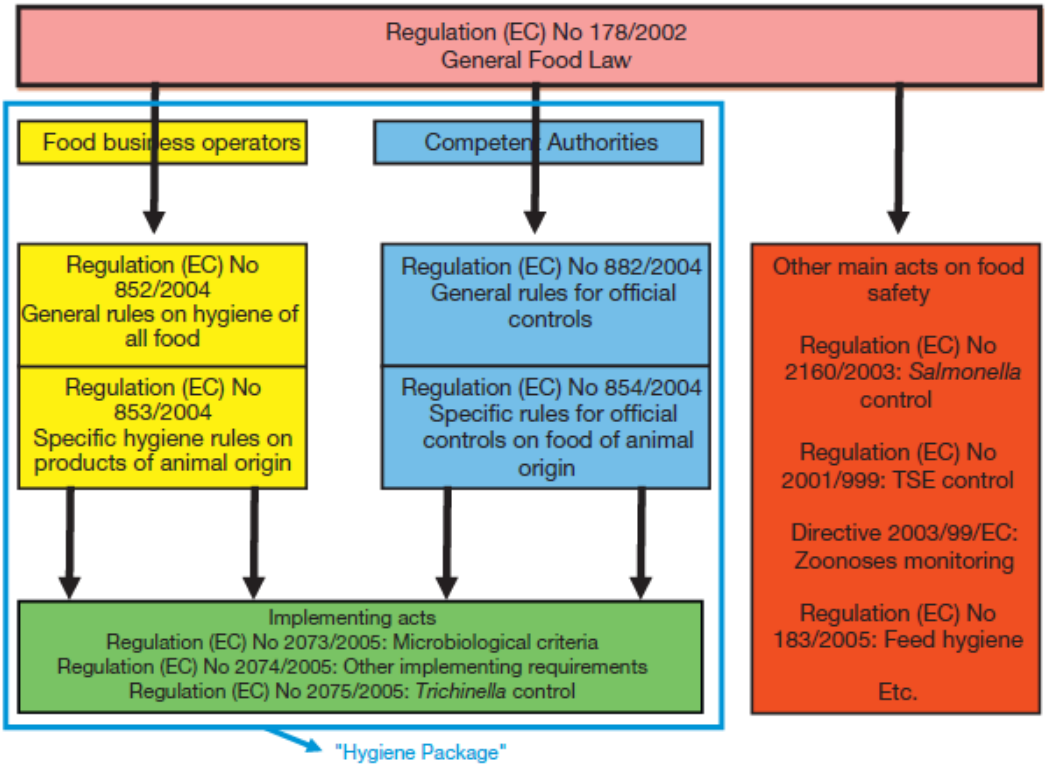


Figure 1.1 Overview of EU legislation on food safety (after Dwinger R.H. and De Smet K., 2016)

The management of microbiological food safety needs knowledge of the current situation and trends regarding the occurrence and spread of pathogens in the food chain. Therefore, member states of the EU are urged to collect data on the occurrence of zoonosis, zoonotic agents, antimicrobial resistance, animal populations and foodborne outbreaks. **Monitoring** is defined as the performance of routine microbiological analysis aimed at detecting microbiological contamination of food from which useful prevalence data may emerge (CEC, 2006). In addition to monitoring, competent authorities perform also surveillance activities. **Surveillance** is defined as the performance of routine microbiological analysis aimed at detecting microbiological contamination of food for the purpose of applying appropriate

control measures (CEC, 2006). Surveillance is mostly applied to follow-up unsatisfactory results and to evaluate implemented control measures. Management of microbiological food safety requires also from competent authorities checking the compliance of food products (i.e. batch control) in case of suspected contaminations (e.g. border controls in case of import or non-satisfactory results of inspection at food business operators (FBOs) on functionality of self-checking systems). Although the competent authority is demanded to assure the food safety by means of official controls, the general food law (i.e. Regulation EC No. 178/2002) puts the responsibility for food safety with FBOs from farm to fork (Anonymous, 2002; Albersmeier et al., 2009; Maudoux et al. 2006). Thus, an organization in the food chain needs to demonstrate its ability to control food safety hazards in order to ensure that food is safe at the time of human consumption. Therefore, FBOs need to develop, implement and maintain a **food safety management system (FSMS)** which consists of procedures based on generic Pre-Requisite Programs (PRP) (covering 'good practices' such as cleaning and disinfection, temperature control, personal hygiene, etc.) and specific structured procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP) (Anonymous, 2004b; CAC, 2003; Jacxsens et al., 2009a; Quinn & Marriott; 2002). In many countries, the company specific FSMS is also recalled a self-checking system due to the fact that the FSMS needs to be planned, executed and checked by the FBO. When non-satisfactory results are obtained on Critical Control Points (CCPs) or internal checks, acting is necessary. Based on this Deming-wheel principle, the FBO has the possibility to assess the safety of their food products and production process in a systematic manner (Jacxsens et al., 2009a; Luning et al., 2009).

1.1.1 Food safety management at the level of competent authorities

The European Food Safety Authority (EFSA) was established, following Regulation EC No. 178/2002, to provide independent scientific advice, information and risk communication in the areas of food and feed safety. To assess risks and to establish baseline values related to zoonosis and zoonotic agents, monitoring programs are established e.g. according to Directive 2003/99/EC for zoonosis (Anonymous, 2003a). As such, the member states of the EU collect data on the occurrence of zoonosis, zoonotic agents, antimicrobial resistance, animal populations and foodborne outbreaks. The overall reporting at EU level on these

monitoring programs is managed by EFSA and is published in the annual European Union Summary Reports in cooperation with the European Centre for Disease Prevention and Control (ECDC) (http://www.efsa.europa.eu/en/panels/zoonoses). The reports illustrate the evolving situation in the EU and identify trends and sources for the pathogens that cause the most common zoonotic infections in humans. If specific needs are identified by the European Commission, EFSA will develop **baseline surveys** in the EU to estimate the prevalence of specific micro-organisms in certain food products in a specific time period. The results of such a baseline survey should help to inform on the consideration of the need for additional risk management strategies. Since the establishment of EFSA in 2002, eight surveys have been carried out in the EU (http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosessurvey). The latest baseline survey estimated the EU prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods at the retail level in 2010-2011 (EFSA, 2013a; EFSA, 2014a).

In the establishment of a framework for controlling and monitoring the production, prevention and management of food safety, although mainly governed by EU Regulation, national authorities should be closely involved. As laid down in the General Food Law (Regulation EC No. 178/2002), competent authorities have to enforce the food law by monitoring and verifying that relevant requirements of the food law are fulfilled by food and feed business operators at all stages of production, processing and distribution. This food law is among others complemented by Regulation EC No. 882/2004 which defines the European Union countries as regards the organization of these controls, as well as the rules which must be respected by the national authorities responsible for carrying out these official controls (Anonymous, 2004a). As such, Regulation EC No. 882/2004 stipulates that the competent authority shall establish and implement a Multi-Annual National Control Plan (MANCP) based on risk analysis, especially with respect to their frequency of sampling and priority of food product/pathogen combination. These MANCPs will also contain procedures based on official sampling and testing for other pathogens and food products than zoonotic agents and animal food products. However, official sampling and testing is only part of the verification process. Compliance to the feed and food law has also to be verified by audits, inspections, border controls, apart from monitoring and surveillance (CEC, 2006).

In Belgium, the Federal Agency for the Safety of the Food Chain (FASFC) is the competent authority for official controls of the safety of the entire food chain, including animal health, plant health and animal welfare. The MANCP (http://www.afsca.be/about/mancp/) includes microbiological analysis of products to check their quality and safety and inspections of self-checking systems at FBOs and products at all stages of the food chain (Maudoux et al. 2006). To acquire a high level of transparency, the FASFC makes the relevant information of the official controls available to the public. It publish every year an annual report on their activities and results of the controls and inspections (http://www.afsca.be/jaarverslagen/). In addition to official controls which are planned in the monitoring program, the FASFC also perform microbiological analysis initiated by other events such as the Rapid Alert System for Food and Feed (RASFF) notifications, consumer complaints, presumptive foodborne outbreaks, actions in collaboration with other national competent authorities (Maudoux et al. 2006). Surveillance allows to gain information when a microbiological issue is identified and to identify potential causes of the problem in order to find potential solutions (ICMSF, 2011).

1.1.2 Food safety management at the level of industry associations

A food sector association or private organizations issuing a quality label or system certificate can perform or ask their members to perform microbiological analysis in the frame of monitoring food safety (i.e. microbiological and chemical food safety) or put forward specifications on hygiene and quality parameters that need to be verified on a regular basis. They can also monitor in the frame of evaluation of implemented interventions to improve the microbiological food quality or safety output (Jacxsens et al., 2010). These monitoring plans focus on hazards related to a food product and not to an individual FBO. This sectoral approach has the advantage that individual companies need to perform fewer analyses themselves. The efforts are in fact evenly distributed across the entire sector, with larger companies performing more analyses than smaller companies. In the end, more results will be available than if individual sampling was performed. In Belgium these sectoral sampling plans are often subjected to approval by the competent authority (FASFC) in order to verify if sampling fulfills some defined sampling and testing requirements for the results of the analysis to be accepted by FASFC. In the Netherlands there is an initiative called Riskplaza

(http://www.riskplaza.nl) which is also a collaborative effort from various sectors to share information about the food safety of ingredients in a common database or platform (accessible by members only). Besides controlling the compliance with existing microbiological guidelines and criteria, the obtained results of analysis can be used to collect baseline data. Food safety is or should not be a competitive issue between food businesses and monitoring of (microbiological) hazards on the level of a food sector, but helps the members of an industry association to control the costs of sampling and analysis in the framework of national or European legislation or their customers' requests. By sharing information, the overall insight in a food safety issue is increased and individual companies can position themselves within the cluster of related companies in the same 'business'.

1.1.3 Food safety management at the level of individual food business operators

As stated in the general food law, a FBO is best placed to devise a safe system for supplying food and ensuring that the food it supplies is safe (Anonymous, 2002). Therefore, FBOs in Europe are obliged to develop, implement and maintain a FSMS (Figure 1.2) to govern food safety and to prevent foodborne outbreaks (Anonymous, 2002; Anonymous, 2004b; CAC, 2003). Such a FSMS includes **Pre-Requisite Programs (PRPs)**, which are the programs related to hygiene and good working conditions in a food producing company (e.g. cleaning and disinfection, pest control, temperature control, etc.). They are tailored to the nature and size of a company and are often also recalled as 'good practices' such as 'good hygienic practices', 'good agricultural practices' or 'good manufacturing practices'. Basic good practices are regulated by Regulation EC No. 852/2004 and 853/2004 within Europe. Important is that these basic (legal) requirements are translated into a company specify PRP, according to a plan, do, check, act principle. For instance, cleaning and disinfection activities are planned (e.g. frequency, when to perform, method of cleaning and disinfection, which chemicals will be used), executed, checked (e.g. visual check, microbial sampling) and act when problems are identified.

A next level within a FSMS is **Hazard Analysis and Critical Control Points (HACCP)**, in which process specific hazards needs to be controlled. Based on the hazard analysis the food company will identify the need for CCPs as steps in the production process where hazards will be prevented, eliminated or reduced to an acceptable level. Typical CCPs are e.g.

pasteurization, fermentation and fast cooling. For those an appropriate monitoring needs to be set-up within a FSMS of the company (Jacxsens et al., 2009a; Luning et al., 2009).

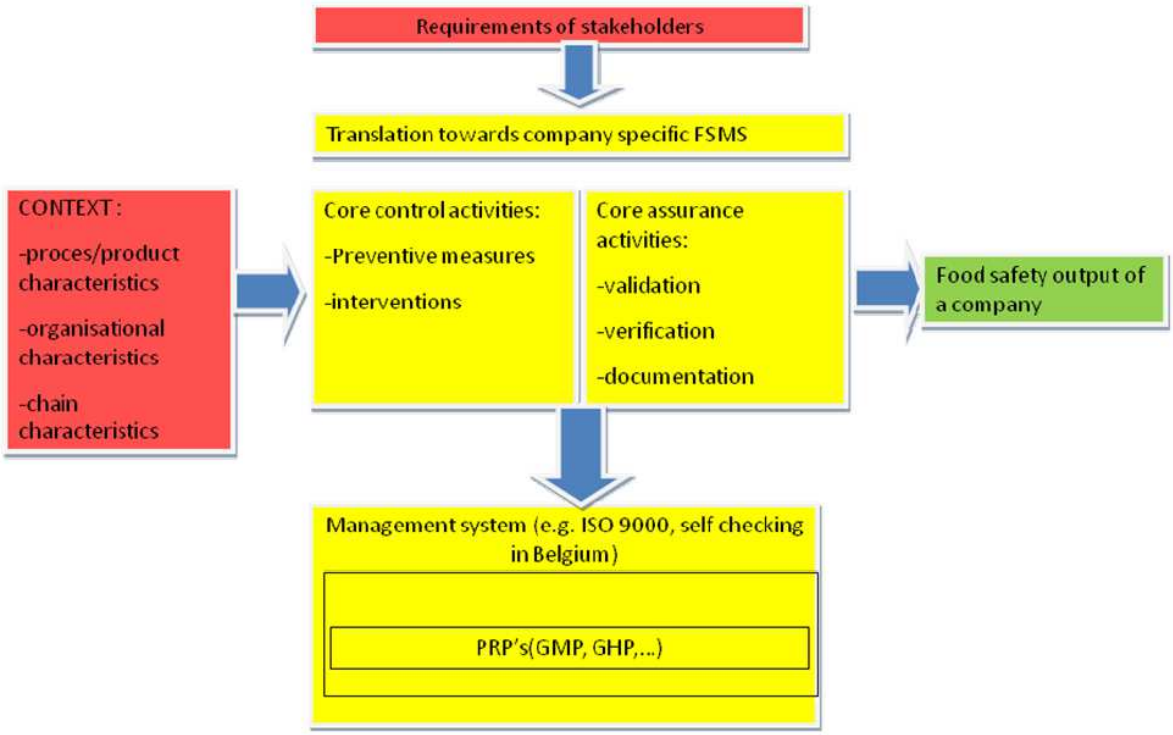


Figure 1.2 A schematic overview of a Food Safety Management System.

A FSMS or self-checking system based on PRPs and HACCP principles, is including both control and assurance activities (Figure 1.2). **Control activities** are aiming at prevention of contamination (e.g. cleaning and disinfection) and outgrowth (e.g. temperature control) or reduction (e.g. heat treatment) of a food safety hazard and are typically related to product and process controls (Luning et al., 2008; Jacxsens et al., 2009a). **Assurance activities** in a food safety management system have the objective to provide evidence (e.g. by sampling, by internal auditing) that products and processes are within set specifications.

Good food safety management should thus be based on evidence that hazards are well under control and that the interplay between initial levels of organisms, reduction, recontamination and growth is supplying a final level or prevalence of the hazard that is appropriate in a food product (Zwietering et al., 2016). Therefore, validation (which is an assurance activity) of the control activities is needed. **Validation** is defined as obtaining

evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome (CAC, 2008). Within the frame of validation, microbial sampling will play a role. For example, in a heating step where time/temperature conditions need to be defined to eliminate pathogens, microbiological sampling and testing will be a tool to evaluate if the set time/temperature conditions are strict enough. Moreover, one of the seven principles of the HACCP system is verifying whether the FSMS is functioning properly. Therefore, verification of the FSMS is needed as well as an assurance activity. **Verification** is defined as the application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended (CAC, 2008). Thus, verification assess whether the system is continuing to function as intended, i.e. has the system or the hazards associated with the food changed so that safety cannot be ensured (Buchanan & Schaffner, 2015; Zwietering et al., 2016). Therefore, food safety management by individual FBOs involves the validation and verification of the implemented system, which can be performed with microbiological analysis next to other tools such as predictive modelling, observations, etc. In addition to microbiological analysis to validate and verify the FSMS, the FBO needs to check whether foods meet the various legal or customary food safety, food hygiene or food quality standards (i.e. batch release). This demands for batch control of raw material or end products are often asked in business-to-business sales. Food safety management involves also the investigation of complaints about food, for example follow-up sampling to establish if the cause of the complaint was an isolated incident or to support root-cause analysis, and environmental sampling to check the hygiene status of food processing equipment and the manufacturing environment.

1.2 Microbiological analysis

Microbiological analysis of foods or food production environment during food production, processing, storage and preparation is an integrated part of the management of microbiological safety in the food chain. However, a microbiological analysis not only deals with the selection of the method of analysis and its performance characteristics, but also concerns the set-up of a sampling plan, selection of microbiological parameters and the

interpretation of test results and subsequent related actions. The latter aspects are highlighted in this PhD thesis.

1.2.1 Sampling: an essential tool in microbiological analysis

In order to benefit from the sampling and testing of food products and the food production environment, the sampling plan, which is an essential tool in controlling quality and safety along the food chain, must be well-planned taking into account also the intended purpose of sampling. As the interpretation of microbiological analysis are strongly dependent on the design of the sampling plan, it is important that the appropriate sampling is used for a defined application, or at least the pros and cons of a selected sampling design are considered and known.

1.2.1.1 Sampling for batch control

Despite a shift from repressive quality control (i.e. end- or finished product testing) to a more proactive food safety management based on PRP and HACCP, batch control is still being used (Zwietering et al., 2016). End product testing may be an essential additional control measure for those (limited number) of foods where PRP and HACCP may be inadequate to provide consumer protection (e.g. raw materials) or when the history of a product is unknown (e.g. at port of entry in case of import) (ICMSF, 2011; Ross et al. 2011). FBOs can use batch control with hold-and-release function if there is a reason to believe that the process is not well under control (e.g. a CCP out of control) or to prove compliance of their food to microbiological criteria set in Regulation EC No. 2073/2005 (Anonymous, 2005; Buchanan & Schaffner, 2015; Jongenburger et al., 2015; Zwietering et al., 2016). Also competent authorities will use batch control in the frame of checking compliance of food products with microbiological criteria (e.g. for acceptance at ports or other points of entry) (Jongenburger et al., 2015). As legal microbiological criteria allow to show acceptability of a food stuff to be set to the market or give guidance on acceptable functioning of the production process, sampling plans for batch control were defined as a consensus between all EU member states in order to ensure harmonized implementation. Two types of sampling plans, namely attributes and variables plans, can be used to support decisions on the acceptability of a batch of food. As attributes sampling plans need no assumptions

about underlying distributions of micro-organisms and because they are the simplest concept to control an alternative characteristic, within EU it was chosen to adopt the attributes sampling plans in the setting of microbiological criteria Regulation EC No. 2073/2005 (some examples mentioned in Table 1.1).

Table 1.1 Examples of sampling plans for batch control as adopted in Regulation EC No. 2073/2005 (from top to bottom in increasing strictness).

Micro-organism	Food category	Type	N	C	m	M
E. coli	Pre-cut fruit and vegetables (RTE)	3-class	5	2	100 cfu/g	1000 cfu/g
L. monocytogenes	RTE foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	2-class	5	0	100 cfu/g	-
L. monocytogenes	RTE foods intended for infants and RTE foods for special medical purposes	2-class	10	0	Abs. in 25g	-
Enterobacteriaceae	Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age	2-class	10	0	Abs. in 10g	-
Enterobacter sakazakii		2-class	30	0	Abs. in 10g	-

The sampling plans in the EC regulation defines the number of sample units to be tested (*n*); the analytical unit size; the analytical reference method; the microbiological threshold limits (*m* and *M*); and the maximum allowable number of sample units (*c*) yielding a positive test result, such as the presence of the organism or the number of micro-organisms above a set limit. A distinguishment can be made between the microbiological threshold limits *m* and *M*. In a two-class sampling plan good quality will be separated from non-acceptable quality or defective quality by determining whether the concentrations of micro-organisms are above (present) or below (absence) some pre-set threshold limit, denoted as the microbiological limit *m*. In a three class-class plan, this microbiological limit *m* will separate good quality from marginally acceptable quality. It is a second microbiological limit *M* which will separate unacceptable (or defective) batches from the (marginally) acceptable batches (Dahms, 2004). Thus, the sampling procedure of these attributes sampling plans involves the selection of a sample with a sample size (*n*) from the batch, the analysis of the sample, and the comparison of the results to the microbiological limit(s) in order to classify a batch of food as (marginally) acceptable or not acceptable (ICMSF, 1986). As with sampling only a

very small part of the batch is analyzed, none of the sampling plans can ensure that every item in a batch is conform to microbiological limits as set in microbiological criteria. (Jongenburger et al., 2015). For any sampling plan there is probability of accepting a batch which may be unacceptable (consumers' risk) or rejecting a batch which may be acceptable (producers' risk) (ICMSF, 1986).

1.2.1.2 Sampling for monitoring and surveillance

Knowledge of the current situation and trends regarding the occurrence and spread of pathogens in the food chain has an important contribution to make in managing food safety. Acquiring this knowledge involves the gathering of information under the terms of monitoring and surveillance. Despite the difference in terms, similar systems of data collection are often used in both monitoring and surveillance. The (big) number of samples to be tested is statistically determined and is based on the confidence level of the conclusions to be made (usually set at 95%, but could also be 90 or 99%) and need to take into account the maximum (tolerable) fraction of the food that may have been contaminated or the (estimated) prevalence of a particular hazard. To improve and co-ordinate the monitoring of zoonotic agents in the EU, some general requirements are laid down in Directive 2003/99/EC on the monitoring of zoonosis and zoonotic agents. These requirements will make it easier to compile and compare the collected data, which will also enable better contribution to risk assessment of zoonotic agents (Anonymous, 2003a).

Monitoring programs in microbiological food safety allow thus to estimate the prevalence of a particular hazard or allow to verify if the hazard is below a defined tolerable set prevalence (e.g. less than 5%, less than 1% or less than 0.1%) with a set accuracy. Depending on the objective of the monitoring program, several statistical methods exist to calculate the required sample size (Evers, 2001). Some examples on approaches taken in setting up monitoring and surveillance studies are described in the technical specification for setting up EU-baseline surveys as reported by EFSA (http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosessurvey) and by Madoux et al. (2006), which sets up sampling strategies in the framework of the Belgian MANCP.

1.2.1.3 Sampling in the framework of validation and verification of FSMS

Management of microbiological food safety is based on a good design of processes, products and procedures. Therefore, microbiological analysis are needed in the framework of validation and verification of the implemented FSMS to provide evidence on this good design.

By validation, it can be demonstrated that particular (process) control measures can adequately prevent, reduce, or eliminate specific hazards. Often, a FBO will perform sampling and testing **to validate** (new) intervention steps or implemented control measures (or considered to be implemented) in the food production process. For information on phenomena like reduction, survival, transfer and growth of microorganisms specific experiments such as challenge tests will be combined with scientific literature or predictive microbiology to validate initial assumptions of sufficient control. Also assumptions on initial levels and prevalence of microbiological contaminants in raw materials and the environment can be validated by collection of data based on sampling and testing.

If control procedures are validated and operational on a continuous basis, the overall expected food safety and food quality to be delivered can be **verified** by finished product testing (and by collection of (molecular) epidemiological information) (Figure 1.3). This finished product testing reflects then the effective integration of all control and assurance steps in the formulation and manufacturing of the food being set to the market (Buchanan & Schaffner, 2015). Verification to make sure that the FSMS is working is defined as principle six in the harmonized HACCP principles from the Codex Alimentarius (CAC, 2003). This verification includes above mentioned sampling and analysis, but can be complemented also by chemical (e.g. water content), physical (e.g. pH, a_w, temperature) or time measurements. The aim of this verification is to test the HACCP-system (i.e. check if the HACCP is followed as it is described and if it is appropriate). In addition to finished product testing, in-line testing may also be useful to verify that the preventive and control measures being designed and implemented as part of the FSMS function as intended (Buchanan & Schaffner, 2015; Zwietering et al., 2016). As such microbiological analysis can be used to verify the adequacy of performance of process controls for ensuring food safety and

(prolonged) shelf life and guarantee that the product and process meets set specifications (ICMSF, 2011).

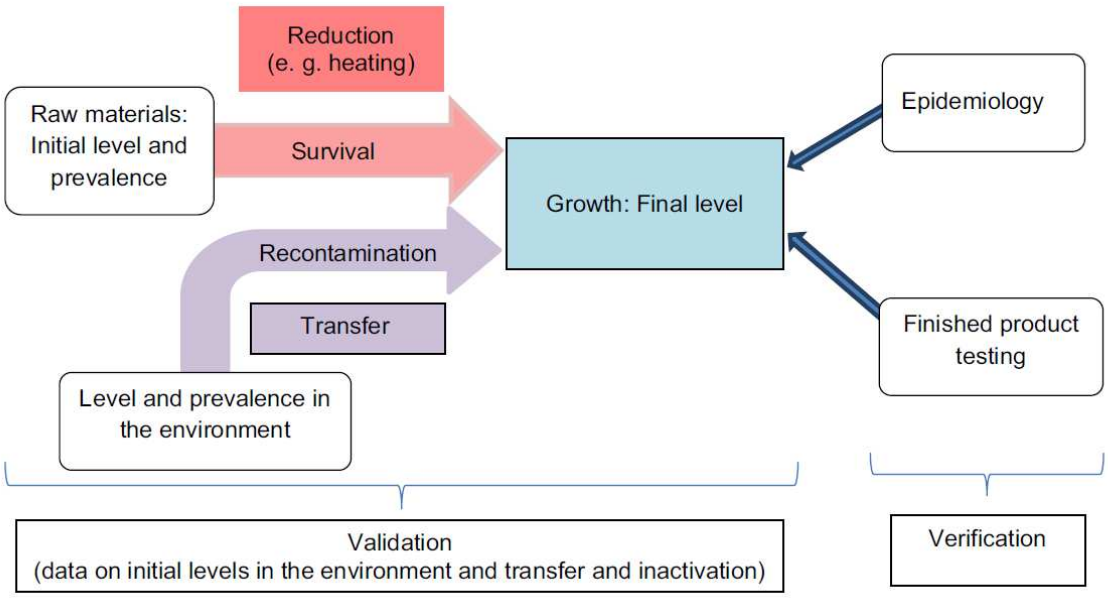


Figure 1.3 Overview of the relevant phenomena in food safety control, indicating the position of validation and verification (after Zwietering et al., 2016).

Although FBOs are obliged to verify the functioning of the HACCP-system and other hygiene control procedures (Anonymous 2004b), they should decide themselves the necessary sampling and testing frequencies. Therefore, the sample size is often determined from the point of view of what is economically acceptable and/or requirements from customers. These **convenience sampling plans** are also known as pragmatic or empirical sampling plans (CAC, 2004). The number and type of samples are mostly selected intuitively, based on the experience and knowledge of the sites most likely to detect a failure in implementation of good practices (such as cleaning and disinfection or personal hygiene) and monitoring of CCPs. This knowledge continues to increase over time, which enables to adjust the sampling plan to improve it further without increasing the analytical costs unnecessarily.

1.2.1.3.1 Microbiological Assessment Scheme (MAS)

Convenience sampling is often used in food industry. However, no procedure to systematically validate and verify the microbiological performance of a FSMS was available, until Jacxsens et al. (2009b) developed a Microbiological Assessment Scheme (MAS) procedure which enables an analysis of actual microbiological performance of a FSMS. The basic idea behind this protocol is that low numbers of micro-organisms and small variations in microbiological counts indicate an effective and well-functioning FSMS (Jacxsens et al., 2010). Therefore, this protocol, which is not a continuous monitoring plan, but rather takes a structured snapshot of the status of the performance of a FSMS can aid the FBOs in their validation of the FSMS during its set-up and aid in their verification process of the functionality of the FSMS (Jacxsens et al., 2015). This type of sampling could be rather designated as a type of *stratified* convenience sampling.

A MAS sampling plan (i) identifies critical sampling locations within the production process (e.g. raw materials, food contact surfaces, finished products), (ii) selects appropriate microbiological parameters to be analyzed, (iii) defines the sampling procedures and analytical methods, and (iv) helps in the interpretation of the results. The goal of a MAS sampling plan is to obtain insight into the maximum microbiological counts, the distribution of microbial contamination, i.e. where to find contamination in the production process (Jacxsens et al., 2009b), and into the dynamics of microbial contamination occurring as a result of the design and application of the control strategies in a FSMS. The obtained results can be evaluated in two ways. Either individual results for each analyzed parameter can be evaluated for each specific sampling location within the production process, or individual results for each analyzed parameter can also be evaluated across sampling locations within the production process. In this latter case, a microbiological safety level is attributed to each type of microbiological parameter to obtain an overall view of microbiological quality, hygiene, and the safety level of products and processes. Each microbiological parameter is given a score from 1 to 3. Level 3 is the best result (legal criteria or guidelines are met, and no improvements are needed); the current level of the FSMS is deemed high enough to cope with any hazards. Level 2 is a moderate result (obtained results exceed legal criteria or guidelines, and improvements are needed in a single control activity of the FSMS), and level 1 is a poor result (obtained results exceed legal criteria or guidelines, and improvements are

needed in multiple control activities of the FSMS). The sum of the levels of all microbiological parameters results in a Microbial Safety Level Profile (MSLP) score (Figure 1.4), which gives an indication of the actual performance level of the FSMS (Jacxsens et al, 2009b; Sampers et al, 2010). When the MSLP score is lower than the maximum achievable value, then improvement of the current FSMS is possible. Examination of the details of the MSLP results for each microbiological parameter, observations during sampling, and discussion with the quality manager of the operation may provide insight into control activities that might be picked out for a further continuous sampling plan in verification of the FSMS. Such a Microbiological Assessment Scheme generating microbiological profiles have been used in food processing companies with a relative modest food product variation, as they focus on the production of a single food stuff such as poultry meat preparations, dairy products or pork or lamb meat preparations (Jacxsens et al. 2009; Sampers et al. 2010; Osés et al., 2012a and 2012b).

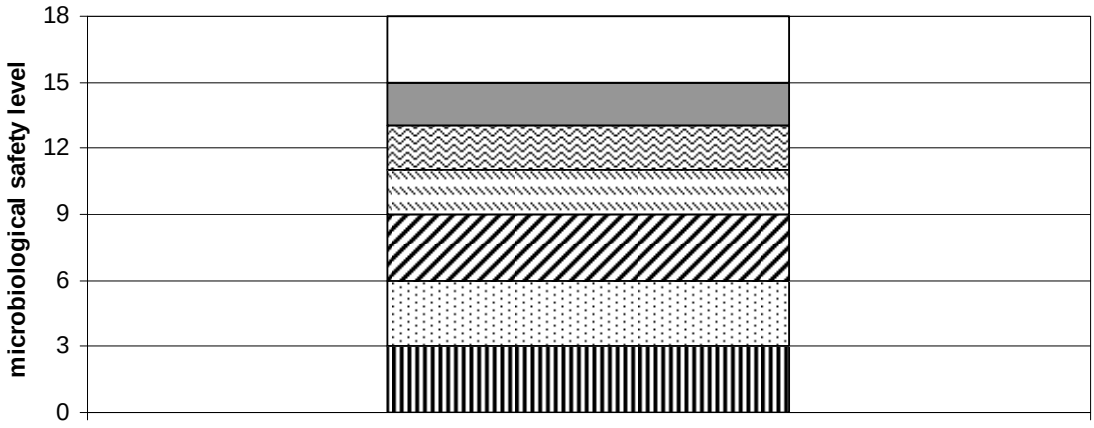


Figure 1.4 Example of a microbial safety profile. The profile is constructed by levels with ▢ total mesophilic counts, ▨ *S. aureus*, ▧ *Enterobacteriaceae*, ▨ *E. coli*, ▩ *L. monocytogenes*, ▥ *Salmonella*. ▢ indicates the remaining microbial safety level according to the maximum of 18, where improvement in the FSMS can be made (after Jacxsens et al., 2009).

1.2.1.3.2 Sampling for environmental control

Also the set-up of sampling plans in the production environment, e.g. to check cleaning and disinfection, is often based on expert knowledge (either in-house or from external consultants) and empirical knowledge based on prior test results from within the food business itself. Such in-house data is preferably put together in a database and subjected to trend observation. These environmental sampling plans are used by FBOs to assess the risk

of product contamination from the environment and may include both pathogen testing as well as hygiene indicator testing and sometimes even quality indicators such as yeasts or molds. Environmental sampling can include sampling of all types of food contact surfaces, including conveyer belts, equipment, utensils but also hands of food handlers to verify whether cleaning and disinfection has been executed properly or protective high care zones function as needed. More elaborated environmental sampling may be set up to investigate a source of contamination after a complaint of foodborne illness, deviating quality or non-compliance to set specifications. Environmental sampling may in particular be helpful in root-cause analysis in order to implement corrective actions (ICMSF, 2002; Pappelbaum et al., 2008; Tompkin, 2004). As it is not intended to assess the probability that a defined batch of food has been contaminated, such programs are not statistically designed sampling plans. Contrary, environmental sampling is based on prior experience and familiarity with the given processing conditions. Environmental sampling plans normally involve an established, routine sampling plan with a defined number of samples and places. In that case, the data can be used to detect trends indicating a potential loss of control and to enable timely corrective actions (Tompkin, 2002). If a non-compliance takes place or another unforeseen event happens, the frequency of sampling can be increased to more intensive sampling (ICMSF, 2002).

It is important in case of convenience sampling at the level of a FBO, no matter whether random or stratified convenience sampling was used, to be aware about the limitations and potential bias that might occur due to convenience sampling. This aspect should also be discussed in making conclusions on the absence (or presence) of biological hazards in food (or the food production environment) based on convenience sampling.

1.2.2 The selection of microbiological parameters

The specific micro-organisms that should be considered for microbiological analysis is dependent on the objectives of the microbiological analysis, the microbiological ecology of the food (or the food production environment), the stage in the food chain where the sample is taken and the (reasonably) foreseen conditions of further processing, storage, distribution and use.

1.2.2.1 Quality indicators

Quality indicators that can be used to assess the overall quality of food products are total viable count (TVC), lactic acid bacteria (LAB), yeast and molds (Lues and Van Tonder, 2007; Jacxsens et al., 2003). Unsatisfactory results of these food spoilage micro-organisms represent, if recommended storage conditions have been respected, less qualitative products which may cause early spoilage and unacceptable sensorial quality, but in general will not cause harm to humans. However, the use of TVC as an indicator of overall quality is debatable for some products. It is for example clear that in fermented products, TVC will always be high due to presence of high levels of lactic acid bacteria (either as starter culture or part of the natural microbiota). Also it is not recommended to use TVC as an indicator of overall quality of fresh produce, because those food products often have high and variable microbiological contamination due to contact with the environment pre-harvest (soil, water) and may be prone to cross-contamination during washing and mixing (Holvoet et al., 2012; Olaimat and Holley, 2012; Tzschoppe et al., 2012). Because TVC is to a lesser extent related to sensorial quality, it is recommended to combine TVC counts always with a judgement of the sensorial quality. For example, deterioration of fresh produce is rather to be judged visually, with notable wilted or discolored leaves due to physiological processes such as anaerobic respiration and enzymatic browning (Caponigro et al., 2010).

1.2.2.2 Hygiene indicators

In addition to quality indicators, hygiene indicators (e.g. *Enterobacteriaceae, E. coli*, *coliforms*) are often chosen in microbiological analysis because they are relatively quick and simple to detect. They can be used to point out failure to comply with general acknowledged codes of hygiene and best practices in food production, that temperature abuse may have occurred and some indicator organism also provide evidence of human or animal fecal contamination (Bayliss et al., 2011). However, *Enterobacteriaceae* provide a good indicator of overall good manufacturing practice (GMP) on the day of production but not throughout the shelf life or at the end of shelf life of some (refrigerated perishable) products. This is due the ability of some *Enterobacteriaceae* to multiply in certain foods, even during chilled storage, which can make interpretation of results more difficult because the numbers present may not always reflect the initial level of contamination (Bayliss et al.,

2011). If analysis of hygiene at the end of shelf life is still preferred, it is better to determine the numbers of *E. coli* as this species is mesophilic (i.e. no growth possible when temperature is <8°C). *E. coli* is also a good indicator of fecal contamination. In addition, high numbers of *E. coli* provide evidence of increased likelihood of finding closely ecologically related enteric pathogens (Bayliss and Petitt, 1997; Mossel, 1982).

1.2.2.3 Foodborne pathogens

When pathogenic bacteria are present in food products, they can cause foodborne diseases. The characteristics for growth and pathogenicity of the most important food infectants (i.e. *L. monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and *E. coli* O157) and food intoxicants (i.e. *B. cereus*, *Cl. perfringens* and *S. aureus*) are listed in Table 1.2. The annual reports of the biological monitoring unit of EFSA and ECDC (http://www.efsa.europa.eu/en/zoonosesscdocs/zoonosescomsumrep) give an overview of reported individual cases of human zoonotic diseases as well of collective foodborne outbreaks. The annual report provides also information about the prevalence and trends of zoonotic micro-organisms in a broad range of food products. Similar information at the national level can be found in the annual activity reports of each EU member states' food safety agency. The information accessible in these reports may help in the selection of the relevant foodborne pathogens to be taken up for microbiological analysis for particular food stuffs. The latest annual report of EFSA, including data from 2009 up to end of 2013, was used to make an overview of the trends in reported zoonotic infections (i.e. reported notification rates) for both the EU and Belgium which is shown in Table 1.3 (EFSA, 2015). The reported collective foodborne outbreaks involving two or more persons in Belgium, as described in the annual activity reports of the FASFC, for the period 2009-2014 are shown in Table 1.4 (FAVV, 2010-2015). It can be concluded, according to these data, that *Campylobacter* infections are the most commonly reported zoonosis in the EU and Belgium, followed by *Salmonella* infections. After several years of an increasing EU trend, the human campylobacteriosis notification rate seems to have stabilized in the EU, however in Belgium there is still an increase in campylobacteriosis going on.

Table 1.2. The most important food pathogens and their taxonomic situation, morphology, growth characteristics, infective dose and (known) virulence genes.

	Family	Morphology		Growth characteristics			Infective dose	Known virulence genes
				T (°C)	pH	Aw	**(cfu)**	
L. monocytogenes	Listeriaceae	G^+	motile rod	0-45	4.4-9.4	0.92	10^3-10^6	*inlA, inlB, prfA, ActA, ami, OpuC operon, prfA*
Salmonella spp.	Enterobacteriaceae	G^-	motile rod	8-45	4.4-9.0	0.95	10-10^6	*invA, spiA, pagC, msgA, sipB, prgH, spaN, orgA, sitC, sifA, sopB and lpfC*
Campylobacter jejuni	Campylobacteraceae	G^-	motile spiril	32-45	4.9-9.0	0.99	500 - 10^6	*flaA, flaB, cdtA, cdtB, cdtC, cdtABC, virB11, cj0588*
E. coli O157	Enterobacteriaceae	G^-	motile rod	8-45	4.4-9.0	0.95	10-10^3	*fliC, stx1, stx2, eae, rfbE, hlyA*
B. cereus	Bacillaceae	G^+	Spore-forming toxin producing rod	4-55	4.5-9.5	0.91	> 10^5/g	*ces, hblA, hblD, hblC, nheA,nheB, nheC, bceT, entFM, cytK*
Clostridium perfringens	Clostridiaceae	G^+	Spore-forming toxin producing rod	10-54	5.1-9.7	0.93	> 10^6/g	*cpe, plc, cpb, cpb2, etx, iap, ibp*
S. aureus	Staphylococcaceae	G^+	Toxin producing coc	6-48	4.2-9.3	0.85	> 10^5/g 1.0 µg (toxin)	*Sea, seb, sec, sed, see, seg, she, sei*

Table 1.3. Reported cases and notification rates per 100,000 of human food infections in the EU and Belgium during the period 2009–2013 (EFSA, 2015).

		2009		2010		2011		2012		2013	
		Cases	**Rate**	**Cases**	**Rate**	**Cases**	**Rate**	**Cases**	**Rate**	**Cases**	**Rate**
EU	*Campylobacter jejuni/coli*	201711	62.80	215397	67.00	223998	69.00	214316	65.90	214779	64.80
	Salmonella enterica.	110179	24.00	101589	22.10	96682	20.90	90883	22.10	82694	20.40
	Yersinica enterocolitica	7578	2.46	6815	2.19	7002	2.23	6506	1.98	6471	1.92
	E. coli O157 and non O157	3580	0.98	3656	1.00	9487	2.58	5680	1.50	6043	1.59
	Listeria monocytogenes	1675	0.37	1663	0.37	1515	0.33	1644	0.41	1763	0.44
Belgium*	*Campylobacter jejuni/coli*	5697	-	6047	-	7716	-	6607	-	8148	-
	Salmonella enterica.	3113	-	3169	-	3177	-	3101	-	2528	-
	Yersinica enterocolitica	238	-	216	-	214	-	256	-	350	-
	E. coli O157 and non O157	96	-	84	-	100	-	105	-	117	-
	Listeria monocytogenes	58	-	40	0.37	70	-	83	0.75	66	0.59

*Data based on sentinel surveillance, therefore no information on the estimated coverage was available. Thus, notification rate cannot be estimated.

Table 1.4 Reported collective foodborne outbreaks (FTI) in Belgium during the period 2009-2014. (FAVV, 2010-2015)

	2009			2010			2011			2012			2013			2014		
	Number of FTI	Number of ill	Number of hospitalized	Number of FTI	Number of ill	Number of hospitalized	Number of FTI	Number of ill	Number of hospitalized	Number of FTI	Number of ill	Number of hospitalized	Number of FTI	Number of ill	Number of hospitalized	Number of FTI	Number of ill	Number of hospitalized
Bacillus cereus	4	53	1	2	11	0	8	87	9	2	24	-	4	30	0	11	46	0
Campylobacter	-	-	-	2	4	0	5	103	1	1	2	-	9	45	11	1	2	0
Clostridium perfringens	4	43	1	-	-	-	-	-	-	-	-	-	2	88	0	1	17	1
Listeria monocytogenes	2	4	2	-	-	-	1	11	11	1	16	7	2	4	0	1	2	1
Norovirus	7	91	0	15	429	25	2	13	0	9	98	4	1	20	5	5	275	0
Salmonella spp.	5	31	3	4	48	14	2	7	2	5	41	3	10	33	15	5	80	5
Shigella spp.	1	58	1	-	-	-	1	37	2	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	2	10	0	-	-	-	2	7	0	2	4	1	4	59	0	3	36	11
E. coli O157 and non O157	-	-	-	2	6	3	3	8	6	5	57	23	10	41	25	1	2	1
Co-infection*	-	-	-	4	409	62	3	189	4	5	42	-	3	31	2	1	3	0
Others and unknown	71	564	12	31	439	9	254	1077	22	300	1166	32	266	961	36	341	1326	45
Total	**96**	**854**	**20**	**60**	**1346**	**113**	**281**	**1539**	**57**	**330**	**1450**	**70**	**311**	**1312**	**94**	**370**	**1789**	**64**

*FTI with more than one causative micro-organism or type of toxin

Infections caused by *L. monocytogenes* and human pathogenic verotoxin producing *E. coli* (i.e. *E. coli* that carry verotoxin genes *vt1* or *vt2* and *eae* gene and are of a recognized pathogenic serotype) are rather limited, but these infections pose a significant risk because of the severity of the complications and the high mortality rate (EFSA, 2015).

1.2.3 Interpretation of microbiological analysis: the use of microbiological criteria

Microbiological analysis inherently contains great variability due to the heterogeneous distribution of micro-organisms in food products but also due to the biological character of the determining parameter and the performance characteristics of the microbiological methods. It is therefore essential to interpret the results of microbiological analysis in their context. As a microbiological analysis is frequently performed to reach a decision on compliance to set criteria or to make a judgement on food quality to share between food business and competent authority or from business-to-business in supplier-customer relationship, it is necessary to set harmonized threshold limits to differentiate acceptable from unacceptable products or processes. However, microbiological analysis may also be used to gather background information (e.g. baseline data) which does not necessarily involve comparison to set threshold limits.

Microbiological limits that include analytical methods and sampling plans are defined as microbiological criteria (ICMSF, 2011). Therefore, if microbiological analyses are performed for making decisions, one can apply existing microbiological criteria. The Codex Alimentarius defines a microbiological criterion as a risk management metric which indicates the acceptability of a food, or the performance of either a process or a food safety control system following the outcome of sampling and testing for microorganisms, their toxins/metabolites or markers associated with pathogenicity or other traits at a specified point of the food chain (CAC, 2013b). Microbiological criteria may be established for overall or sanitary quality as well as safety concerns and are used in setting legal microbiological standards, guidelines and purchase specifications (CAC, 1997; ICMSF, 2011). These microbiological criteria, and in particular the stated threshold limits mentioned are inevitably prone to debate. Nevertheless, the setting of microbiological criteria and

threshold limits to distinguish between acceptable and not acceptable food stuffs (or processes) is necessary and can be very useful for the food industry when well applied. Depending on the origin of the microbiological criteria, a differentiation can be made between standard, guideline and specification (ICMSF, 2011).

1.2.3.1 Microbiological standards

A microbiological standard is defined by the International Commission on Microbiological Specification for Foods (ICMSF) as a microbiological criterion contained in international, national and regional laws and regulations (ICMSF, 2011). Examples of microbiological standards are the food safety criteria and process hygiene criteria laid down in Regulation EC No. 2073/2005 which are used to verify implementation of general and specific hygiene measures mentioned in Regulation EC No. 852/2004. Food safety criteria define the acceptability of a product or a batch of foodstuff placed on the market. Exceeding food safety criteria may lead to product recalls and potentially punitive actions. It should be clear that if a batch does not comply with the microbiological criterion, the batch should not be subjected to repeat testing to confirm (or reject) the prior result obtained, unless the criterion specifies otherwise or unless this is needed for investigational purposes. The batch should neither redefined by breaking the batch into sub-batches to retest each of these (CAC 2013b; ICMSF, 2002). Regulation (EC) No 2073/2005 also sets down process hygiene criteria to judge the acceptable functioning of the production process. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law.

1.2.3.2 Microbiological guidelines

A microbiological guideline is defined by ICMSF as an internal, advisory criterion established by a processor, a trade association or sometimes governments (ICMSF, 2011). The term guidelines is often used if microbiological threshold limits are defined for particular food and microbiological parameter combinations but no actual sampling plan (n, c) is defined neither a method for analysis. Failure to meet guidelines serves as an alert to the processor. In case there is still an action defined when the threshold limit is exceeded, one may use the

term “action limit”. In Belgium, the food safety agency has defined and published on its website a list of action limits (http://www.favv-afsca.fgov.be/thematischepublicaties/inventaris-acties.asp). These type of microbiological guidelines are also often used to interpret results of in-process samples from food products or production environment samples tested for pathogens or hygiene indicators. Often no wide-spread accepted microbiological guidelines to assess results of environmental sampling (in particular for hygiene indicators) are available. As such, convenience sampling is usually first executed to collect baseline data under conditions when the production facility is considered under control in order to set in-house threshold limits. A shortlist of a number of booklets or reports with microbiological criteria or guidelines are provided in Table 1.5. Results obtained from testing against microbiological guidelines assist in trend analysis. In that case, results that deviate significantly from the trend may indicate a tendency towards a situation which is out of control and highlights the need for attention before control is lost.

1.2.3.3 Microbiological specifications

A microbiological specification (i.e. purchase specification) is defined by ICMSF as an agreement between the vendor and the buyer of a product as a basis for sale (ICMSF, 2011). These specifications may include pathogens, indicator organisms or spoilage organisms and can be considered as “license to sell” and fit into commercial agreements. Failure of the vendor to meet specifications can be used a basis for product rejection, even if they are not hazardous or unwholesome at the time of testing. Products not complying with specifications should be investigated to determine the cause. It is often noticed that microbiological specifications set in business-to-business trade are more stringent than microbiological standards or action limits set by national competent authorities. In Table 1.5 the microbiological criteria compiled by the “Federation des entreprises du commerce et de la distribution” in France, is an example of microbiological specifications.

Table 1.5. Microbiological criteria established by processors, trade associations or governments.

Title	Compiled by	Category	Link
Microorganisms in Foods 2. Sampling For Microbiological Analysis: Principles and Specific Applications	International Commission on Microbiological Specifications for Foods.	All types	http://www.icmsf.org/pdf/icmsf2.pdf
Microorganisms in Foods 8. Use of Data for Assessing Process Control and Product Acceptance	International Commission on Microbiological Specifications for Foods.	All types	ISBN 978-1-4419-9373-1
Guidelines for the Microbiological Examination of Ready-To-Eat Foods	Food Standards Australia New Zealand	Ready-to-eat foods	http://www.foodstandards.gov.au/publications/pages/guidelinesformicrobi1306.aspx
Microbiological Guidelines for Ready-to-eat Foods	Hong Kong Food and Environmental Hygiene Department. Centre for Food Safety	Ready-to-eat foods	http://blpd.dss.go.th/micro/ready.pdf
Guidance Note No.3: Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market	Food Safety Authority of Ireland	Ready-to-eat foods	https://www.fsai.ie/food_businesses/micro_criteria/guideline_micro_criteria.html
Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market	Health Protection Agency, UK	Ready-to-eat foods	https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/363146/Guidelines_for_assessing_the_microbiological_safety_of_ready-to-eat_foods_on_the_market.pdf
Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale	PHLS advisory committee, UK	Ready-to-eat foods	Commun Dis Public Health 2000; 3: 163-7
La qualité microbiologique des aliments: Maîtrise et critères	Centre national d'études et de recommandations sur la nutrition et l'alimentation (CNERNA)- Centre national de la recherche scientifique (CNRS), France	All types	ISBN 2-84054-040-1
Critères microbiologiques applicables à partir de 2015 aux marques de distributeurs, marques premiers prix et matières premières dans leur conditionnement initial industriel	Federation des entreprises du commerce et de la distribution en France	All types	http://www.fcd.fr/documentation/index/annee/2015/mois/1
Sectorial guides and Codes of Practice	Belgian Federal Agency for the Safety of the Food Chain	All types	http://www.afsca.be/autocontrole-nl/sectorspecifieketools/
Microbiologische richtwaarden en wettelijke microbiologische criteria	Lab Food Microbiology and Food Preservation, Ghent University, Belgium	All types	ISBN 978-90-5989-385-6
Inhoudelijke criteria bij het opstellen of herzien van een hygiënecode	Voedsel en Waren Autoriteit, Netherlands	Food products which undergo reduction step	https://www.nvwa.nl/zoekresultaten?zoekterm=Inhoudelijke+criteria+bij+het+opstellen&domein=S_nvwa_documenten&sort=relevance
Microbiologische criteria, Praktijkgids Waar & Wet	WFC Food Safety, Netherlands	All types	ISBN 978-90-12-39247-1

Overall in any microbiological criterion (legal standard, guideline or specification) it is important that they are set for these food and micro-organism combinations where i) it is expected to improve the degree of food safety or food quality for the consumer (and no other effective tools are available), ii) their application is practical, iii) the microbiological parameters involved are widely accepted and relevant to a particular food (and its conditions of production, processing, or distribution) and iv) the objective of microbiological analysis is fully understood and supported by the stakeholders involved (or both parties involved in the agreement of specifications).

1.3 Microbiological Analysis for ensuring safety in the food supply chain

Ensuring the safety of foods in the supply chain is the major objective of food safety management. As microbiological analysis play a major role into this, various applications using microbiological analysis for validation and verification of the food safety management system are described in the current PhD thesis. Some background on the main challenges and available guidelines for using microbiological analysis in ensuring safety in the food supply chain that will be further elaborated in the research chapters of the PhD thesis is highlighted below.

1.3.1 Sampling and testing for ensuring safety in food service operations

Food processing companies have in general a relative moderate variety of food products and complex production processes in their scope and worked out (extensive) environmental and product testing programs in the frame of verification of their FSMS, recalled as stratified sampling plans (Gonzàlez-Miret et al., 2001; Jacxsens et al., 2015, Luning et al., 2015; Osimani et al., 2013). However, the application of microbiological testing programs in food service operations (FSO) is rather exceptional and still lags behind (Luning et al., 2013). FSO are those businesses, institutions, and companies responsible for any meal prepared outside the home (e.g. restaurants, school and hospital cafeterias, catering operations). Many FSO are non-profit institutional organizations with a restricted budget allocated to sampling and microbiological analysis. Therefore, to verify their FSMS, food service operations rely on the

sampling of the competent authority, which is occasionally performed in the framework of official inspection and monitoring programs. Food service operations are business-to-consumer organizations and although being prone to official controls, they receive less pressure from customers or buyers than food processing companies from their customers (often high demanding retailers) to demonstrate the performance of their FSMS. However, in FSO various types of often raw (at-risk) materials are used and a wide variety of final products are served, including hot meals, cold sandwiches, and salads. This multitude of products, processes and personnel involved makes it very important to have a well-functioning FSMS to assure food safety as requested from all food business operations in Regulation (EC) 852/2004 on the hygiene of foodstuffs.

The lack of good food safety management in FSO may result into microbiological contamination and growth due to contaminated ingredients, dirty food contact materials, poor personnel hygiene practices, inappropriate storage temperatures and insufficient cooking (Hertzman and Barrash, 2007; Jones et al., 2008a and 2008b; Käferstein, 2003). This may result in a foodborne outbreak (EFSA, 2015; Lianou and Sofos, 2007; Tuominen and Maijala, 2009). Indeed, FSO with a history of association with foodborne outbreaks or consumer complaints about food safety issues have significantly more frequent problems with personnel hygiene and inadequate raw material storage than do other kitchens (Tuominen and Maijala; 2009). In addition, food handlers were epidemiologically linked to 80% of the norovirus outbreaks reported in Belgium (Baert et al., 2009) and food handlers' malpractices contributed to 97% of foodborne illnesses associated with FSO (Worsfold and Griffith, 2003).

The application of microbiological verification testing programs in FSO is also lagging behind due to important differences between the food industry and FSO at the level of organization (e.g. staff turnover, structure and size), technology (e.g. capacity for analysis, level of automation) and production (e.g. product variation, process variation) (Doménech et al., 2011). This ensures that microbiological verification testing programs in FSO need flexibility in their application, which challenges the set-up and implementation of a sampling plan (Buchanan and Schaffner, 2015). Besides, in Belgium no guidelines for the elaboration of a sampling plan are taken up in the self-checking guide for the sector of food service operations and health care institutions (FAVV, 2006a; FAVV 2008a). Therefore, if quality

managers in FSO perform microbiological analysis in the framework of self-checking, they usually select food products and sampling frequencies on ad hoc basis (Luning et al., 2013). They also tend to focus on end products such as served hot meals or sandwiches. However, apart from end product testing, it is also interesting to obtain information on the microbiological quality and safety of incoming raw materials (to contribute to the verification of supplier selection) and/or half fabricates (to verify the well-functioning of adherence to procedures). These observations were the main drivers to set-up research as described in **CHAPTER 2 and 3** of the current PhD thesis.

1.3.2 Challenge testing for assessing the growth potential of *L. monocytogenes*

Listeria monocytogenes is the main pathogen of concern in ready-to-eat foods with prolonged shelf life under refrigeration. It is a Gram-positive bacterium and it is in comparison to Gram-negative zoonotic pathogens (e.g. pathogenic *E. coli*, *Salmonella* spp., *Campylobacter*) more resistant towards unfavorable preservation factors. The pathogen is known to be cold tolerant, which allows it to grow (slow) under refrigeration (Table 1.2). Therefore, *L. monocytogenes* is a parameter of interest in preserved products (whether or not heat treated) with a long shelf in the refrigerator (e.g. smoked fish, soft and semi-soft cheeses, ready-to-eat meat products, etc.) and to a lesser extent, in fresh foods (vegetables/ meat/ fish) with a short shelf life (up to 5 days) (EFSA, 2015; Lianou and Sofos, 2007). Infections caused by *L. monocytogenes* can cause among others bacteremia, septicemia, meningitis, but also spontaneous abortion and neonatal infections in pregnant women. Luckily, listeriosis infections are rather limited, however an increasing trend of listeriosis in the EU over the period 2009-2013 is observed (Table 1.3)(EFSA, 2015). There is also an increase in reported cases of listeriosis reported in Belgium in 2014 versus the previous years (Institute of Public Health, Brussels, personal communication). The cause of this increase is at the moment not yet clear, but leads to a constant attention from the EU member states towards this pathogenic germ. It has been reported that a growing segment of the population is more susceptible to the risk of listeriosis, i.e. adult aged ≥ 65 years or immuno-suppressed persons such as cancer patients (Scallan et al. 2015; Goulet et al. 2012). Besides an increasing number of at risk products for *L. monocytogenes* (ready-to-eat products with a long shelf in the refrigerator) are being marketed and consumed, also the

demand for food products with less salt is increasing, which is attributed to an increasing awareness of the society considering nutrition and health. However, salt is an important factor in food preservation (reduction of a_w-value) causing growth inhibition of *L. monocytogenes*. As the pathogen may occasionally be present in low levels in these type of refrigerated food products with extended shelf life, the FBO should be able to control and restrict the growth during the shelf-life (FAO/WHO, 2004).

To ensure food safety of these at risk food products, Annex II of Regulation EC No. 2073/2005 specifies that FBOs manufacturing these type of products should obtain knowledge on the growth of *L. monocytogenes* in the products during shelf-life under reasonably foreseeable storage conditions. An estimate of the growth potential of *L. monocytogenes* during storage can be based on specifications of physico-chemical characteristics of the product and available scientific data from literature (Anonymous, 2005). However, the growth potential in the product is preferably assessed through "challenge tests" where the pathogen is artificially inoculated and monitored on the product in the lab by simulating the expected conditions during storage, during distribution and during storage at the consumer's home. Guidelines for carrying out challenge tests are issued by the EU Reference Laboratory for *Listeria monocytogenes* (EU CRL, 2014). As prescribed in these EU guidelines, the growth potential can be obtained by calculating the difference between the median concentration of *L. monocytogenes* of the three replicates at the end of storage and the median concentration of *L. monocytogenes* of the three replicates at the start of the challenge. On the other hand, the growth potential can also be calculated by predictive models using a validated maximum specific growth rate. Assessing the growth potential in the frame of Regulation EC No. 2073/2005, allows to classify a food product into the category "RTE foods able to support the growth of *L. monocytogenes*" or "RTE foods unable to support the growth of *L. monocytogenes*", or also allows to more accurately quantify the behavior of *L. monocytogenes* in the food product under consideration (EU CRL, 2014). This information is important for both the food business operator and the competent authority to determine which threshold limit is applicable (whether tolerance for *L. monocytogenes* up to 100 CFU/g or rather absence per 25 gram) when putting these RTE foods to the market under set shelf life conditions. Nevertheless the protocol of challenge testing is not yet a set protocol and still prone to debate (Alvarez-

Ordonez et al., 2014). As the factors impacting on the behavior of *L. monocytogenes* in a non-standardized traditional product such as soft and semi-hard farm made cheese needs further study, this was the main driver to assess the behavior of *L. monocytogenes* in a number of Belgian cheeses and at the same time scientifically assess the modalities of the above mentioned EU CRL challenge testing recommendations **(CHAPTER 6).**

1.3.3 Inactivation studies to validate the efficacy of heat treatment of foods

A heat treatment plays an important role in food processing and food preparation to reduce the number of (pathogenic) micro-organisms and ensure safety of foods. As the destruction of micro-organisms depends on the heating time and heating temperature, it is important to know the required time-temperature combination for inactivation. The traditional approach uses two parameters, namely the D-value and the z-value, to quantify this time-temperature combination (Bean et al., 2012). The definition of related heat processing variables used in assessing the lethality of a thermal treatment is provided in Table 1.6 and further explained.

Table 1.6 Definitions of related heat processing variables.

D-value	(i.e. decimal reduction time) is the time necessary at a specific temperature to reduce the number of organisms to 1/10 of the original value. It is thus the time required for a log-cycle reduction in the number of micro-organisms.
z-value	(i.e. thermal resistance) is a constant value and is defined as the number of degrees Celsius required to bring about a ten-fold change in the decimal reduction time (D)
L	i.e. lethal rate, the time needed to achieve an equivalent heat treatment, compared to T_{ref} at a different temperature.
P-value	(i.e. process lethality value) is a process value used to express the lethality

The D-value is determined at a specific temperature; hence the D_T notation. Although it is a simplification, in the scientific underpinning of heat treatments one usually estimates the heating time required at a certain temperature to achieve a certain number of log reduction by a deterministic calculation multiplying the D_T-value of the target organism under

consideration with the number of required reductions. As *L. monocytogenes* is known to be the most heat resistant vegetative pathogen, this is usually the main target organism for determining whether a heat treatment is sufficient to eliminate the 'big four' zoonotic non-sporeforming pathogens (*Salmonella, Campylobacter jejuni/coli*, pathogenic *E. coli* and *L. monocytogenes)*. For example, *L. monocytogenes* has an average D_{70} = 0.27 min (i.e. D-value at 70°C). Throughout the years, it has been established that a 6 log reduction of *L. monocytogenes* has been sufficient to obtain safe foods. Thus, to obtain a 6-log reduction, a heating time of 1.62 min (i.e. 0.27 min * 6) at 70°C will be sufficient. However, due to strain variability, impact of food product composition etc., a safety margin is applied. Therefore, it is generally accepted that a heating time of 2 min at 70°C is sufficient to have a 6-log reduction of *L. monocytogenes*. The 2 min at 70°C has been referred to in food processing and preparation as a 'safe harbor' (ILSI, 2012). The z-value enables the calculation of the D-value at different temperatures. For example, following the definition of a z-value and a z=7.5°C, 0.27 minutes at 70°C will give the same pathogen log reduction as 2.7 minutes at 62.5°C (=70°C-7.5°C). Although, this deterministic D-/z- approach is fairly rudimentary, it is widely used in the food industry as a generally accepted and practical system. However, this D-/z- approach can only be used for isothermal heating processes. If the heating process is non-isothermal the inactivation can be approximated by summating the lethal rates (*L*) between the measuring intervals (Gaze, 2006). The lethal rate for every interval is calculated with following formula:

$$L = t \cdot 10^{\frac{(T - T_{Tref})}{z}}$$

with T_{ref} the reference temperature (e.g. 70°C), *T* the measured temperature (e.g. 60°C), and *t* the heating time. As an example, if *z* is 7.5°C, then 1 minute at 60°C corresponds to 0.046 min at 70°C. Thus for a non-isothermal heating process, the process lethality (*P)* is appromixated by summating the lethal rates obtained by measured successive discrete (usually 1°C accurate) temperatures during a set time interval (e.g. 1 sec). A *P*-value is mostly written as $P^{z}_{T_{ref}}$ with *Tref* the reference temperature and *z* the z-value of the target organism. As such, a $P^{7.5}_{70}$ =2 means that the (non-isothermal) heating process applied was equivalent (i.e. can give the same inactivation) as an isothermal treatment of 2 minutes at 70°C.

To demonstrate that a particular heating process can adequately reduce or eliminate specific hazards, it should not be merely calculated but preferably the estimated (and targeted) inactivation of e.g. 6 log reduction should also be validated by experimental studies. For the validation of this control measure it may be adequate to demonstrate the absence of the micro-organism after treatment (in a stated number of samples) or a particular level of reduction. However, there is a need for harmonized experimental design protocols for heat resistance testing and a need for a harmonized validation procedure for heat treatment studies (Condron et al., 2015).

A minimum heat treatment of 2 minutes at 70°C or equivalent would be needed to provide a 6 log reduction to ensure safe cooking. However there is concern on the effective heat transfer and the Belgian culinary habit of pan-frying of meat was not found to be actually validated by experimental set-up or microbiological analysis of the meat before and after pan-frying to confirm this 6 log reduction. Therefore it was decided to validate the procedure of meat pan-frying in **CHAPTER 5** which is assumed to be a critical control step in restaurants (and at home) to prevent food infections occurring from the occasional presence of *Salmonella, L. monocytogenes*, pathogenic *E. coli* or *Campylobacter* in fresh meat and meat preparations. In doing this, it also contributed to the intention for setting-up a standardized procedure for validation of inactivation treatments in food processing and preparation in the food supply chain.

1.4 Conclusion

Microbiological analysis are being used by many stakeholders and may serve many objectives to manage the food safety and food quality along the food chain. This introduction illustrates that microbiological analysis not only deals with the laboratory work (e.g. weighing the sample, execution of the method of analysis), but also involves the need to think about the sampling plan, selection of relevant microbiological parameters for a food product and interpretation of the obtained results using appropriate threshold values. Thus microbiological analyses are rather complex and need expertise to gain the maximum outcome of the results and to contribute to good food safety management. Besides, there is

a need to empower micro and small-sized enterprises (e.g. FSO) in diagnosing and improving their FSMS themselves. However, they mostly have not the expertise on setting up sampling and testing schemes and may lack the resources to perform microbiological analysis. Food business operations that are involved in business-to-consumer activities such as FSO serve food directly to the consumer and were frequently involved in foodborne outbreaks. As such, control of food safety and food quality with adequate food safety management is especially important for these sectors. Therefore, convenience sampling plans are needed to guide those involved into business-to-consumer activities, in the validation and verification of their FSMS. Besides, a validation of the effectiveness of critical practices (e.g. heat treatment of meat, storage of cheeses) for those sectors is needed. Therefore, this PhD study aimed to develop a systematic approach for microbiological verification testing in FSMS, especially if multiple food types are handled and various processing conditions are part of the food business' activities. The proposed systematic approach is based on risk categorization to underpin focused sampling. As such the risk-based approach consists of selection of critical sampling locations, sampling frequency, microbiological parameters and methods of analysis. In the present PhD study on the use of microbiological analysis, also challenge testing with artificially inoculated samples was used to provide an insight on the concern of microbiological safety in two sectors.

CHAPTER 2

MICROBIOLOGICAL PERFORMANCE OF A FOOD SAFETY MANAGEMENT SYSTEM IN A FOOD SERVICE OPERATION

Published as:

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F., and Uyttendaele, M. (2012). Microbiological performance of a food safety managment system in a food service operation. *Journal of Food Protection* **75**, 706-716.

Abstract

The microbiological performance of a food safety management system (FSMS) in a food service operation (FSO) was measured using a microbiological assessment scheme (MAS) as a vertical sampling plan throughout the production process, from raw materials to final product. The assessment scheme can give insight into the microbiological contamination and the variability of a production process and pinpoint bottlenecks in the FSMS. Three production processes were evaluated: a high-risk sandwich production process (involving a raw meat preparation), a medium-risk hot meal production process (starting from undercooked raw materials), and a low-risk hot meal production process (reheating in a bag). Microbiological quality parameters, hygiene indicators, and relevant pathogens (*L. monocytogenes*, *Salmonella*, *B. cereus* and *E. coli* O157) were in accordance with legal criteria and/or microbiological guidelines, suggesting that the FSMS was effective. High levels of total aerobic bacteria (>3.9 log CFU/50 cm^2) were noted occasionally on gloves of food handlers and on food contact surfaces, especially in high contamination areas (e.g. during handling of raw material, preparation room). Core control activities such as hand hygiene of personnel and cleaning and disinfection (especially in highly contaminated areas) were considered points of attention. The present sampling plan was used to produce an overall microbiological profile (snapshot) to validate the FSMS in place.

2.1 Introduction

Some individuals tend to eat out of home, often at food service operations such as cafeterias, canteens, fast food outlets, bars, and restaurants (Nyachuba and Donnelly, 2010; Vandevijvere et al., 2009). A national food consumption survey in Belgium revealed that in 2004 more than 35% of the population consumed more than 25% of their daily energy intake out of the home (Lachat et al., 2010). Eating out at food service operations seems to be no longer reserved for special occasions. However, food service operations can be involved in foodborne disease outbreaks associated with a variety of pathogens, e.g. *Salmonella*, *Campylobacter* spp., *E. coli* O157, *L. monocytogenes*, *B. cereus*, *Cl. perfringens*, and *S. aureus* (EFSA, 2009-2010; Lianou and Sofos, 2007).

Kitchens with a history of association with foodborne outbreaks or consumer complaints about food safety issues have significantly more frequent problems with personnel hygiene and inadequate raw material storage than do other kitchens (Tuominen and Maijala; 2009). Food handlers were epidemiologically linked to 80% of the norovirus outbreaks reported in Belgium (Baert et al., 2009). Food handlers' malpractices contributed to 97% of foodborne illnesses associated with food service operations (Worsfold and Griffith, 2003). The main causes of microbiological contamination and growth, which occur in food service operations mainly because of a lack of a well-functioning food safety management sytem (FSMS), are contaminated ingredients, dirty food contact materials, poor personnel hygiene practices, inappropriate storage temperatures and insufficient cooking (Hertzman and Barrash, 2007; Jones et al., 2008a and 2008b; Käferstein, 2003).

In food service operations, various types of raw (at-risk) materials are used and a wide variety of final products are served, including hot meals, cold sandwiches, and salads. This multitude of products, processes and personnel involved makes it very important to ensure safe food service and to have a well-functioning FSMS, as requested from all food business operations in Regulation (EC) 852/2004 on the hygiene of foodstuffs. The microbiological quality and safety of foods is largely affected by the performance of the FSMS (Jacxsens et al., 2009 and 2010). The application of good manufacturing practices and good hygiene practices as parts of a hazard analysis critical control point (HACCP) plan and the use of ISO (International Organization for Standardization, Geneva, Switzerland) method 9001:2008

can improve product quality and safety (Kokkinakis et al. 2008; Kokkinakis and Fragkiadakis, 2007). Periodic verification of the HACCP plan is recommended.

Adequate process controls and periodic verification are more effective than control of only final products (Ropkins and Beck, 2000). Swanson and Anderson (2000) stated that testing of final products is equivalent to finding a needle in a haystack, particularly in food service operations where a wide variety of final products are generated as output for the consumers. Moreover, sampling and microbiological analysis often are perceived as costly. A microbiological assessment scheme (MAS) developed by Jacxsens et al. (2009b) includes minimal sampling and analysis, but uses a systematic approach to assess the microbiological performance of a company-specific FSMS. This sampling plan, when accompanied by observations at the time of sampling and discussion of results with the quality manager of the company, provides an overview of the microbiological quality, hygiene, and safety level of products and processes at a food business operation. Such information may help managers to identify bottlenecks in the core control activities of an implemented FSMS (Luning et al., 2011a; Sampers et al., 2010). With this approach, samples are collected throughout the process from raw materials to final products at critical locations on three different days (in a time period of multiple weeks) and analyzed for multiple microbiological parameters. A microbiological profile of the production process can then be established. The microbiological analyses to assess the FSMS performance are aimed at obtaining contamination profiles, which provide insight into the maximum microbiological counts and the distribution of the microbiological contamination. The sample analysis is not meant to guarantee food safety but rather provides verification of the preventive measures taken in the food service operation. The principle of the MAS is that a better performing FSMS would be better able to realize products with lower contamination levels and less variation in contamination loads. MASs already have been applied in poultry meat preparation processing plants (Sampers et al., 2010), a pork processing company (Jacxsens et al., 2009b), the lamb chain (Osés et al, 2012), and various dairy and meat processing plants in Europe (Jacxsens et al, 2010). In the present study, a MAS was developed for a food service operation and was used in a vertical manner throughout the production process from raw materials to final products. Three different production processes in the food service

operation were evaluated to measure the microbiological performance of the FSMS implemented in this food service operation.

2.2 Materials and Methods

2.2.1 Characterization of the food service operation

The food service operation selected for this case study comprised 8 restaurants and 11 cafeterias at a university that were separated in different building areas in Ghent, Belgium. In the restaurants, hot meals, soups, sandwiches, and salads were served, whereas in the cafeterias no hot meals were available. In this catering establishment, 650 000 hot meals and 29 000 sandwiches were served each year. Each restaurant had its own regeneration kitchen where undercooked frozen or previously prepared foods delivered from the supplier as frozen (cook and freeze) or chilled (cook and chill) products were (re)heated and the cooking process was completed. Three main production processes were used in the food service operation: (i) preparation of a sandwich and/or salad (process A), (ii) production of a hot meal starting with unprocessed raw or undercooked frozen ingredients and preparation out of packaging (possibility of post-processing contamination) (process B), and (iii) production of a hot meal starting with cooked products in reheating bags (no post-processing contamination can occur) (process C). The main difference between process B and process C was the utilization of raw material in process B with its overall higher microbiological load and higher risk of pathogen contamination.

2.2.2 Microbiological Assessment Scheme

The MAS was developed as described by Jacxsens et al. (2009b) and applied vertically throughout a production process. The MAS (i) identifies critical sampling locations within the production process, (ii) selects appropriate microbiological parameters to be analyzed, (iii) defines the sampling procedures and analytical methods, and (iv) helps in the interpretation of the results. Samples were collected at various critical locations in the process A, B, and C processing lines from raw materials to final products and then analyzed

to assess the microbiological performance of the core control activities in the implemented FSMS. The same samples were collected and analyzed for the same parameters during three visits on three days in one restaurant of the food service operation to provide information on the maximum load and the distribution of the microbiological load at each sampling location in the production process. The visits took place in March 2009, February 2010, and March 2010. During each visit, 33 samples were collected and analyzed. This resulted in a total of 99 samples throughout the survey: 36 food samples, 21 swabs for detection of *L. monocytogenes*, and 42 swabs for enumeration of hygiene indicators and determination of the total viable bacteria count (TVC). A total of 147 analyses of quality parameters, 216 analyses of hygiene indicators, and 132 analyses of foodborne pathogens were performed. The number of samples was small, but they were collected as part of a periodical verification of the preventive measures implemented in the food service operation rather than as indicators of food safety.

Samples were collected within the production processes on locations where loss of control will lead to unacceptable food safety problems due to contamination with or growth and/or survival of microorganisms (Jacxsens et al, 2009b). Those locations are referred to critical sampling locations (CSLs) and are illustrated in Figure 2.1. In this study seven CSLs were identified. Samples were collected from at-risk (i.e. raw) materials at point of receipt (CSL 1) (e.g. vegetables and frozen fish) to determine the initial contamination level of microorganisms and verify appropriate supplier selection. Samples from intermediate products (CSL 2) (i.e. after regeneration and cooling) were collected throughout the production line where manual operations were performed and/or physical intervention processes occurred to verify good hygienic practices to control microbiological growth and potential cross-contamination. Samples were collected from final products (CSL 3) after assemblage (sandwiches and salads) or at the buffet where the food was kept warm for prolonged periods in a hot water bath (hot meal components) to verify the final storage and handling conditions at the serving counter and thus maintain good microbiological quality. Sampling in the production environment was conducted by taking swabs of gloves and hands (CSL 4 and 5) of personnel that manipulated the food products (e.g. cutting vegetables or opening vacuum-packed bags of cooked chilled foods after final heat treatment) or taking swabs of food contact surfaces (CSLs 6 and 7) (e.g. chopping boards,

knives, and spoons) throughout the production processes. These environmental samples were linked to samples of intermediate or final food products (Figure 2.1).

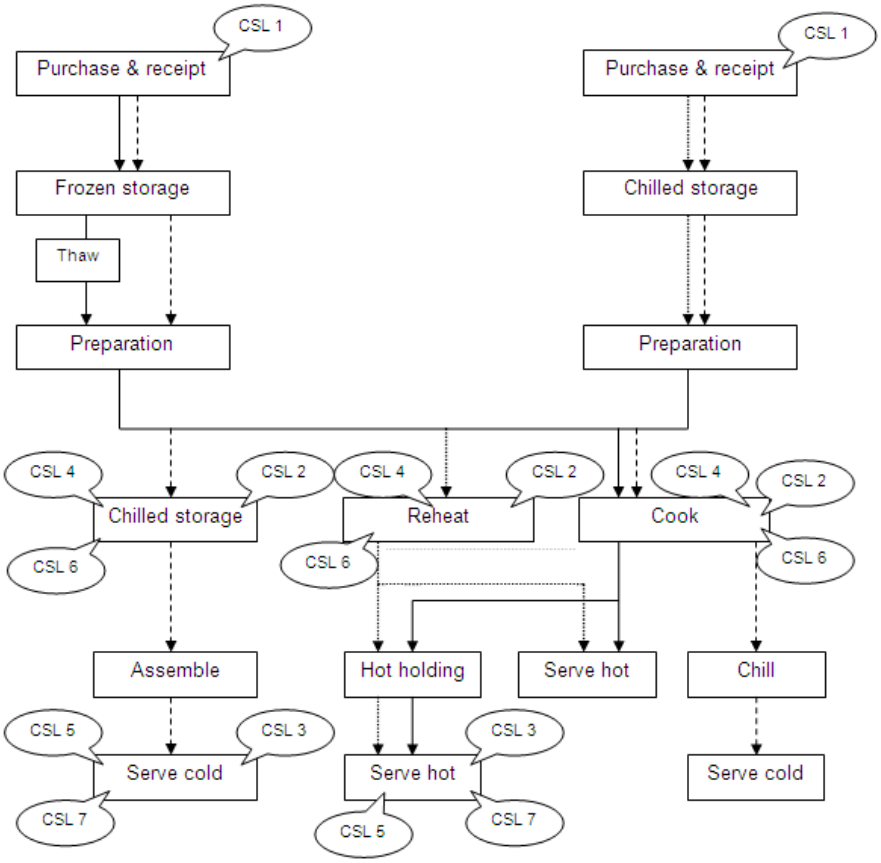


Figure 2.1. Schematic overview of the main production processes in a FSO. Critical sampling locations (CSLs) within the processes were identified: CSL 1, raw materials; CSL 2, intermediate materials; CSL 3, final food products; CSLs 4 and 5, gloves and/or hand of workers; CSLs 6 and 7, food contact surface. Production of a sandwich (process A); production of a hot meal starting with unprocessed raw or undercooked frozen ingredients (process B); production of a hot meal starting with cooked products with "reheating in bag" (process C).

The microbiological parameters selected for this study differed depending on the type of food product (Table 2.1). Parameter selection was based on European legal criteria (Anonymous, 2005), national action limits (FAVV, 2010a), and knowledge of the microbiological ecology of foods. Total aerobic bacteria, yeasts, and molds were selected as indicators of overall quality. Lactic acid bacteria (LAB) are taken up as an indicator of spoilage by gram-positive bacteria. *Pseudomonas* can be taken up as an indicator of spoilage by gram-negative bacteria, especially for meat and fish. However, it was decided not to analyse them as a selection has to be made and LAB are more common in use.

Tabel 2.1. Overview of critical sampling locations (CSLs) encompassing food products and production environment with the corresponding analyzed microbiological parameters and legal requirements or microbiological guidelines.

	Process A			Process B				Process C			All processes		
	Parameter		Criteria (log CFU/g)	Parameter		Criteria (log CFU/g)		Parameter	Criteria (log CFU/g)		Parameter		Criteria[b] (log CFU/16 cm²)
Parameter / CSL	1,2	3	1,2,3	1	2,3	1	2,3	2,3	2	3	4,5	6,7	4,5,6,7
Total viable bacteria[b]	+	+	m= 5 M= 8	+	+	m= 5 M= 7	m= 3 M= 6	+	m= 3 M= 6		+	+	Good, ≤ 1; average, ≤ 1.8; bad, ≤ 2.5; intolerable: > 2.5
Lactic acid bacteria[b]	+	+	m= 3 M= 7	+		m= 2 M= 7							
Yeasts[b]	+	+	m= 3 M= 5	+		m= 2 M= 5							
Molds[b]	+	+	m= 3 M= 4	+		m= 2 M= 3							
B. cereus		+	m= 3[c] M= 4[c]		+		m= 3[b] M= 4[b]	+	m= 2[b] M= 5[b]				
Sulphite-reducing clostridia[b]					+		m= 2 M= 5	+	m= 2 M= 5				
L. monocytogenes[a]	+	+	Absent in 25g or 2 log CFU/g at end of shelf life	+	+	Absent in 25g or 2 log CFU/g at end of shelf life		+	+			+	Absent on tested surface
Salmonella	+	+	absent in 25g[a]	+	+	absent in 25g[a,b]		+	+				
E. coli O157[b]	+		absent in 25g										
Enterobacteriaceae		+	m= 2.7[c] M= 3.7[c]	+	+	m= 2[b] M= 3[b]	m= 1.69[b] M= 2.69[b]	+	m= 1[c] M= 2[c]	m= 1.69[b] M= 2.69[b]	+	+	Good, ≤ 1; average, ≤ 1.8; bad, ≤ 2.5; intolerable: > 2.5
E. coli	+	+	m= 2[a] M= 3[a]	+	+	m= 2[b] M= 3[b]	m= < 1[b] M= 1.69[b]	+	m= 1[c] M= 2[c]	m= <1[b] M= 1,69[b]	+	+	Absent on tested surface
S. aureus	+	+	m= 2[b] M= 3[b]	+	+	m= 2[b] M= 3[b]		+	+	m= 2[b] M= 3[b]	+	+	Absent on tested surface

[a] CSL 1, raw materials at point of receipt; CSL 2, intermediate products; CSL 3, final products; CSLs 4 and 5, hand and gloves; CSLs 6 and 7, food contact surfaces. +, analysis was conducted for the parameter. M, maximum level of bacteria per test volume considered acceptable (food with values above this level in any sample are considered marginally acceptable or unacceptable); M, maximum level of bacteria per test volume considered marginally acceptable (food with values at or above M is unacceptable).[b] According to microbiological guidelines of the LFMFP-UGent (Uyttendaele et al. 2010). [c] According to action limits of the FASFC (FAVV, 2010a).[d] According to EU regulation 2073/2005.

Enterobacteriaceae, *E. coli*, and *S. aureus* were selected as hygiene indicators. The presence of the foodborne pathogens *Salmonella*, *L. monocytogenes*, *E. coli* O157:H7, *B. cereus*, and sulfite-reducing *clostridia* (an indicator for *C. perfringens*) was evaluated when appropriate for the food type.

For food products, 300 g was aseptically collected with a sterile spoon or forceps and transferred to a sterile sampling bag. Food contact surfaces and gloves and hands were swabbed in a deliminated area of 50 cm^2 using a sterile rayon swab pre-moistened in 7 ml of sterile peptone water (for microbiological enumeration) or 5 ml of demi-Fraser enrichment medium (for detection of *L. monocytogenes*). For knives, an area of 10 cm^2 was swabbed because of the limited surface area. The food samples and the moistened swabs were transported in a cool box at ≤ 4°C to the laboratory. Microbiological analyses were performed in the laboratory within 6 h of sample collection. For enumeration, 10 g of each food sample was homogenized for 2 min in 90 ml of sterile peptone water. For detection of *Salmonella* and *L. monocytogenes*, a 25 g subsample was weighed in a stomacher bag and homogenized for 2 min in 225 ml of the respective (semi)selective medium, i.e. buffered peptone water for the detection of *Salmonella* and demi-Fraser for the detection of *L. monocytogenes*. Swab samples were vortexed for 10 s and incubated for detection of specific organisms. Tenfold serial dilutions were made in sterile peptone water for microbiological enumeration. For each microorganism, standardized methods (ISO) or alternative (rapid) methods validated according to ISO 16140:2003 were applied. The reference method ISO 4833:2003 (plating on plate count agar and incubating for 72 h at 30°C) was used for the enumeration of aerobic mesophilic bacteria. ISO 15214:1998 (plating on MRS agar with an overlayer and incubating for 72 h at 30°C) was used for the enumeration of lactic acid bacteria. Yeast extract glucose chloramphenicol selective medium (Bio-Rad, Hercules, CA), an AFNOR (Association Française de Normalisation, Paris, France) validated method (NF V08-059 2002), was used for enumeration of yeasts (incubation for 72 h at 22°C) and molds (incubation for 120 h at 22°C). Coli-ID chromogenic medium (bioMérieux, Marcy l'Etoile, France) (24 h of incubation at 44°C), an AFNOR validated method (BIO 12/5-01/99), was used for enumeration of *E. coli*.

ISO 21528-2:2004 (plating on VRBG agar with an overlayer and incubating for 24 to 48 h at 37°C) was used for enumeration of *Enterobacteriaceae*. ISO 6888-1:1999/Amd 1:2003 (plating on BP agar and incubating for 24 to 48 h at 37°C) was used for enumeration of *S. aureus*. Because low numbers were expected for *S. aureus*, 1 ml of inoculum was spread on three plates. Enumeration of *B. cereus* was performed with ISO 7932:2004 (plating on MYP agar and incubating for 24 h at 30°C). Tryptose sulfite cycloserine selective medium with an overlayer (24 h of incubation at 37°C), an AFNOR validated method (XP V 08-061 1996), was used for enumeration of sulfite-reducing anaerobic bacteria. ISO 16654:2001 (an immunomagnetic separation method and isolation on CT-SMAC) was used for detection of *E. coli* O157:H7. The detection of *L. monocytogenes* was performed using VidasLMO2 (bioMérieux), an AFNOR validated enzyme-linked fluorescent assay (ELFA) (BIO 12/11-03/04). When positive results were obtained, *L. monocytogenes* was enumerated from the same sample according to ISO 11290-2:1998/Amd 1:2004. *Salmonella* was detected using Vidas Easy SLM (bioMérieux), an AFNOR validated ELFA (BIO-12/16-09/05).

2.2.3 Data analysis and interpretation: microbiological safety level profiles.

Data were manipulated with MS Excel (Microsoft, Redmond, WA) to develop graphics and tables to illustrate visually the levels and distribution of microbiological contamination during the three sampling periods. No means, standard deviations, or statistical analyses were needed to evaluate the variability in this food operation service because the microbiological analyses for measuring the FSMS performance were designed only to obtain microbiological safety level profiles (MSLPs). These profiles provide insight into the maximum microbiological counts and the distribution of microbial contamination, i.e. where to find contamination in the production process (Jacxsens et al., 2009b), and into the dynamics of microbial contamination occurring as a result of the design and application of the control strategies in a FSMS. The results for each process were evaluated in two ways. Individual results for each analyzed parameter were evaluated for each specific sampling location within the production process. The results obtained at each location (e.g., CSL 1) were compared with defined legal criteria (Anonymous, 2005; Anonymous, 2009). In the absence of legal criteria, microbiological values established by the Laboratory of Food Microbiology and Food Preservation of the University of Ghent (LFMFP-UGent) (Uyttendaele

et al., 2010) or the action limits established by the FASFC version June 2010 (FAVV, 2010a) were used for comparison (Table 2.1). The score attribution system is summarized in Table 2.2. When the legal requirements or the guidelines are exceeded (score 0) for a specific microorganism in a specific sampling location, the specific control activity in the FSMS at that location is not working properly. Corrective action(s) is then needed to change this non-compliance situation and improve the current FSMS performance.

Table 2.2. Score attribution system [a]

	Food products		**Food contact surfaces**
Score	**Legal criteria, action limits of FASFC**	**LFMFP-UGent**	**LFMFP-UGent**
3	R ≤ m Absent in x g[b]	R ≤ target	R ≤ 10 CFU/16 cm² Absent on surface[c]
2	m < R < M	Target < R ≤ tolerance	10 CFU/16 cm² < R ≤ 69 CFU/16 cm²
1	R = M	tolerance < R ≤ use by date or best before date	69 CFU/ 16 cm² < R ≤ 350 CFU/16 cm²
0	R > M Present in x g[b]	R > use by date or best before date	R > 350 CFU/16 cm² Present on surface[b]

[a] R, result in CFU per gram (food products) or CFU per 16 cm² (food contact surfaces); m, maximum level of bacteria per test volume considered acceptable; M, maximum level of bacteria per test volume considered marginally acceptable (food with values at or above M in any sample is unacceptable).
[b] Specifically for *L. monocytogenes*, *E. coli* O157, and *Salmonella*.
[c] Specifically for *E. coli*, *L. monocytogenes*, and S. aureus

Individual results for each analyzed parameter also were evaluated across sampling locations (CSL 1 to CSL 7) within the production process. A microbiological safety level was attributed to each type of microbiological parameter to obtain an overall view of microbiological quality, hygiene, and the safety level of products and processes at the food service operation. Each microbiological parameter was given a score from 1 to 3. Level 3 is the best result (legal criteria or guidelines are met, and no improvements are needed); the current level of the FSMS is deemed high enough to cope with any hazards. Level 2 is a moderate result (bacterial values exceed legal criteria or guidelines, and improvements are needed in a single control activity of the FSMS), and level 1 is a poor result (bacterial values exceed legal criteria or guidelines, and improvements are needed in multiple control activities of the FSMS). The sum of the levels of all microbiological parameters results in an MSLP score, which gives an indication of the actual performance level of the FSMS (Jacxsens et al, 2009b; Sampers et al, 2010). When the MSLP score is lower than the maximum achievable value, then improvement of the current FSMS is possible. Examination of the

details of the MSLP results for each microbiological parameter, observations during sampling, and discussion with the quality manager of the operation may provide insight into the points of attention and result in useful recommendations for follow-up.

2.2.4 Hand hygiene

In the present case study, hand hygiene was identified as a point of attention. To evaluate the general hand hygiene of the food handlers, extra swab samples of gloves and hands were collected during a 6-month period and analyzed for TVC and the hygiene indicators *E. coli*, *S. aureus* and *Enterobacteriaceae*. A sterile rayon swab pre-moistened in 7 ml of sterile peptone water was used to swab a deliminated area of 50 cm^2 and then placed aseptically into its tube. The swabs were stored and transported in a cool box at ≤ 4°C. Microbiological analyses were performed in the laboratory within 6 h of sampling. A total of 93 extra samples were collected from employees working at different restaurants and performing different activities in the food service operation. Descriptive statistical analysis of the data was performed with MS Excel.

2.3 Results and Discussion

The 99 samples collected during the initial three visits were analyzed for multiple parameters (147 analyses of quality parameters, 216 analyses of hygiene indicators, and 132 analyses of foodborne pathogens) (Table 2.3), and a microbiological safety level was established for each microbiological parameter over all sampling locations within the production process to evaluate the current microbiological status of the FSMS (Figure 2.2). The overall MSLP score for process A (the production of a sandwich or salad) was 31 of a possible 33 (11 microbiological parameters with a maximum microbiological safety level of 3), and for process B (production of a hot meal starting with unprocessed raw or undercooked frozen ingredients) the score was 32 of a possible 33. For process C (production of a hot meal starting with cooked products and reheating in a bag) the score was 23 of a possible 24. The maximum for this process was only 24 because no at-risk raw material was present (i.e. products were cooked in bags) and no samples of raw material

Table 2.3. Detailed results of the microbiological assessment scheme [a]

			Food safety indicators (log_{10} CFU)					Hygiene indicators (log_{10} CFU)			Overall indicators (log_{10} CFU)			
Process	**CSL**	**Sample**	***E.coli* O157**	***Salmonella***	***L. monocyt-ogenes***	***B. cereus***	**SRC[b]**	***E. coli***	***Enterobac-teriaceae***	***S. aureus***	**TVC[c]**	**LAB[d]**	**Yeast**	**Molds**
A	1	Raw material	A	A	A	NA	NA	<1.0	NA	<1.0	3.7 –4.2	2.4 –3.3	2.7 –3.5	<2.0 –2.3
		Raw material	A	A	A	NA	NA	<1.0	NA	<1.0	6.0 –6.3	3.1 –5.5	4.4 –4.6	2.0 –2.6
		Raw material	A	A	A	NA	NA	<1.0	NA	<1.0	4.2 –4.9	2.4 –2.6	3.0 –3.2	2.0 –2.9
	2	Raw material in cool bar	A	A	A	NA	NA	<1.0	NA	<1.0	3.8 –5.3	2.3 –3.1	3.2 –3.9	<2.0 –3.0
		Raw material in cool bar	A	A	A	NA	NA	<1.0	NA	<1.0	5.9 –6.9	2.9 –5.1	4.3 –4.6	<2 –2.9
		Raw material in cool bar	A	A	A	NA	NA	<1.0	NA	<1.0	4.3 –6.8	2.3 –3.9	3.1 –5.0	2.0 –2.7
	3	Sandwich	NA	A	P (<2 log CFU)	<2.0	NA	<1.0 –1.0	2.5 –3.5	<1.0	4.7 –5.4	3.2 –4.7	3.3 –4.9	<2.0 –3.0
	4	Gloves	NA	NA	NA	NA	NA	A	<1.0	<1.0	2.1 – 4.3 **(2/3)[e]**	NA	NA	NA
	5	Gloves	NA	NA	NA	NA	NA	A	<1.0 –2.4	<1.0	2.1 –4.3 **(2/3)[e]**	NA	NA	NA
	6	Gastronorm	NA	NA	A	NA	NA	A	<1.0	A	<1.0 –1.9	NA	NA	NA
	7	Cutting board (in use)	NA	NA	A	NA	NA	A	<1.0	A	<1.0 –5.3 **(1/3)[e]**	NA	NA	NA
		Knife (in use)	NA	NA	A	NA	NA	A	<1.0	A	<1,0 –5.8 **(1/3)[e]**	NA	NA	NA
B	1	Raw material	NA	A	A	NA	NA	<1.0	<1.0	<1.0	2.8 –4.1	<1.0 –2.7	<2.0 –2.9	<2.0 –2.0
	2	After regeneration	NA	A	A	<2.0	<1.0	<1.0	<1.0	<1.0	<1.0 –3.9	NA	NA	NA
	3	In buffet	NA	A	A	<2.0	<1.0	<1.0	<1.0	<1.0	<1.0	NA	NA	NA
	4	Gloves	NA	NA	NA	NA	NA	A	<1.0	<1.0	<1.0 –2.3	NA	NA	NA
	5	Gloves clean	NA	NA	NA	NA	NA	A	<1.0	<1.0	<1.0 –1.7	NA	NA	NA
		Gloves	NA	NA	NA	NA	NA	A	<1.0	<1.0	<1.0 –1.2	NA	NA	NA
	6	Gastronorm	NA	NA	A	NA	NA	A	<1.0	A	<1.0 –3.9 **(1/3)[e]**	NA	NA	NA
	7	Spoon	NA	NA	A	NA	NA	A	<1.0	A	<1.0 –1.9	NA	NA	NA
C	2	After regeneration	NA	A	A	<2.0	<1.0	<1.0	<1.0	<1.0	<1.0	NA	NA	NA
	3	In buffet	NA	A	A	<2.0	<1.0	<1.0	<1.0	<1.0	1.0 –1.6	NA	NA	NA
	4	Gloves	NA	NA	NA	NA	NA	A	<1.0	<1.0	2.3 –4.1 **(1/3)[e]**	NA	NA	NA
	5	Gloves	NA	NA	NA	NA	NA	A	<1.0	<1.0	0 –1.5	NA	NA	NA
	6	Gastronorm	NA	NA	A	NA	NA	A	<1.0	A	<1.0	NA	NA	NA
	7	Spoon	NA	NA	A	NA	NA	A	<1.0	A	<1.0	NA	NA	NA

[a] Absent in 25g sample or on 50 or 10 cm²; P, present in 25g sample or on 50 or 10 cm²; NA, parameter not analyzed for this product or CSL. Results are in log CFU/g for food products and in log CFU/50 cm² for environmental samples. Values with < symbol are below quantification limit.

[b] SRC, sulfite-reducing clostridia; [c] TVC, total viable count; [c] LAB, lactic acid bacteria; [e] Values in parentheses are the number of samples exceeding legal criteria or guidelines/number of samples tested.

were collected; therefore, no analyses of lactic acid bacteria, yeasts, and molds were conducted. For each process, the maximum MSLP score was nearly obtained (Figure 2.2), indicating that the current FSMS has no major flaws in its ability to produce safe and hygienic food products. However, continuous verification of the implemented HACCP system by microbiological analysis of raw materials and final products, regular supplier audits, and continuous training of personnel in good hygienic practices is recommended. Results of other studies have indicated that inherent barriers to effective implementation of HACCP systems exist in catering companies (Garayoa et al., 2011), and more effort should be made to apply HACCP principles (Marzano and Balzaretti, 2011). In a study of catering establishments, Marzano and Balzaretti (2011) found that a percentage of samples did not meet microbiological reference standards for *L. monocytogenes*, *S. aureus*, *E. coli*, and total coliforms.

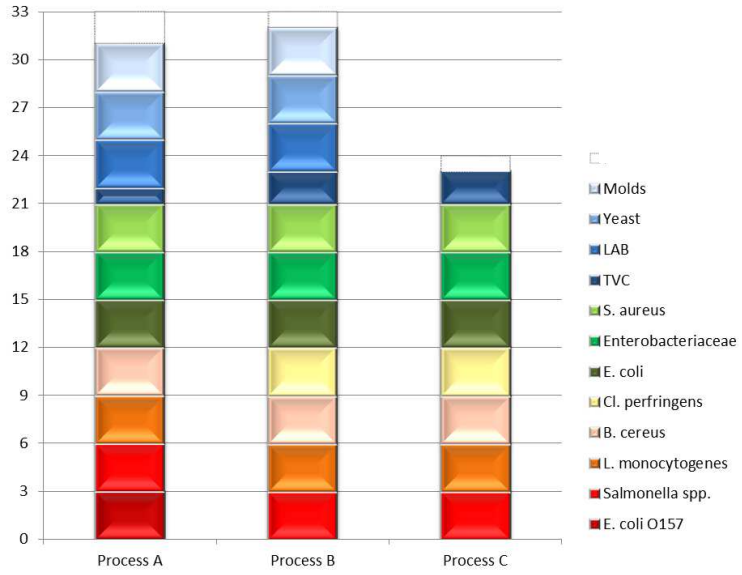


Figure 2.2. Microbial safety level profile for three production processes in the catering establishments: process A, sandwich production process; process B, production of hot meal starting with raw or undercooked material; process C, production of a hot meal by reheating in a bag. For each parameter, a microbiological safety level was determined: 1, low; 2, moderate; 3, high. Dashed line indicates the maximum score that could be obtained for each process.

Evaluation of the details of each type of production process in the present study revealed no *E. coli* O157 or *Salmonella*. The results for the spore-forming pathogens *B. cereus* and sulfite-reducing *clostridia* were below the detection limit of the analytical method (2 log

CFU/g) at all sampling times. Only in the sandwich production process (process A) was *L. monocytogenes* detected in a sandwich made with a spread of raw meat prepared with Worcestershire sauce during the first visit (Table 2.3). The level of *L. monocytogenes* in this sample was < 2 log CFU/g and thus did not exceed the European Commission Regulation 2073/2005 criterion for *L. monocytogenes* in ready-to-eat foods at the time of consumption (Anonymous, 2005). Upon reporting of this *L. monocytogenes* result to the quality manager of the food service operation, steps were taken by the catering establishment for follow-up sampling of a second batch of this sandwich spread. This second sample also was contaminated with *L. monocytogenes*, indicating that the source of *L. monocytogenes* was most likely located at the supplier (Christison et al., 2008; Martinez-Tomé et al., 2000). Further investigation at the supplier of the sandwich spread revealed *L monocytogenes* on the equipment in the production area. This pathogen can adhere to surfaces and form biofilms on various materials in food processing facilities (BCCDC, 2010). Based on this information, the food service operation changed to a new supplier for this type of sandwich spread, and no *L. monocytogenes* was found in this spread during the two subsequent visits. This example illustrates the impact of supplier selection on the performance of the FSMS.

The number of samples analyzed for pathogen detection in the present MAS was limited. The objective of this sampling plan was to provide an overall microbiological profile (snapshot) to evaluate the current FSMS. Statistically based on-going monitoring of pathogens in raw materials and final products should complement the MAS as a sampling plan to allow verification of proper implementation of HACCP principles on a continuous basis. However, in particular for pathogenic organisms with low prevalence (< 1 to 5 %), inherent limitations in sampling schemes determine the level of detectable contamination.

All results obtained for *Enterobacteriaceae*, an indicator of overall good manufacturing practices, and *E. coli*, as a fecal hygiene indicator, in each process line were satisfactory. *Enterobacteriaceae* were present in the sandwich production process at 2.5 to 3.5 log CFU/g. These results are within acceptable levels as defined by the action limits of the FASFC (Table 2.1). The presence of *Enterobacteriaceae* in this sandwich probably was due to the presence of vegetables, which generally carry high levels of *Enterobacteriaceae* (Lianou and Sofos, 2007). For the personal hygiene indicator *S. aureus*, no unacceptable levels were found. For the quality parameters of lactic acid bacteria, yeasts, and molds (Table 2.3) in

processes A and B, all results were within microbiological reference standards. However, high TVCs (> 4.3 log CFU/50 cm^2) were found on gloves (CSLs 4 and 5) of food operators and on food contact surfaces (CSL 7) in process A during various visits (Table 2.3). Microbiological reference standards for TVCs were exceeded on gloves, which is inevitable in the present context because of manipulation of raw materials with high microbiological loads, i.e., vegetables. The TVCs of the analyzed food products (ready-to-eat sliced mixed lettuce, tomatoes, and cucumber) in the sandwich production process were 3.7 to 6.9 log CFU/g. High TVCs on gloves can be controlled by changing gloves on a regular basis to avoid cross-contamination (Simoes et al., 2010). TVCs for food contact surfaces, i.e. the cutting board and knife, also exceeded the microbiological guidelines. Further investigation revealed that these utensils were not replaced during the service period, a total of 4 h. However, Schaffner et al. (2004) found that changes in the bacterial population on a plastic cutting board for a 5 min interval ranged from a decrease of 4 CFU/cm^2 to an increase of 13 CFU/4 cm^2. When a cutting board is used for 60 min, it usually will become contaminated with more than 50 CFU/4 cm^2. As a consequence, cleaning or a change of cutting boards during the food service period is recommended to reduce the probability of biofilm formation and cross-contamination (Oliveira et al., 2010; Rayner et al., 2004; Schaffner et al., 2004).

In process B (production of a hot meal starting with unprocessed raw or undercooked frozen ingredients), high TVCs (> 3.9 log CFU/50 cm^2) also were found on food contact surfaces (CSL 6), namely an inox serving tray, due to contact with raw frozen fish filets, which is a high-risk raw material with high microbiological loads. These results indicated that improvements in some of the core control activities in the FSMS of the food service operation were needed, e.g., cleaning and disinfection of food contact surfaces, switching gloves and washing hands on a regular basis, and cleaning the utensils properly during service (Shojaei et al., 2006). Schaffner et al. (2004) found that surfaces with high mean microbiological levels and a high percentage of samples with bacteria that were ‘‘too numerous to count’’ tended to be plastic. Doménech- Sánchez et al. (2011) found that 26.0 % (n= 4611) of the surfaces analyzed had microbiological levels higher than the recommended standards of < 1.3 log CFU/cm^2.

In process C (production of a hot meal starting with cooked products and reheating in a bag), TVCs of 2.3 to 4.1 log CFU/50 cm^2 were found on the gloves of a food operator (CSL 4) in the regeneration area. This finding was in contrast to the very low levels of bacteria on the gloves of a food operator on the buffet line, which were < 1.0 to 1.5 log CFU/50 cm^2 (Table 2.3). This difference may be due to the highly contaminated surfaces of cupboards, refrigerators, ovens, etc. in the regeneration room. On the buffet line, food operators serve customers with clean (thus less contaminated) utensils. This contrast illustrates the difficulty inherent in using the TVC as an indicator. For trend monitoring, appropriate guidelines for food contact surfaces and gloves should be developed taking into account the various activities that occur at different locations, direct or indirect contact with foods, and the type of foods handled. Because hand hygiene was identified as a point of attention, extra samples were collected from gloves and hands of food handlers working in different kitchen areas to evaluate general hand hygiene. For interpretation of the TVCs for assessing hand hygiene, guidelines were based on hygiene scores for surfaces after cleaning and disinfection in food processing companies because of a lack of guidelines specific for food contact surfaces in food service operations or for hand hygiene (Strohbehn et al., 2008; Uyttendaele et al., 2010). According to these guidelines, 43 % of the gloves and/or hand samples collected in the regeneration area, 57 % of samples from the (buffet) counter, 59 % of the samples from the sandwich bar, and 25 % of the samples from the self-service counter would be classified as unsatisfactory (> 3 log CFU/50 cm^2) based on the TVCs on gloves and/or hands (Figure 2.3A).

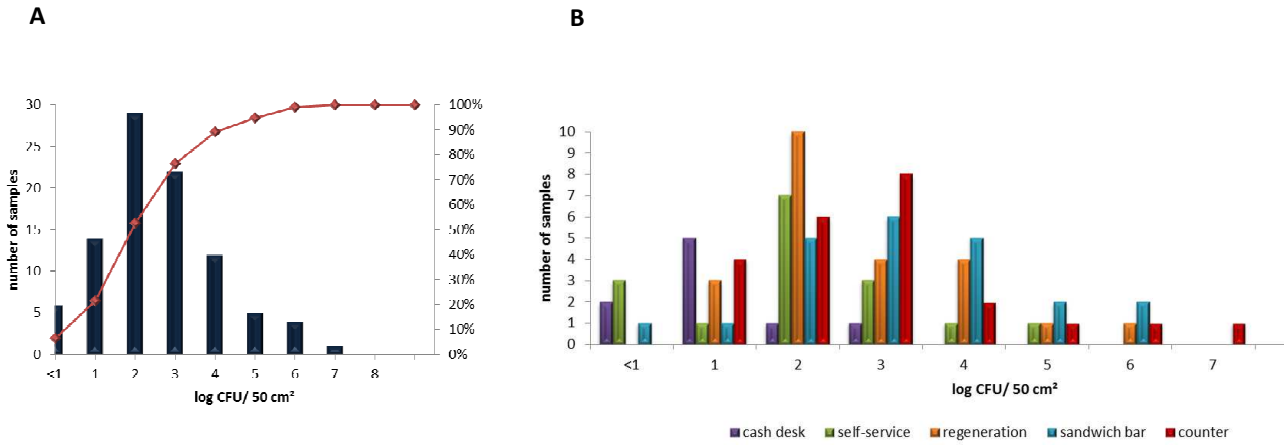


Figure 2.3. Distribution of bacterial contamination on hands/or gloves of all food handlers (A) and of food handlers performing a specific food activity (B).

However, the hygiene indicator *E. coli* was absent in all analyzed samples, and *S. aureus* was detected in only one sample. *Enterobacteriaceae* were detected in 18 (19 %) of the 93 samples (Table 2.4).

Table 2.3. Detection of hygiene indicators on hands and/or gloves of food handlers performing specific activities in institutional catering.

Location	No. of samples tested	No. of samples positive for [a]:		
		E. coli [a]	*Enterobacteriaceae*	*S. aureus* [b]
Regeneration area	23	0	4	1
Counter	23	0	6	0
Cash desk	9	0	1	0
Sandwich bar	22	0	6	0
Self-service	16	0	1	0
Total	93	0	18	1

[a] Samples were considered positive when the level of micro-organisms was at or above the detection limit of the method, which was 1 log CFU/50cm² for *E. coli* and Enterobacteriaceae and 2 log CFU/50 cm² for S. aureus.

The results of the supplementary samples collected from hands and/or gloves of food handlers indicate a difference in total microbiological contamination of the hands and/or gloves of food handlers performing different activities (Figure 2.3B). For example, a significant difference ($P < 0.05$) in TVC on the hands and/or gloves was found between food handlers working at the cash desk and food handlers working at the counter, sandwich bar, or regeneration area. A significant difference ($P < 0.05$) in TVC on the hands and/or gloves also was found between food handlers refilling the self-service counter and food handlers working at the (buffet) counter or sandwich bar. No significant difference ($P < 0.05$) in TVC on the hands and/or gloves was found between food handlers working in the regeneration area and those working at the counter, sandwich bar, or self-service counter. No significant difference in TVC on the hands and/or gloves was found between food handlers working at the counter and those working at the sandwich bar and between food handlers working at the cash desk and those refilling the self-service counter. A lower level of total microbiological contamination (1.0 log CFU/50 cm^2) was established for activities at the cash desk, and a higher level (2.0 log CFU/50 cm^2) was established for activities at the self-service counter and the regeneration area. The highest microbiological contamination (3.0 log CFU/50 cm^2) was noted for activities at the (buffet) counter, and the sandwich bar (Figure 2.

3B). Results of these supplementary samples supported the recommendation that specific microbiological guidelines should be formulated for hands and/or gloves for each location. These guidelines can be developed after establishing an overall baseline by taking daily, weekly, or monthly samples at each location and performing trend analysis. This analysis can allow identification of problem areas in the process that need more attention (Little and Sagoo, 2009). With these baseline results, in particular by sampling contact materials and hands when food handlers are using good manufacturing practices (e.g. changing gloves between handling raw and cooked products, hand washing according the protocol, and changing cutting boards during service when there is no time to clean them), a microbiological guideline can be set. Schaffner et al. (2004) recommended the use of microbiological modeling and Monte Carlo simulation as tools for evaluating cutting board polices and setting appropriate sanitary microbiological criteria.

In this study of the food service operation, the period between the first and the last visit covered one year. During this year, a snapshot of the performance of the FSMS was taken at each visit, allowing validation of the preventive measures implemented in the FSMS. By comparing the MSLPs, which reflect performance, conclusions can be reached concerning decline, stabilization, or improvement in performance. During the first and last visits, the operation was less busy, whereas during the second visit it was very busy; however, the MSLPs of the processes remained stable (comparable results) across all visits (Figure 2.2), which is an indication of a stable and well-performing FSMS. Those kinds of results may not be obvious in food service establishments because of high staff turnover, laborious production processes, and a complex variety of (raw) material and food products (Walker et al., 2003). The positive assessment of the FSMS performance also was due to proper supplier selection and to outsourcing of basic steps of food preparation. Proper supplier selection (according to microbiological specifications and auditing) results in the delivery of high-quality food products, thus putting less pressure on the FSMS of a food service operation (Luning et al., 2011a). High-quality raw materials are less likely to have undesirably high initial contamination levels or allow growth or survival of pathogens; therefore, they contribute to a low-risk environment. According Luning et al. (2011b), the concept behind FSMS diagnosis is that companies operating in a high-risk context need an advanced FSMS to achieve high levels of food safety. In a moderate-risk context, an average

FSMS may be sufficient for adequate food safety, whereas in a low-risk context even a basic FSMS would be sufficient to maintain acceptable microbiological quality, hygiene, and safety. When the food safety services operation obtains some power in supplier relationships, the risks associated with inadequate raw materials may be reduced by evaluating the current specifications and systematically auditing the FSMS of the supplier (Luning et al., 2011). These steps lead to systematic quality improvement, which is beneficial for both consumer and supplier, because food safety will be enhanced. Therefore, to have a better control on food safety in a food service operation, microbiological specifications must be present for all incoming food products (Luning et al., 2011; Tanik, 2010). The hot meals in the operation in the current study were almost all produced from bagged cooked chilled foods that had been partially prepared by the supplier and subjected to only reheating in bag on the food service premises. That type of food product has already been subjected to a (mild) heat treatment at the supplier, which results in a less contaminated product entering the food service operation. Therefore, outsourcing of basic steps in food preparation means there will be a smaller amount of high-risk material (e.g., raw meat and poultry, raw vegetables) entering the food service operation, and the pressure on the FSMS of the operation is reduced (Luning et al., 2011). Reheating in a bag also reduces the risk for introduction and dispersion of microbiological contamination in the production environment and thus fewer critical control points in the FSMS (Luning et al., 2011; Seward, 2000). Even with medium-risk food products, which are still raw in the center but have been subjected to some heat treatment (e.g. baking or frying) on the outside, there is a lower chance of contaminating the kitchen environment than when completely raw materials are processed. At the time of slaughter, the muscle tissue of a healthy animal is essentially sterile, but the surface of the meat becomes contaminated during slaughter and subsequent handling (James and Evans, 2006). The same is true for the majority of fruits and vegetables. If bacteria on the surface of raw foods could be eliminated, for example by a heat treatment, then the risk of cross-contamination during processing would be reduced (James and Evans, 2006). Raw vegetables are considered ready-to-eat products and have been subjected to an industrial washing process at the producer, which leads to a product with less contamination (Vandekinderen et al., 2009).

2.4 Conclusion

The MAS provides information on the microbiological contamination and variability of a production process and allows identification of the weak points in a FSMS. In the present case study, core control activities in the FSMS needed attention. In particular, hand hygiene of personnel (e.g. effective hand washing techniques and frequency of hand washing) and cleaning and disinfection of processing areas, especially in highly contaminated locations, needed to be monitored and followed up. The TVCs in samples from food contact surfaces and gloves did not meet food safety standards, especially in highly contaminated areas, e.g. where raw material was handled. However, these TVCs were difficult to interpret because widely accepted specific guidelines for food contact surfaces and gloves in use do not presently exist. Appropriate guidelines for food contact surfaces and/or gloves are needed that account for the various activities that take place at different locations, the direct or indirect contact with foods, and the types of food being handled. These guidelines should be based on trend analysis of information gathered in a baseline study conducted while workers are following good manufacturing practices. In this study, values for all hygiene parameters, i.e., *E. coli*, *Enterobacteriaceae*, and *S. aureus*, were in compliance with legal criteria and/or microbiological reference standards, and pathogens were not detected at unacceptable levels. Therefore, the current FSMS at this institutional catering facility can be considered validated. However, the elaboration of a statistical annual sampling plan used for continuous monitoring and surveillance as well as verification of the functioning of the HACCP plan was recommended.

CHAPTER 3

MICROBIOLOGICAL SAMPLING PLAN BASED ON RISK CLASSIFICATION TO VERIFY SUPPLIER SELECTION AND PRODUCTION OF SERVED MEALS IN FOOD SERVICE OPERATION

Published as:

Lahou, E., Jacxsens, L., Van Landeghem, F., and Uyttendaele, M. (2014). Microbiological sampling plan based on risk classification to verify supplier selection and production of served meals in food service operation. *Food Microbiology* **41**, 60-75.

Abstract

Food service operations are confronted with a diverse range of raw materials and served meals. The implementation of a microbiological sampling plan in the framework of verification of suppliers and their own production process (functionality of their prerequisite and HACCP program), demands selection of food products and sampling frequencies. However, these are often selected without a well described scientifically underpinned sampling plan. Therefore, an approach on how to set-up a focused sampling plan, enabled by a microbiological risk categorization of food products, for both incoming raw materials and meals served to the consumers is presented. The sampling plan was implemented as a case study during a one-year period in an institutional food service operation to test the feasibility of the chosen approach. This resulted in 123 samples of raw materials and 87 samples of meal servings (focused on high risk categorized food products) which were analyzed for spoilage bacteria, hygiene indicators and foodborne pathogens. Although sampling plans are intrinsically limited in assessing the quality and safety of sampled foods, it was shown to be useful to reveal major non-compliances and opportunities to improve the FSMS in place. Points of attention deduced in the case study were control of *L. monocytogenes* in raw meat spread and raw fish as well as overall microbial quality of served sandwiches and salads.

3.1 Introduction

European regulations (EC No. 852/2004 and EC No. 178/2002) and public health authorities put pressure on food business operators to develop and implement a food safety management system (FSMS) to govern food safety and to prevent foodborne outbreaks. Such a FSMS consists of generic prerequisite programs (PRP) and specific structured procedures based on the principles of Hazard Analysis and Critical Control Point (HACCP) (CIES, 2007; CAC, 2003; Jacxsens et al., 2009a; Quinn and Marriott, 2002). One of the seven principles of the HACCP system is verifying whether the FSMS is functioning properly. Such verification can be elaborated with a microbiological sampling plan (Anonymous, 2003, 2005; Anonymous, 2005; Gonzales-Barron et al., 2013). However, no strict requirements related to the sampling plan have been set at an European level. Neither are any sampling guidelines for verification of the system taken up in the food service operation's self-checking guide at the Belgian level (FAVV, 2006a and 2008a). Therefore, quality managers in food service operations mostly select food products and sampling frequencies without a well described scientifically underpinned sampling plan. Besides, they tend to focus on end products such as served hot meals or sandwiches. However, apart from end product testing, it is also interesting to obtain information on the microbiological quality and safety of incoming raw materials (to contribute to the verification of supplier selection) and/or half fabricates (to verify the well-functioning of adherence to procedures). Ropkins and Beck (2000) showed in their study, in which they conducted a review of HACCP in the EU, America, Australia, New Zealand and developing countries, that adequate process control by periodic verification is more effective than control of end products only. Swanson and Anderson (2000) reported that testing on final products is analogous to finding a needle in a haystack. Well implemented testing of incoming raw materials and hygiene in the process flow is also important in a preventive systems' approach and may provide more security than mere end product testing (ICMSF, 2002). Various authors have thus recommended microbiological assessment of critical control points in a FSMS (e.g. Cormier et al., 2007; Gonzàlez-Miret et al., 2001; Kvenberg and Schwalm, 2000; Martins and Germano, 2008; Ropkins and Beck, 2000; Swanson and Anderson, 2000). As such, Jacxsens et al. (2009b, 2010), Sampers et al. (2010 and 2011) and Osés et al. (2012) previously elaborated focused microbiological sampling plans to validate a FSMS in the meat, poultry and dairy processing

industry. Their microbiological assessment scheme describes which type of samples are preferably taken in a production site with relative modest food product variation and takes a dominantly vertical process approach throughout the processing line from raw materials over half fabricates to end products including environmental sampling.

These studies mainly focused on food processing industries which function in a business-to-business transaction of foods. Food service operations are business-to-consumer operations and although being prone to official controls, have less pressure from customers or buyers than food processing companies, to demonstrate the performance of their FSMS in place. We have previously elaborated the vertical microbiological assessment scheme in a food service operation to validate the FSMS in place (Lahou et al., 2012). However, this vertical microbiological assessment scheme which focuses on sampling throughout the processing line is rather a tool for validation and identification of bottlenecks within the operation of a processing company or food service operation. In addition to sampling within the processing line a year-around sampling plan focusing on both incoming raw materials and outcoming products (i.e. served meals including hot dishes, cold sandwiches and salads within a food service operation) is recommended to contribute to the verification of supplier selection and the overall functioning of the FSMS in place. However, setting up and implementing a sampling plan in food service operations is seen as a challenge because, apart from usually many staff members being involved, there are also a variety of incoming raw materials being handled during the meal preparation processes and a wide variety of served meals offered to consumers (Airey and Greaves, 2005; Griffith, 2000). Still, this latter part of the food chain especially needs attention because it serves food directly to the consumer.

In addition, eating out-of-home is increasingly popular, for example 35 % of the Belgian population is defined as substantial out-of-home eaters, which means that they consume on average at least 25 % of their daily energy (e.g. a lunch meal) outside the home (Vandevijvere et al., 2009). As we are aware that in many food service operations, in particular in non-profit institutional food service operations, there is a restricted budget allocated to sampling and microbiological analysis, it is recommended to set up a focused sampling plan to gain the maximum outcome from the samples taken and attributed resources. Therefore, the aim of this study is to elaborate a focused sampling plan based on risk categorization of both incoming raw materials and meals (including hot meals, salads

and sandwiches) served to consumers in food service operations while taking into account the limitation of resources for microbiological analysis. The approach is illustrated and applied on an actual case study of the non-profit institutional food service operation at Ghent University, Belgium.

3.2 Materials and methods

3.2.1 Definition of the case study

The food service operation selected for this case study includes eight restaurants and eleven cafeterias from Ghent University, separated over different locations in Ghent, Belgium. In this food service operation approximately 660 000 hot meals and 400 000 sandwiches are served each year. The HACCP system that has been implemented at this food service operation is consistently applied across all meals, restaurants and cafeterias. The inventory list of all incoming raw materials and the list of all meals scheduled to be served on various days at the food service operation was obtained from the quality manager of the food service operation.

3.2.2 Food categorization and risk attribution

The chosen approach to develop a focused sampling plan for verification of the FSMS in food service operation was first the classification of all food products on the inventory lists into various food categories and more specific in various food types (Table 3.1). The same procedure was applied on foods served to the consumer. Food categories are based upon the common origin. Food types are defined as a group of food products processed/preserved in a similar way, with similar intrinsic characteristics and a similar microbiological ecology. The different food types were then subjected to a risk score attribution system. This score system is based on the principles of risk attribution used in the ICMSF attributive sampling plans and the approach adopted by the Belgian Federal Agency for the Safety of the Food Chain (FASFC) to program analysis in the frame of detecting contamination (ICMSF, 2002; Maudoux et al., 2006). A similar approach for the

model variables was used in a qualitative risk ranking approach of the European Food Safety Authority (EFSA, 2012). In the presented risk attribution system three parameters are attributed to each food type within a food category based upon i) epidemiological association of the food type with reported foodborne outbreaks, ii) the reported prevalence of foodborne pathogens and level of hygiene indicators in the food type, and iii) the potential of microorganisms (pathogens but also spoilage microflora) to grow or survive during storage and/or further processing. For each parameter one out of four scores can be attributed (0, 1, 2 or 3) to the food type (Tables 3.1 and 3.2). By summation of the attributed score of each parameter, a minimum score of zero and a maximum score of nine could be obtained for each food type within a food category. It is assumed that a score zero up to three is a low risk food type, a score four up to six is a moderate risk and a score seven up to nine as a high risk food type. To collect the data for scoring the three parameters a literature study was carried out in the ICMSF books (ICMSF, 1986, 2002, 2005) complemented with the European Community summary reports on trends and sources of zoonoses published by the European Food Safety Authority (EFSA, 2006 and 2007). To verify the general situation for the regional situation, the annual reports on zoonotic agents in Belgium of the Federal Agency for the Safety of the Food Chain (FASFC) and the Belgian report on zoonoses and zoonotic agents were consulted (Dierick and Botteldoorn, 2007; FAVV, 2004, 2006a,b, 2007, 2008b,c, 2009a). The pathogens, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni/coli*, *Bacillus cereus* and *Clostridium perfringens* (or sulphite reducing clostridia (SRC)) were considered and targeted for data collection. Additional, for pork meat *Yersinia enterocolitica* and for shellfish *Vibrio parahaemolyticus* were considered. Setting up the scoring system, including the gathering of all data for scoring the three parameters, was performed over a ca. one-year period with bimonthly discussion between the authors on the outcome of the scoring and was also validated with authors' expert opinion from a track record built in education, research and extension services in food microbiology and food preservation.

Table 3.1. Categorization and risk attribution of raw materials in the inventory list of the food service operation.

Food Category	Food Type	Examples of food products	Epidemiological association score 0 = no collective food toxi-infections between 1998-2008 score 1 = 1 collective food toxi-infection between 1998-2008 score 2 = >1 and ≤ 5 collective food toxi-infections between 1998-2008 score 3 = > 5 collective food toxi-infections between 1998-2008	Prevalence score 0 = accidental prevalence (< 0.1%) score 1 = low prevalence (>0.1% and ≤1%) score 2 = moderate prevalence (>1% and ≤10%) score 3 = high prevalence (>10%)	Possibility to grow/survive score 0 = no growth/survival and inactivation/inhibition score 1 = growth/survival and inactivation/inhibition score 2 = no growth, but survival possible and no inactivation/inhibition score 3 = growth/survival and no inactivation/inhibition	Risk category (sum of scores)
Milk and dairy products	Heat processed (thermized/ pasteurized) dairy products	Milk based desserts, ice cream, chocolate mousse, butter	2	3	1	6
	Fermented or acidified dairy products	Gouda cheese, Emmental cheese, cream cheese	2	2	1	5
	Dried	Milk powder	2	2	1	5
(Red) Meat and meat products	Fresh/ undercooked (raw) meat	Beef steak	2	3	3	8
	Heat processed meat products	Cooked ham, wiener schnitzel, Italian schnitzel, merguez, meat loaf, fried beef burger	2	2	1	5
	Fermented meat products	Salami	2	2	2	6
Poultry and poultry products	Heat processed poultry products	Cooked turkey/chicken breast, fried chicken sausage, fried chicken leg, turkey meat loaf	3	1	1	5
Fish and seafood products	Raw (frozen) fish and seafood	Pollock fillet, hake fillet, white fish fillet, salmon steak, tuna steak, cod fillet, salmon fillet, fish cubes	2	2	3	7
	Heat processed fish and seafood	oceans gold, fish sticks	1	2	1	4
	Marinated and acidified fish and seafood	Marinated anchovy	1	0	2	3
Eggs and derivates	Heat-processed eggs and derivates	Cooked eggs	3	0	1	4
Vegetables and fruits	Unprocessed fresh vegetables and fruits	Orange, apple, banana, nectarine, pear	1	2	2	5
	Pre-cut fresh vegetables and fruits (ready-to-eat)	Mixed pre-cut lettuce, pre-cut cucumber slices, pre-cut tomatoes, grated carrots, grated red cabbage, grated white cabbage, pre-cut chicory	3	1	3	7
	Heat processed vegetables and fruits (juices)	Orange juice	0	0	0	0
	Fermented/acidified vegetables	Pickles, olives	0	0	2	2
	Dried vegetable products (a_w < 0.6)	Seasonings, dried herbs	2	3	2	7
	Canned (ambient stable) vegetables and fruits	Asparagus	1	0	0	1
Composite foods	Ready-to-cook, ready-to-reheat	cauliflower in cheese sauce,	2	2	1	5

	refrigerated food products	braised chicory, braised red cabbage, salsify in milk sauce, nasi goreng, lasagna, macaroni, thai noodles, béchamel, curry, pepper sauce, meat sauce, meat stew, babi pangang, goulash, soups, meatballs in tomato sauce, vol-au-vent, fried chicken legs, fish stew, baked cordon blue, vegetarian bolognaise, quorn, mixed grill brochette, tika masala, …				
	Ready-to-cook, ready-to-reheat frozen food products	Spinach with herb cheese, sprouts with bacon, leek in cream sauce, broccoli with cream, nasi rolls, lasagna florentine, tortellini quattro formaggi, pancake rolls, moussaka, mashed potatoes, vegetarian spring rolls, swiss steak, tirolerschnitzel, gyros, …	2	2	1	5
	Ready-to-eat refrigerated food products with substantial raw ingredients	Feta salad, taboulé, Mexican salad, greek salad, celery salad, couscous salad, Bulgarian salad, beet salad	3	2	2	7
	Ready-to-eat emulsified or non-emulsified sauces mixed with raw materials	raw meat spread	1	3	3	7
	Ready-to-eat emulsified or non-emulsified sauces mixed with heat treated materials	Egg sandwich spread, ham sandwich spread, tuna sandwich spread, chicken sandwich spread, fish sandwich spread, shrimp sandwich spread, crab sandwich spread, salmon sandwich spread	2	3	1	6
	Ambient stable acid products, emulsified and non-emulsified sauces	Ketchup, mayonnaise, vinegar, mustard, tartar, yoghurt vinegar, tabasco	2	2	1	5
Chocolate, bakery and confectionary products	Dry & sugared low moisture (a_w < 0.85)	Pre-baked and pre-packed French baguettes	2	2	1	5
	Dry & sugared low moisture (a_w < 0.65)	Chocolate paste, honey, ginger bread, sugar	2	2	1	5
Beverages	Alcohol with < 10% alcohol	Beer, wine	0	0	0	0
	Nonalcoholic, carbonated	Soda water	0	0	0	0
	Nonalcoholic, non-carbonated	Bottled water	0	0	3	3

Table 3.2. Example of the motivation of the risk characterization for food category meat and meat products.

Food type	Epidemiological Association	Motivation score 0 = no collective food toxi-infections between 1998-2008 score 1 = 1 collective food toxi-infection between 1998-2008 score 2 = >1 and ≤ 5 collective food toxi-infections between 1998-2008 score 3 = > 5 collective food toxi-infections between 1998-2008	Prevalence	Motivation score 0 = accidental prevalence (< 0.1%) score 1 = low prevalence (>0.1% and ≤1%) score 2 = moderate prevalence (>1% and ≤10%) score 3 = high prevalence (>10%)	Possibility to grow/survive	Motivation score 0 = no growth/survival and inactivation/inhibition score 1 = growth/survival and inactivation/inhibition score 2 = no growth, but survival possible and no inactivation/inhibition score 3 = growth/survival and no inactivation/inhibition	Risk category (sum of scores)
Fresh/ undercooked (raw) meat	2	2003: In France a total of 14 human cases *Salmonella enterica* subsp. *enterica* serotype Newport were reported. All cases reported have eaten horsemeat consumed as ground meat (11 cases, and consumed raw by at least 6 cases) or steak (3 cases). (Espié et al., 2003) 2005: In Belgium, contamination at a slaughterhouse resulted in one *S.* Ohio outbreak with 60 known cases. At the same time, an increase of *S.* Ohio was observed among results from the nationalmonitoring programme of pork products. PFGE typing confirmed the clonal relationship between the human isolates and those isolated from pork products. Further epidemiological investigations confirmed the link to the slaughterhouse. (EFSA, 2007) 2008: In Switzerland, 150 cases were infected by S. Typhimurium. Molecular typing of clinical and food isolates revealed that pig meat or products thereof were probably responsible for the infections. About 34% of the cases were infected with the same strain detected in the quality control of pork at a company, on a pig carcass from a slaughterhouse and in an imported (from Germany) spare rib sample. (EFSA, 2010)	3	The prevalence of *Salmonella* on bovine carcasses is between 0,1% and 7,5% (EFSA, 2007) After slaughter, *Campylobacter* can be found in 19 to 70% of sheep carcasses, in 2 to 32% of bovine carcasses, in 20 to 97% of veal carcasses and in 20 to 60% of pork carcasses. (ICMSF, 2005)	3	Listeria can grow in MAP packaged meat products and at a temperature of 4°C. *Salmonella* can grow under specified conditions at 10°C. (ICMSF, 2005)	8
Heat processed meat products	2	2000: 26 cases of listeriosis were identified in France by the National Reference Centre for Listeria. Results of a case control study carried out by the Institut de Veille Sanitaire and district health departments showed that the consumption of pork tongue in jelly was associated with infection with the outbreak strain. (de Valk et al., 2000) 2004: The United Kingdom reported a large *E. coli* O157 outbreak affecting 134 people. Cooked meat sandwiches from a single shop were identified as the source. (EFSA, 2006) 2006: In Portugal, sandwiches with cooked meat	2	*Listeria* has an average prevalence of 2.7% in pork meat and 3.5% in cooked bovine meat (EFSA 2007b) In 2007 the prevalence of Listeria in cooked meat is 2% (FAVV 2008)	1	Listeria can grow in MAP packaged meat products and at a temperature of 4°C. *Salmonella* can grow under specified conditions at 10°C. (ICMSF, 2005). However, growth is only possible when a post-contamination takes place, because a heat inactivation step is involved in the production of these food types.	5

		served at a school picnic affected 25 people, all of whom were hospitalised. *E. coli* was laboratory confirmed to be the causative agent. (EFSA, 2007) 2007: Belgium reported a *S. aureus* outbreak from frozen hamburgers at a summer camp. At least 15 children and adults had severe symptoms of nausea, vomiting and diarrhoea shortly after eating lunch. The Dutch inspection revealed that the cooling system used to rapidly cool the cooked hamburgers was contaminated with *S. aureus* and may not have been properly cleaned. (EFSA 2009). 2001: In Spain, 181 cases have been reported in an outbreak of Escherichia *coli* O157:H7 infection. Preliminary enquiries suggested that the vehicle of infection was sausage served by a catering company on 18 September 2000. The catering company supplied 10 schools, one factory, and a home for elderly people. Cases arose at schools where the sausages were not reheated; at the remaining schools the sausages had been reheated. (Hernández et al., 2001)					
Fermented meat products	2	2005: Germany reported a nation wide outbreak involving 487 cases of gastroenteritis due to *S.* Bovismorbificans during the winter of 2004/2005. Consumption of raw minced pork and short-time fermented raw-pork sausage were strongly associated with infections in a case-control study. (EFSA, 2007) 2006: A small but severe outbreak was reported by Norway. The outbreak was caused by verotoxigenic *E. coli* O103:H25 involving 17 persons. The source of infection was laboratory confirmed to be a traditional Norwegian sausage (morrpoelse) made from sheep (EFSA, 2007) 2007: In Germany, an increase in cases with *Salmonella* Panama infections was detected and more than 30 young children were infected. A case-control study implicated consumption of short-fermented minisalami sticks as the likely source of infection (EFSA, 2009) 2007: In Denmark, an outbreak with verotoxigenic Escherichia *coli* (VTEC) O26:H11 occurred with 20 laboratory confirmed cases. The outbreak strain was confirmed microbiologically in both the raw meat and the sausages obtained from private households who had bought the sausage but which had not yet been consumed (EFSA 2009).	2	Listeria has a prevalence of 16% (2000), 8,6% (2001) and 10% (2003) (FAVV, 2004) in dry sausage In 2006, the prevalence of dry sausage in Germany was 9.6% (EFSA 2007)	2	*E. coli* O157 can survive the fermentation process. (ICMSF, 2005) Listeria can grow in these types of products if the fermentation process fails, with normal fermentation Listeria can not grow (ICMSF, 2005)	6

3.2.3 Elaboration of the sampling plan

The risk attribution, described in the above paragraph, was used for selection of food types to be taken up in the sampling plan because the range of incoming (raw) materials and foods served in the food service operation is broad. Assumptions made in the development of the approach are i) the sampling plan should only focus on high risk categorized food types (score 7, 8 or 9), ii) for low risk food types (score 0, 1, 2 or 3) it should be sufficient to set microbiological specifications to the supplier and iii) for moderate risk food categories (score 4, 5 or 6) reports of microbiological analysis should be asked regularly from suppliers to verify compliance of the microbiological specifications agreed upon. To determine the amount of samples to be taken for each high risk food type, the following approach is proposed. In case of more than one food product within a food type (presence of variety) and/or when each year more than two suppliers can be involved in the delivery of the food product(s) within a food type, the approach based on binomial distribution as described by Evers (2001) and ICMSF (1986) is proposed. Table 3.3 lists the number of samples needed, to detect a positive result at an indicative level of the fraction positive samples within a range of 0.1 % - 10 % and a probability of 90 %, 95 % or 99 %. It is proposed to link the probability and the allowed fraction of positive samples to the specific risk score of the food type. The idea behind this approach is that when the risk of the food type to cause food safety problems or to be associated with unacceptable quality is lower, a smaller amount of samples is needed to be taken in the frame of verification (ICMSF, 1986). However, in case of only one food product within a food type (no variety) and if yearly no more than two suppliers are involved in the delivery of this food product, it is proposed to take one sample/month. The idea behind this approach is that those food types containing only one specific food product should be diminished in sample size to balance the ratio samples/supplier with the other food categories where multiple food products are included within a food type supplied by multiple suppliers. Moreover, upon discussion with the quality manager it was revealed that the frequency of consumption of those food types containing only one specific food product, is only half of the food products within the other food types.

Table 3.3. Number of samples required for a certain confidence level in order to detect a maximum fraction of contamination given all samples are negative (Evers, 2001; ICMSF, 1986).

Fraction positive samples (in %)	Confidence level		
	90%	95%	99%
0.1	2 300	2 991	4 593
0.2	1 150	1 496	2 299
0.3	767	998	1 533
0.4	575	748	1 149
0.5	460	598	919
0.6	384	499	766
0.7	329	427	656
0.8	288	374	574
0.9	256	332	510
1	230	299	459
2	115	149	229
3	77	99	152
4	57	74	114
5	46	59	91
6	38	49	75
7	33	42	64
8	29	37	56
9	25	33	50
10	23	29	45

3.2.4 Implementation of the sampling plan at the selected food service operation

The sampling plan was implemented during a one-year period (September 2009 to October 2010) to test the feasibility of the chosen approach. According to the above mentioned approach and taking into account the food service operation's limitation of resources for microbiological analyses, it was decided to use for food products with risk score 7 a probability of 95 % and an indicative maximum set fraction of positive samples of 10 % which means that 29 samples have to be taken for those food types. For food products with a risk score 8 it is proposed to use a probability of 95 % and an indicative maximum set fraction of positive samples of 5 % which means that 59 samples have to be taken for those food types. For food products with a risk score 9 it is proposed to use a probability of 95 % and an indicative maximum set fraction of positive samples of 2 % which means that 149 samples have to be taken for those food types. However, food products with a risk score 9 were absent in the case study and therefore could not be sampled. In case of undercooked meat, dried vegetable products (aw < 0.6) and emulsified or non-emulsified sauces mixed with raw ingredients, only one food product is present within this particular food type and it is delivered by maximum two suppliers each year. Therefore a total of 12 samples (one

sample/month) for each of these food types was taken according to the assumed approach resulting in a maximum set fraction of positive samples of 22 % (with a probability of 95 %) (Table 3.4). In this case study a total of 123 samples of incoming raw materials and 87 samples of meal servings (end products or its components) at the serving counter were taken and analyzed for multiple parameters (spoilage flora, hygiene indicators and pathogens) (Tables 3.4 and 3.5). After aseptically sampling, the food samples were transported in an icepack cooled, insulated cool box at ≤ 4°C to the laboratory. Microbiological analyses were performed in the laboratory within 6 h of sample collection. For enumeration, 10 g of each food sample was homogenized for 2 min in 90 ml of sterile peptone water. For detection of *Salmonella* and *L. monocytogenes*, a 25 g subsample was weighed in a stomacher bag and homogenized for 2 min in 225 ml of the respective (semi)selective medium, i.e. buffered peptone water for the detection of *Salmonella* and demi-Fraser for the detection of *L. monocytogenes*. Tenfold serial dilutions were made in sterile peptone water for microbial enumeration. For each parameter standardized methods (ISO) or alternative (rapid) methods validated according to ISO 16140:2003 were applied. A modified ISO 4833:2003 method (plating on PCA and 120 h incubation at 22°C) was used for the enumeration of aerobic psychrotrophic count; ISO 15214:1998 (plating on MRS with overlayer and 72 h incubation at 30°C) for the enumeration of lactic acid bacteria); YGC selective medium (Bio-Rad, USA) (72 h incubation at 22°C), an AFNOR validated method (n° NF V08-059:2002), was used for the enumeration of yeast; Coli-ID chromogenic medium (Biomérieux, France) (24 h incubation at 44°C) an AFNOR validated method (n° BIO 12/5 e 01/99) for the enumeration of *E. coli*; ISO 21528-2:2004 (plating on VRBG with overlayer and 24 - 48 h incubation at 37°C) for the enumeration of Enterobacteriaceae; ISO 6888-1:1999/Amd1:2003 (plating on BP and incubation 24 - 48 h at 37°C) for the enumeration of *Salmonella* aureus. Enumeration of B. cereus was performed with ISO 7932:2004 (plating on MYP and 24 h incubation at 30°C). The detection of *L. monocytogenes* was performed using VidasLMO2 (Biomérieux, France), an AFNOR validated Enzyme-Linked Fluorescent assay (ELFA-) method (n° BIO-12/11-03/04). When positive results were obtained, enumeration of *L. monocytogenes* was performed on a sample of the positive food product according ISO 11290-2:1998/Amd1:2004. Detection of *Salmonella* spp. was performed using Vidas Easy SLM (Biomérieux, France) also an AFNOR validated ELFA-method (n° BIO-12/16-09/05).

Table 3.4. Overview of high risk categories of raw materials with their analysed parameters and microbiological criteria and guidelines

Food Category	Food Type	Examples of food products	Risk Category	Number of proposed samples	Number of analyzed samples	Parameters analyzed	Microbiological criteria & guidelines Acceptable levels at used by date/best before date
(Red) Meat and meat products	Fresh/undercooked (raw) meat	Beef steak	8	59	12	TVC	10^7 CFU/g
						LAB	10^7 CFU/g
						Yeast	10^5 CFU/g
						E. coli	10^3 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g[a]
						L. monocytogenes	10^2 CFU/g[a]
Fish and seafood products	Raw (frozen) fish and seafood	Pollock fillet, hake fillet, white fish fillet, salmon steak, tuna steak, cod fillet, salmon fillet, fish cubes	7	29	29	TVC	10^7 CFU/g
						LAB	10^7 CFU/g
						Yeast	10^5 CFU/g
						E. coli	10^3 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g
						L. monocytogenes	10^2 CFU/g[a]
Vegetables and fruits	Pre-cut fresh vegetables and fruits (ready-to-eat)	Mixed pre-cut lettuce, pre-cut cucumber slices, pre-cut tomatoes, grated carrots, grated red cabbage, grated white cabbage, pre-cut chicory	7	29	29	TVC	10^8 CFU/g
						LAB	10^7 CFU/g
						Yeast	10^5 CFU/g
						E. coli	10^3 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g[a]
						L. monocytogenes	10^2 CFU/g[a]
	Dried vegetable products ($a_w < 0.6$)	Seasonings, dried herbs	7	29	12	TVC	10^4 CFU/g
						LAB	10^4 CFU/g
						Yeast	10^3 CFU/g
						E. coli	10^2 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g
						L. monocytogenes	10^2 CFU/g[a]
						B. cereus	10^4 CFU/g
Composite foods	Ready-to-eat refrigerated food products with substantial raw ingredients	Feta salad, taboulé, Mexican salad, greek salad, celery salad, couscous salad, Bulgarian salad, beet salad	7	29	29	TVC	10^7 CFU/g
						LAB	10^7 CFU/g
						Yeast	10^5 CFU/g
						E .coli	10^3 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g
						L. monocytogenes	10^2 CFU/g[a]
						B. cereus	10^5 CFU/g
	Ready-to-eat emulsified or non-emulsified sauces mixed with raw materials	raw meat spread	7	29	12	TVC	10^7 CFU/g
						LAB	10^7 CFU/g
						Yeast	10^5 CFU/g
						E. coli	10^2 CFU/g
						Coagulase positive Staphylococci	10^3 CFU/g
						Salmonella spp.	Absence in 25g
						L. monocytogenes	10^2 CFU/g[a]

Table 3.5. Overview of high risk meal servings with their analysed parameters and microbiological criteria and guidelines.

Food Category	Food Type	Risk category	Number of proposed samples	Number of analyzed samples	Parameters analyzed	Microbiological criteria & guidelines
						Acceptable levels at used by date/best before date
Composite Foods	Salads	7	29	29	TVC	10^8 CFU/g
					LAB	10^7 CFU/g
					Yeast	10^5 CFU/g
					E.coli	10^3 CFU/g
					Coagulase positive Staphylococci	10^3 CFU/g
					Salmonella spp.	Absence in 25g
					L. monocytogenes	10^2 CFU/g[a]
	Sandwiches	7	29	29	TVC	10^8 CFU/g
					LAB	10^7 CFU/g
					Yeast	10^5 CFU/g
					E.coli	10^3 CFU/g
					Coagulase positive Staphylococci	10^3 CFU/g
					B. cereus	10^5 CFU/g
					Salmonella spp.	Absence in 25g
					L. monocytogenes	10^2 CFU/g[a]
	Hot meals/ constituents	7	29	29	TVC	10^6 CFU/g
					E.coli	10^3 CFU/g
					Enterobaccteriaceae	10^3 CFU/g
					Coagulase positive Staphylococci	10^3 CFU/g
					B. cereus	10^5 CFU/g
					Salmonella spp.	Absence in 25g
					L. monocytogenes	10^2 CFU/g[a]

[a] **according COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs**

3.2.5 Interpretation of results and feedback on elaborated sampling plan

Data processing was carried out with Microsoft Office Excel in order to develop graphics and tables to visualize the concentrations and the distribution of microbiological contamination. The results were compared with defined legal criteria (Anonymous, 2005, 2009). In the absence of legal criteria, microbiological threshold values established by the Laboratory of Food Microbiology and Food Preservation of Ghent (LFMFP-UGent) (Uyttendaele et al., 2010) were used for comparison (Tables 3.4 and 3.5). When the results of the analysis exceed the threshold levels at end of shelf life (used by date/best before date), the results are indicated as unsatisfactory. After the one-year study, the results were evaluated and discussed with the quality manager of the food service operations and suggestions for further fine-tuning of the sampling plan were provided.

3.3 Results

3.3.1 Food categorization and risk attribution

Table 3.1 gives an overview of the identified food categories and food types on the inventory list of the food service operation and their attributed risks based on the scoring system. The major food categories are based upon origin/species/commodity of the food and encompass: milk and dairy products; (red) meat and meat products; poultry and poultry products; fish and seafood products; eggs and derivates; vegetables and fruits; composite (multi-ingredient) foods; chocolate, bakery and confectionary products; and beverages. Low risk food types (scores 0-3) present in the food service operation are marinated and acidified fish and seafood (e.g. anchovy); heat processed vegetables and fruits juices (e.g. orange juice); fermented/acidified vegetables (e.g. pickles); canned (ambient stable) vegetables and fruits (e.g. canned asparagus) and those food types belonging to the food category beverages (e.g. carbonated and non-carbonated bottled water). Moderate risk food types (scores 4-6) are heat processed (thermized/pasteurized) dairy products (e.g. milk based desserts); fermented or acidified dairy products (e.g. Gouda cheese); dried milk and dairy products (e.g. milk powder); heat processed meat products (e.g. cooked ham); fermented meat products (e.g. salami); heat processed poultry products (e.g. cooked turkey breast); heat processed fish and seafood (e.g. fish sticks); heat-processed eggs and derivates (e.g. cooked eggs); unprocessed fresh vegetables and fruits (e.g. oranges); ready-to-cook/reheat refrigerated composite food products (e.g. nasi goreng); ready-to-cook/reheat frozen composite food products (e.g. spinach with herb cheese); emulsified or non-emulsified sauces mixed with heat treated materials (e.g. mayonnaise based chicken sandwich spread); ambient stable acid products (e.g. ketchup); dry and sugared low moisture ($a_w < 0.85$) chocolate, bakery and confectionary products (e.g. prebaked and pre-packed French baguettes) and dry and sugared low moisture ($a_w < 0.65$) chocolate, bakery and confectionary products (e.g. chocolate paste). High risk food types (scores 7-9) are undercooked meat (e.g. beef steak); raw (frozen) fish and seafood (e.g. hake fillet); pre-cut fresh vegetables and fruits ready-to-eat (e.g. mixed pre-cut lettuce); dried vegetable products ($a_w < 0.60$) (e.g. seasonings); ready-to-eat refrigerated composite food products with raw ingredients (e.g. feta salad) and ready-to-eat emulsified or non-emulsified sauces

mixed with raw materials (e.g. raw meat spread). The high risk meal servings (or its components) can be categorized as salads, sandwiches and hot meals (Table 3.5) and are the result of the main production processes present in food service operation (Lahou et al., 2012).

3.3.2 Implementation of the elaborated sampling plan for verification of supplier selection and microbial quality and safety of end products

3.3.2.1 Raw materials

The concentrations and variability of the total viable count (TVC), lactic acid bacteria (LAB) and yeasts as well the microbiological threshold values as recommended in the guidelines of LFMFP-UGent (Uyttendaele et al., 2010) for the end of shelf life are shown in Figure 3.1. As the samples were analyzed, somewhere between production and end of shelf life, when they were supposed to be eaten, exceeding the threshold value of end of shelf life (i.e. worst case scenario) for these parameters may result in early spoilage and unacceptable sensorial quality, but in general will not cause harm to human health. TVC, LAB and yeasts relate to overall quality and unsatisfactory samples represent, if temperatures are respected (< 4°C), less qualitative products which may not in accordance with the specifications of the suppliers. The samples of the food type “pre-cut fresh vegetables and fruits (ready-to-eat)”, such as mixed lettuce, mixed bell peppers, red and white cabbage, are high (> 10^6 CFU/g) in overall microbial contamination (TVC) and high (> 10^4 CFU/g) in spoilage flora such as LAB and yeasts. However, ready-to-eat, raw, pre-cut vegetables often have high and variable microbial contamination due to contact with the environment pre-harvest (soil, water) or because they are prone to cross-contamination during washing and mixing in fresh-cut processing (Holvoet et al., 2012; Olaimat and Holley, 2012; Tzschoppe et al., 2012). Therefore, sensorial quality of fresh produce is rather linked to physiological processes such as anaerobic respiration or enzymatic browning and is often better assessed visual (Jacxsens et al., 2002). It is to a lesser extent related to levels of microbial quality indicators such as TVC, although yeasts and LAB are specific spoilage bacteria which can influence taste and odor of these products (Jacxsens et al., 2003).

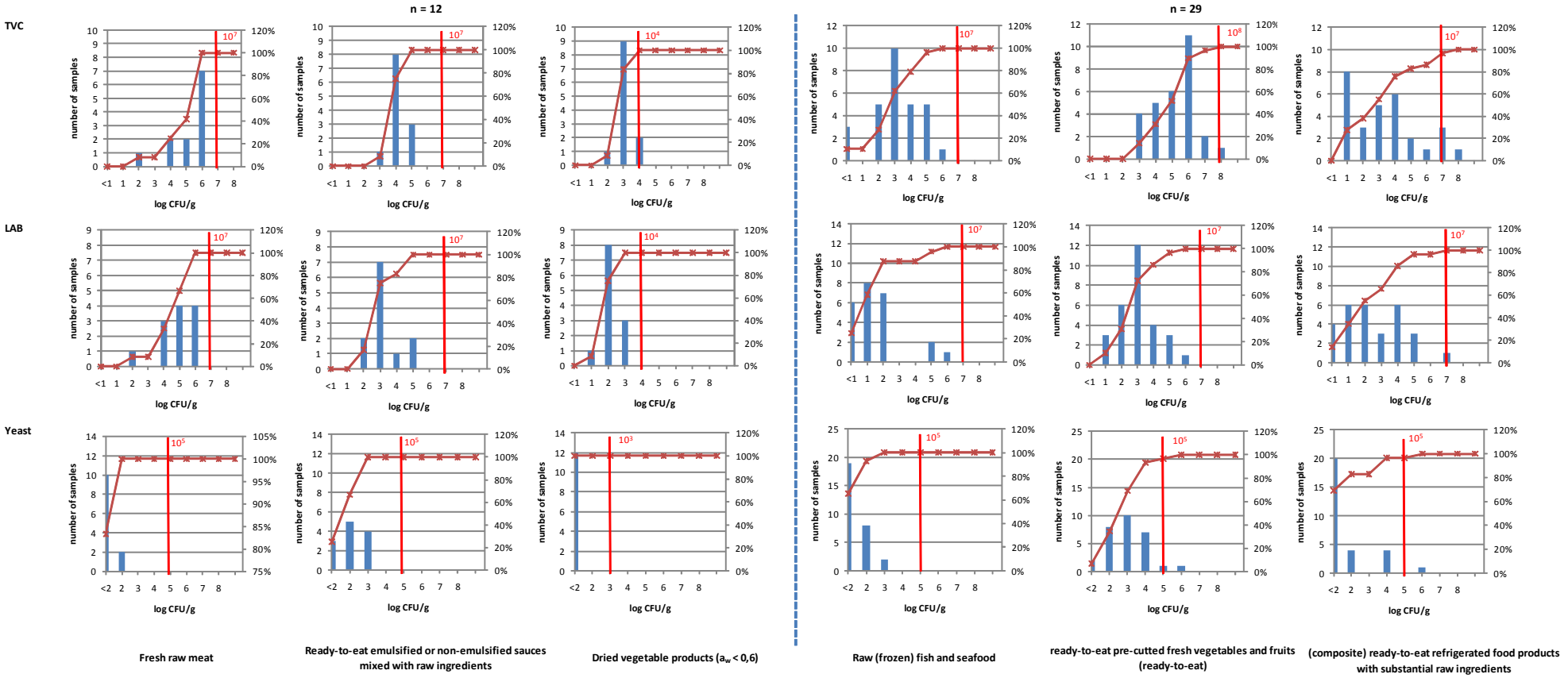


Figure 3.1. Overview of concentrations (in log CFU/g) and variability of spoilage indicators of high risk raw materials used in food service operation. On the right axis the cumulative percentage is shown, while the microbiological reference value at end of shelf life is shown as a vertical line in the graph. N, number of samples; TVC, total viable count; LAB, lactic acid bacteria.

Dried vegetable products such as dried spices are high (> 10^4 CFU/g) in TVC, but this is generally known. Vitullo et al. (2011) linked the presence of these high aerobic mesophilic bacteria to poor initial quality, cross-contamination or conditions that promote microbial growth. Also variety in levels of TVC of raw (frozen) fish and fresh/ undercooked meat (beef steak) were observed, where many samples of the meat and one sample of raw fish showed high counts (> 10^6 CFU/g) of TVC. Whereas assumed that good manufacturing and hygiene practices during slaughter and storage/distribution would be better able to realize products with lower contamination levels and less variation in contamination loads, a more strict control and interaction with suppliers may provide improvement (Jacxsens et al., 2009a). Emulsified or non-emulsified sauces mixed with raw materials show higher variation of LAB and two samples (n= 12) showed high microbial counts (> 10^5 CFU/g). In these food products LAB are the main spoilage flora (and thus coincides with TVC). Overall, high microbial counts (>10^5 CFU/g) for TVC were observed for three samples (n = 12) just before use and consumption. Therefore no direct safety problem is assumed but no further prolonged storage of these products is recommended to prevent exceeding the microbiological threshold values and quality deviation. All results of the hygiene indicators are summarized in Table 3.6. The presence of enumerable *E. coli* was observed in 3 of the 88 samples (once in raw meat spread, once in lettuce and once in cucumber), although the analysis only indicated an estimate of mere presence of *E. coli* at ca. 10 CFU/g (detection limit of the enumeration method). *S. aureus*, an indicator of hygiene related to contamination of foods by humans, was not detected (< 10^2 CFU/g) in any of the samples (n= 88) analyzed. Results of the food safety analyses are summarized in Table 3.6. *Salmonella* spp. (n= 112) were not detected. *L. monocytogenes* was detected in 11 of the 115 samples, in particular in (RTE) raw meat spread (5 of 12 samples) and raw fish (6 of 27 samples). All positive raw meat spread samples were below 10 CFU/g (detection limit of enumeration method) and thus below the maximum acceptable level of 10^2 CFU/g at the time of consumption as stated in Regulation EC No. 2073/2005 for RTE foods. Raw fish samples contained low numbers of the pathogen (< 10 CFU/g) as well and if they were supposed to be eaten raw, they would have been within acceptable limits of the Regulation EC No. 2073/2005. Therefore, the presence of *L. monocytogenes* in these fish samples merely indicates that there is a “pressure” for *L. monocytogenes* contamination in this product (from fish catching or production environment) and indicates in this case there might be a better hygiene.

Table 3.6. Detection and unsatisfactory results of hygiene indicators and pathogens.

Food type	Score	No. of samples to be taken[a]	*E. coli*[c]	*Enterobacteriaceae*[c,2]	*S. aureus*[d,2]	*B. cereus*[d]	*Salmonella* spp.[5]	*L. monocytogenes*[1]
Fresh/undercooked (raw) meat	8	12	0/12[2]	-	0/12	-	0/12	0/12
Ready-to-eat emulsified or non-emulsified sauces mixed with raw materials	7	12	1/12[1]	-	0/12	-	0/12	5/12
Dried vegetable products ($a_w < 0.6$)	7	12	0/12[1]	-	0/12	2/12[2]	0/12	0/12
Raw (frozen) fish and seafoods	7	29	0/29[2]	-	0/29	-	0/24	6/27
Pre-cut fresh vegetables and fruits (ready-to-eat)	7	29	2/23[2]	-	0/23	-	0/23	0/23
(Composite) ready-to-eat refrigerated food products substantial raw ingredients	7	29	0/29[2]	-	0/29	2/6[4]	0/29	0/29
Total raw materials		**123**	**3/88**	**-**	**0/88**	**4/18**	**0/112**	**11/115**
Sandwiches	7	29	0/29[2]	-	1/29	-	0/29	1/29
Salad bar	7	29	0/29[2]	-	0/29	0/6[4]	0/29	4/29
Hot meals	7	29	0/29[2]	0/29	0/29	0/29[4]	0/29	0/29
Total end products		**87**	**0/87**	**0/29**	**1/87**	**0/35**	**0/87**	**5/87**
Total detected[b]		***210***	***3/175***	***0/29***	***1/175***	***4/53***	***0/199***	***16/202***
Total unsatisfactory[e]		***210***	***0/175***	***0/29***	***0/175***	***0/53***	***0/199***	***0/202***

[a] Samples analyzed can deviate for hygiene indicators and/or pathogens depending on the type of food product sampled.
[b] Samples are indicated as detected when micro-organisms are present (after enrichment) or above the detection limit of the method (in case of enumeration).
[c] Detection limit of 10 CFU/g
[d] Detection limit of 100 CFU/g,
[e] Samples are unsatisfactory when the results exceed following threshold values at end of shelf life [1] $> 10^2$, [2] $> 10^3$, [3] $> 10^4$, [4] $> 10^5$, or when [5] presence/25g.

However, the presence of *L. monocytogenes* should be avoided in incoming materials because this increases the pressure on the FSMS of the food service operation. For example, proper cleaning and disinfection procedures should be applied in the food service operation to control cross-contamination of *L. monocytogenes*. *B. cereus* was enumerated in 4 of 18 samples (in particular in pasta salad, potato salad and seasonings), but only in low levels indicating the mere presence of the *B. cereus* at levels near the detection limit of the method (10^2 CFU/g) and thus in compliance with good hygienic practices and far below the overall accepted safety level of 10^5 CFU/g (Uyttendaele et al., 2010). Although the presence of low levels of *L. monocytogenes* and *B. cereus* does not immediate raise public health concerns and are in compliance with current national action limits or EU legislation. Still the mere presence of these pathogens demands continuous attention of implementation of adequate control measures in the processing line of the meals preparation, e.g. low temperatures and short storage times, are necessary to prevent the growth of these pathogens in those raw materials. Proper cleaning and disinfection of infrastructure, equipment and utensils is needed to prevent the persistence and formation of biofilms by these pathogens.

3.3.2.2 Served meals

The concentrations and variability of the quality indicators (TVC, LAB, yeasts) and the microbiological threshold values as recommended in the microbiological guidelines of LFMFP-UGent (Uyttendaele et al., 2010) are indicated in Figure 3.2. The results for the quality indicators of the raw materials, especially of the ready-to-eat, pre-cut vegetables are also reflected in the results of the spoilage indicators of the sandwiches and salad bar, which is mainly composed of those raw fresh-cut pre-packed vegetables. Higher TVC count of the vegetables leads inevitably to higher TVC count in the served meals, especially sandwiches and salads. Therefore, TVC is not considered a good indicator of overall quality and good practices during production of those served meals. For hot meals, as expected due to prior heat treatment, mainly TVC levels of < 10^3 CFU/g were observed. High variability for LAB was observed, which is a reflection of the variable quality of the raw materials used. In 4 of 58 sandwich and salad samples unacceptable levels (> 10^7 CFU/g) of LAB were

observed. Unacceptable levels (> 10^5 CFU/g) for yeasts were observed in 4 of 29 salad samples.

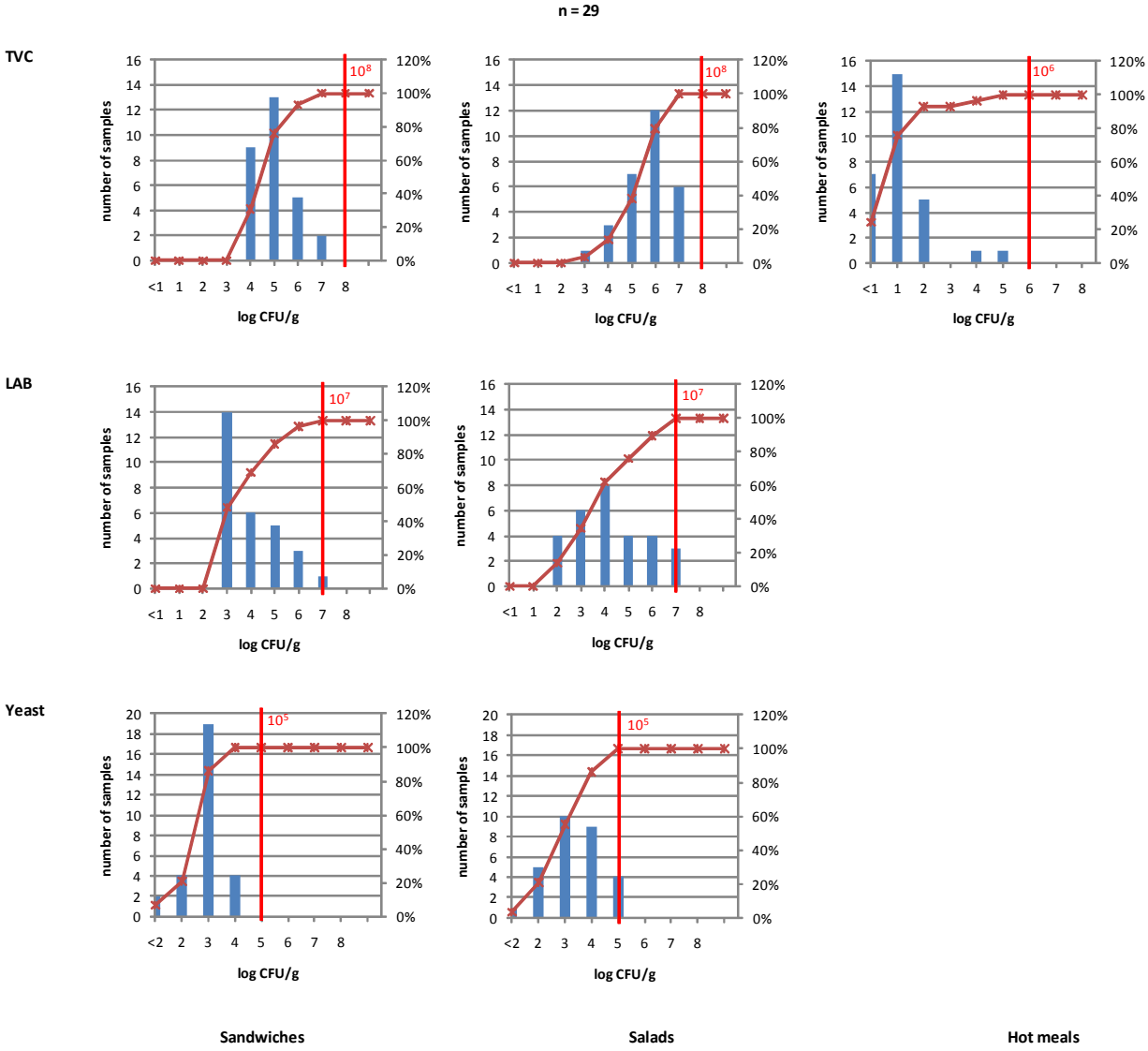


Figure 3.2. Overview of concentrations (in log CFU/g) and variability of spoilage indicators of high risk meals produced in food service operation. On the right axis the cumulative percentage is shown, while the microbiological reference value at end of shelf life is shown as a vertical line in the graph. n, number of samples; TVC, total viable count; LAB, lactic acid bacteria.

Results of the hygiene indicators and pathogens are summarized in Table 3.6. *E. coli* was not detected, meaning it was below the detection limit of the enumeration method (10 CFU/g) in all 87 samples. Also *Enterobacteriaceae* are below the detection limit of the enumeration method (10 CFU/g) in all 29 samples. *S. aureus* was enumerated in 1 of 87 samples analyzed (presence estimated at 10^2 CFU/g). This indicates that there is no major problem with the

overall hygiene in the food service operation. As for the pathogens, *L. monocytogenes* could be detected in 5 of the 87 samples (in particular in sandwiches and salads with raw meat spread), but if present the maximum acceptable level of 10^2 CFU/g at the time of consumption (Anonymous, 2005) was not exceeded. Upon enumeration of *L. monocytogenes* in the positive samples, none of the samples exceeded the level of 10 CFU/g (detection limit of the enumeration method). Contamination of *L. monocytogenes*, which seems to have taken place at the manufacturer operation, could not be reduced because no heating step takes place for this meal type in the food service operation. Therefore as a corrective action, intensification of sampling for this particular food type by supplier is needed and contact needs to be taken with the supplier to inquire on the performance of his GMP and HACCP plan to control contamination of *L. monocytogenes*. The low concentration of the pathogen in the sandwiches and salads indicates that on the occasion of this sampling event well controlled storage conditions are applied in the food service operation not enabling outgrowth to numbers above the limit of 100 CFU/g at the time of consumption. As well, no *L. monocytogenes* was detected in hot meals which indicates that separated storage facilities and a forward flow among raw materials and hot meals are present in the food service operation reducing the risk of cross-contamination.

3.4 Discussion

Categorizing all food items on the inventory list of the institutional food service operation, by itself, is a challenging issue. Many approaches exist to set up food categories, but the methodology behind them depends on the goal (EFSA, 2008, 2010; Greig and Ravel, 2009; Painter et al., 2009). Many of those approaches are made in the frame of consumption surveys and are thus mainly based upon the nutritional value and composition of foods. In the present study the food categorization is made to underpin the development of a focused microbiological sampling plan to contribute to the verification of supplier selection and the functioning of the prerequisite and HACCP program in an institutional food service operation. The chosen approach for food categorization is based upon commodity type and the processing and preservation method applied. This type of food categorization has previously been used to define food categories in ISO 16140 (the technical protocol for

validating alternative methods in the field of microbiological analysis of food, animal feeding stuffs). However, the processing method is not always taken into account. Processing methods are important in reducing the risk of a particular food type and therefore it is of interest to be taken up in the food categorization of this study. Moreover, composite foods are typical products used or produced in food service operations and therefore were assigned to an individual food category in this study. Composite foods are defined as those food products containing ingredients from more than one of the seventeen commodities. A distinction is made on the level of this category for the different food types based on ready-to-eat and ready-to-reheat/ready-to-cook (Daelman et al., 2013). A sampling plan for either incoming raw materials (to contribute to the verification of supplier selection) or food dishes and sandwiches served to consumers (to verify hygienic practices and control measures to provide sensorial acceptable and safe foods) is preferably elaborated on the basis of a risk classification of food products. This enables to focus sampling of food products and to maximize information on the performance of the FSMS within the given limitation of resources. A scoring system was used which is based upon i) epidemiological association of the food type with reported foodborne outbreaks, ii) the reported prevalence of foodborne pathogens and level of hygiene indicators in the food type, and iii) the potential of microorganisms (pathogens but also spoilage microflora) to grow or survive during storage and/or further processing. However, as with any system used, bias can occur in the scoring of parameters for risk attribution e.g. due to the lack of notification, investigation or publication of foodborne outbreaks (Greig and Ravel, 2009; O'Brien et al., 2006). Also bias in prevalence data may occur because of restrictions/limitations in the number of samples taken and thus uncertainty associated to the obtained prevalence data. Therefore general knowledge derived from scientific literature and some standard information from books was supplemented with regional data taken from the annual EU baseline reports and the national monitoring programs. It is recommended that risk attribution to the food categories and food types is revised on a regular basis and kept up to date with available information on the hazards in foods. For example in the present proposal, Norovirus and the wide group of pathogenic verotoxin producing *E. coli* (VTEC) strains have not been included as microbiological parameters. Reported outbreaks in the EU or Belgium with non-O157 human pathogenic VTEC strains and its association with a food type are scarce. Norovirus outbreaks are often linked to institutional catering and food

handlers being identified as the source of infections but control measures would probably be more set in the processing line related to training of staff members handling food to pay attention to good hygienic practices and in particular hand washing. In addition Norovirus and the wide range on human pathogenic VTEC strains were not taken up in the sampling plan as well because the (ISO) standard methods of analysis are only published in 2013 and not yet established routine methods. Both Norovirus and VTEC detection use PCR (i.e. Polymerase Chain Reaction) methods (ISO/TS 15216:2013 and ISO/TS 13136:2012) which are currently still costly in execution, performance characteristics have not been fully established and relevance of the outcome of these PCR methods for public health risk is still debated. Still in due time, when more information on epidemiological association and methods are getting accessible and affordable also Norovirus and human pathogenic VTEC might be taken up in the sampling plan. Therefore, the present risk classification of food types and selection of microbiological parameters is not definite, but will need updates when more information gets available. Adaptations to the proposal will also be needed if applied in a food service operation in perhaps another country as in the present study there was a regional focus on EU and Belgium for the scoring of epidemiological association. Upon implementation of the food categorization and scoring of food items on the inventory list in the selected food service operation, it was noticed that only a limited number of food types were assigned a risk score of 7 or 8 and that no food types of risk score 9 were present in the inventory list. After feedback with the quality manager it became clear that the food service operation has reoriented their business since 2000 from on-site cooking to regeneration of half-fabricates. Their main processes can be identified as (i) the preparation of a sandwich and/or salad from pre-cut ready-to use vegetables, (ii) the preparation of a hot meal starting from unprocessed raw or (undercooked) frozen raw materials and preparation out of packaging (possibility of post-processing contamination) and (iii) production of a hot meal starting with cooked products in reheating bags (no post-processing contamination can occur) (Lahou et al., 2012). It was a strategic decision, partly as the consequence of the elaboration of the HACCP plan at that time, to reduce the risks by limiting the introduction of unprocessed (raw) high risk food products into the kitchen environment. This explains also the long list of composite foods and pre-cut fruits and vegetables (RTE). The limited number of class 7 and 8 risk food products which are currently present, are these food components for which no alternative (lower risk) product is

available to be taken in without affecting the sensorial characteristics. For example, fully processed (cooked) fish or beef steak is not available or purchased as such because it will be too dry to eat after a reheating step before serving. Another point of attention noted when discussing the sampling plan with the quality manager of the food service operation are the difficulties sometimes established in setting and control of specifications for the various at risk food categories, as was set for average and low risk food products. It was noted that incoming raw materials in the selected food service operation were not always directly supplied from manufacturers, but were bought via middlemen from wholesale business, which sell a wide range of food items. Therefore joint decision making on a set of specifications and in particular acquiring proof of compliance testing on the particular raw material (e.g. for at risk food categories of risk score 4 (or higher) as suggested in the sampling plan) was perceived to be difficult. This stresses thus the need to verify supplier selection and to perform analysis of the purchased raw materials, in particular for the high risk food categories (of risk score 7, 8 and 9) as suggested in this study, in frame of on-site control. The verification of suppliers' selection can be performed by visual controls for quality when food products are delivered, by complaints of customers, but also by sampling food products and analyzing them for different microbiological parameters (the frequency and type of parameters analyzed depending upon the food type). Sampling is providing objective results and reveals some information on the quality and safety of the products (which cannot be visually checked) and thus contributes to the verification of supplier selection. Therefore, once a supplier is selected and contracted and is delivering food products to the food service operation they (and their goods) should be evaluated in the framework of the food services' FSMS. Besides, good microbiological quality of incoming raw materials contribute to good microbiological quality end products when the prerequisite programs and HACCP system is performing well (Jacxsens et al., 2009b). This was noted in the outcome of the microbiological sampling of end products which was executed in the present study. However, as shown in this case study, it is not recommended to use TVC as an indicator of overall quality of incoming fresh produce. Because TVC is to a lesser extent related to sensorial quality, it is recommended to judge the quality of fresh produce visually, based on physiological processes such as anaerobic respiration and enzymatic browning. However, LAB and yeasts can still be assessed on fresh produce because these are specific spoilage bacteria which can influence the taste and odor of these

products. Besides, TVC is also no reliable indicator of good practices during the production of cold served end products, such as sandwiches and salads. However, TVC can still be useful to judge good practices during the production of hot meals. Although sampling plans have intrinsic limitations in assessing the quality and safety of the foods sampled, it was shown useful to reveal major non-compliances and opportunities to improve the FSMS in place. Points of attention deduced in the current case study were the overall microbiological quality of sandwiches and salads served, as well as the control of *L. monocytogenes* in raw meat spread and fish. The overall quality of sandwiches and salads is related to the quality of the raw materials used to prepare these. As seen in this case study, the spoilage flora were high in numbers on the samples of the incoming fresh produce. This resulted in unacceptable levels of LAB and yeast in the served sandwiches and salads. Therefore it is important to have a high flow-through of these raw materials, but also to import good quality raw materials, to avoid a diminished quality of sandwiches and salads served to the consumer. Besides, as noted in the results, the analyses confirmed the presence of *L. monocytogenes* in 7.9 % (n= 202) of the analyzed samples, but enumeration of samples of positive food products showed always levels < 10 CFU/g which is lower than the maximum tolerable level (10^2 CFU/g) in the EU for RTE food products at time of consumption. Nevertheless under good hygienic practices one would seek to prefer absence of *L. monocytogenes* in 25 g. *L. monocytogenes* was present in 9.6 % (n= 115) of the analyzed raw materials indicating the potential introduction of the pathogen in the production environment which may lead to contamination of other raw materials or attachment on food contact surfaces. Therefore supplier control, storage conditions and cleaning and disinfection are important and need to be controlled to avoid the entrance of this pathogen. The importance of supplier control is illustrated by the results from Table 3.6. *L. monocytogenes* was present in six samples of the food samples analyzed from the category “raw (frozen) fish and seafoods” which are delivered by three suppliers (two bigger suppliers and one smaller supplier). From the six positive fish samples, four food products were delivered by one supplier (n= 6), the other two positive samples were delivered by the biggest supplier (n= 18). Therefore, if analyses reveal that four out of six (67 %) analyzed samples delivered by one supplier contained *L. monocytogenes* in 25 g, while the other supplier delivers two positive samples out of eighteen (11 %) food samples, it can be deduced that the first supplier has more problems to compliance its specifications. Thus

from the results it is seen that one supplier deliver more contaminated products than the other one. Therefore, when contracts have to be renewed, these complaints can be taken into account. Performing a pressure on the supplier to improve the quality of their food products, if not, they may lose a customer (psychological pressure) because a food service operation should avoid the potential introduction of the pathogen to reduce the risk on cross-contamination. Overall, although microbiological analyses are useful as can be deduced from the findings in the present case study above, microbiological sampling cannot assure food safety and food quality on its own, but can only be used in the evaluation whether a FSMS is providing the assumed control (Van Schothorst et al., 2009). The presented sampling plan in particular aims at process control and not immediately batch control. In fact, at the frequency of sampling suggested in this study the sampling of high risk foods (score 7) provides 95 % confidence that no more than 10 % of samples are unsatisfactory. With these low numbers of samples, and sampling plans only detecting gross errors, positive results should be seen as a very severe outcome. Satisfactory samplings show only that defectives are below 10 % which is, taking into account the large numbers of meals served per year (ca. 1 000 000) not sufficient, but then positive samples show that there is a serious violation against good hygienic or good manufacturing practices. So, it is acknowledged that this sampling plan will only pick up the major non-compliances and thus major (systematic) failures in the functioning of the preventive systems approach. However, upon discussing with the quality manager, it was established that the food service operation will react on each unsatisfactory sample that they detect and take corrective actions. If it is noticed that if a particular supplier does not respond to these complaints as a result of the sampling plan or when he can not comply with the imposed specifications, it can be decided by the food service operation to remove this supplier from the supplier list, as happened in the case of the raw meat spread supplier. This creates a pressure on the supplier to improve their FSMS, which will gradually lead to an improvement of food safety and quality of food products. The same reasoning can be applied to judge the results of their own produced end products when on-going verification with the sampling plan is performed. When a systematic failure is present in their food management system, an increasing number of unsatisfactory samples will be detected which will generate the awareness of a systematic failure or non-adherence to good hygienic practices which is communicated to the personnel and should lead to corrective actions. In addition, in our case study, the mere

presence of a sampling plan raised awareness and vigilance with personnel and provided incentives to the suppliers on the adherence to good hygienic practices and appropriate control measures. Moreover, Powell et al. (2011) stated that individuals who, among others, dedicate resources to evaluate supplier practices and focus on food safety risks within an organization, contribute to a good food safety culture. A food safety culture is the way in which an organization or group approaches food safety, in thought and in behavior, and is a component of a larger organizational culture (Powell et al., 2011). The implementation of a focused sampling plan was a starting point but needs continuation to build a track record of results. The sampling plan is prone to updates and modifications depending upon the increase in available information on hazards in foods, the changes in the type of meals served and suppliers, the performance of the HACCP system and the resources of the food service operation. With regard to the current frequency of sampling, there was a basic statistical input on indicative numbers to be taken of incoming raw materials and served meals to monitor microbiological safety and quality but further modifications on this elaborated sampling plan may be taken and can be adopted to be fit for purpose. In this case study a total of 210 samples was taken over a 10 month period (catering facility closed 8 weeks), which corresponds to 21 samples/month including raw materials (12 samples) and served meals (9 samples). This number of samples was a starting point and at the time considered accountable within the resources of the food service operation in particular if knowing that the majority of food service operations perform no microbiological analysis or only to a very limited extent. Besides, upon continuation and with the track record of the obtained results, the sampling plan will and can be further tailored and be dynamic. For example the quality manager may have prior information available from interactions with the supplier or the personnel or audit reports, e.g. results of sampling and analysis of previous years, complaints from consumers or concerns picked up from outbreak reports in the media, knowledge on structural problems at some supplier or within defined steps in the food service operation, etc. None of the latter information was available for the current case study, but if present it can be used to focus and adapt the sampling plan. It is clear that with limited resources and a restricted number of samples for analysis the sampling plan is not able to guarantee food safety and quality. Limitation in resources, in particular in combination with a wide variety of food types typically handled in food service operation, will evidently restrict the statistical power of sampling, enabling only the detection of gross

errors in compliance to specifications or performance of the FSMS. Still, it does mean that if under these conditions positive (i.e. non-compliant) samples are noted, there is a situation out of control and an urgency to take corrective actions. However, if no sampling and analysis is done at all, there is no knowledge gathered on the microbiological status of either incoming raw materials or meals served and issues like the one of the *L. monocytogenes* in the meat spread in the present case study would go undetected. Also if no sampling and analysis is performed, there is no output to suppliers or staff members, involved in food preparation, that food safety and quality is actually controlled upon and that adherence to a proper functioning FSMS is of importance. Thus the aim of the elaboration of the sampling plan in the present study is rather a pragmatic one. Although the main goal is to detect major non-compliances in food safety and food quality of delivered raw materials or meals served, also additional benefits can be obtained. The mere implementation of the sampling plan and communication on this to suppliers probably provides a pressure on the supplier to deliver goods in accordance to set specifications. In addition, having a set of actual results available of taken samples and communication on this to the staff members may increase awareness and highlight the importance of food safety and quality within the food service operation management. Apart from a preventive GMP and HACCP systematic approach, the presence of commitment by all involved to food safety culture is still the major leads in providing safe and qualitative food to the consumers.

3.5 Conclusion

A proposal on microbiological risk categorization of incoming raw materials and food served to consumers is provided to set up a focused sampling plan in food service operations. It was shown upon application of the focused sampling plan at our university (non-profit) food service operation that the approach taken can deduce major non-compliances and systematic failures in “best practices” of both suppliers and the food service operation itself. Moreover, it may serve to build a systematic track record of the well-functioning of assurance activities. This is important to serve as a baseline in case of complaints or external audits and it will be complementary to systematic visual checks on hygiene, registration of times and temperatures at critical control points and training of personnel to guarantee safe

foods. However, the sampling plan is prone to updates and modifications depending upon the increase in available information on hazards in foods, changes in the type of meals served and suppliers, the performance of the HACCP system and resources of the food service operation.

CHAPTER 4

EVALUATION OF THE FOOD SAFETY MANAGEMENT SYSTEM IN A HOSPITAL FOOD SERVICE OPERATION TOWARD LISTERIA MONOCYTOGENES

Partially redrafted after:

Lahou, E., Jacxsens, L., Verbunt, E., and Uyttendaele, M. (2015). Evaluation of the food safety management system in a hospital food service operation toward *L.monocytogenes* *Food Control* **49**, 75-84.

Abstract

The unique aspects of a hospital environment, such as the multitude of dietary needs and thus the variety of meals to be served and incoming (raw) materials to be used, challenge the development and application of appropriate control and assurance measures to guarantee food safety. Besides, *Listeria monocytogenes* is considered a risk for most food service operations producing and serving ready-to-eat foods. Therefore the Food Safety Management System (FSMS) of a hospital food service operation (FSO) has been evaluated toward *L. monocytogenes* with an extensive questionnaire in the preset of this case study. In addition, 49 samples of food products and 145 environmental samples were taken and analyzed for *L. monocytogenes* to verify the implemented control measures. From this case study, it becomes clear that incoming (raw) materials, produced final products and their immediate supply to patients/consumers are high risk situations. This was demonstrated by the presence of *L. monocytogenes* in six incoming (raw) materials (n= 19) and one final product (n= 9). These risky situations are in need to be mitigated by the implementation of proper control measures, e.g. intensified supplier control, low storage temperatures, cleaning and disinfection to control cross-contamination. However major improvements can be made on the hygienic design of equipment and facilities and on the level of the sampling design. In terms of assurance activities, such as setting up a sampling plan, only a basic level was obtained for the validation and verification of their FSMS. This case study illustrates that the combination of data from the questionnaire together with data of the sampling result in an overview on the performance of the current FSMS and that major non-compliances and possibilities for improvement in the system can be defined.

4.1 Introduction

The foodborne pathogen *L. monocytogenes* is the causative agent of listeriosis, a severe disease with high hospitalization rates and mortality rates ranging from 16 to 30 % (Cairns and Payne, 2009; Denny and McLauchin, 2008; EFSA, 2009-2012; Gandhi and Chikindas, 2007). The FAO/WHO reported yearly incidence rates of 0.3 - 7.5 cases per million people in Europe (FAO/WHO, 2004). Although rates of listeriosis have remained stable, a changing pattern of human listeriosis can be observed in Europe (EFSA, 2009-2012; Gillespie et al., 2006; Goulet et al. 2008). Listeriosis is now affecting the elderly (> 65 years) population more often and pregnant women less frequently (Metelmann et al., 2010; Muñoz et al., 2011). It has been shown that the majority of these elderly persons are suffering from underlying diseases and therefore most listeriosis infections are occurring in immune-compromised elderly persons, which form part of the hospital population (FAO/WHO, 2004; Gillespie et al., 2010; Muñoz et al., 2011). Moreover, such vulnerable persons are more likely than healthy individuals to be affected by low numbers of a pathogen and are more likely to suffer severe consequences of infection (Lianou and Sofos, 2007; Lund and O'Brien, 2009). Lund and O'Brien (2009) summarize in their review foodborne *L. monocytogenes* outbreaks in health care settings between 1997 and 2008. From these data it can be concluded that hospitals were involved in six outbreaks of *L. monocytogenes* infection and that cases could be linked to consumption of ready-to-eat (RTE) foods. Surveillance and epidemiological data also revealed an association between food handling at retail and food service establishments and the incidence of foodborne illness (Lianou and Sofos, 2007). However, no studies about the prevalence of *L. monocytogenes* in food handling areas of hospital food service operations were retrieved, although data on the distribution and transmission of *L. monocytogenes* in hospital food-processing environments and retail can be found in literature (Hoelzer et al., 2011; Lund and O'Brien, 2009; Rodriguez et al., 2011). These and other studies on the potential transmission of *L. monocytogenes* within retail and food service operations revealed that the potential sources of the organism include the environment (utensils and equipment), food handlers and incoming raw or processed products that have been contaminated after a lethal treatment at the manufacturing facility (Lianou and Sofos, 2007; Tompkin, 2002). Besides, high prevalence data of *L. monocytogenes* in RTE products and on food contact surfaces at retail and commercial food

service operations can be found (Hoelzer et al., 2011; Lianou and Sofos, 2007). This necessitates the implementation of appropriate control and assurance measures to prevent foodborne outbreaks within a hospital. However, this is challenged by the unique aspects of retail and food service operations, such as variety of meals to be served and incoming (raw) materials to be used. Therefore, the purpose of this study was to evaluate the current implemented control and assurance activities toward *L. monocytogenes* within a hospital food service operation. This was performed using an extensive questionnaire, which can be used as a self-assessment tool, combined with additional samples of incoming (raw) materials, meal components, final products and (non-) food contact surfaces. The combination of the data from the questionnaire together with the data of the sampling, results in an overview on the efforts taken to prevent *L. monocytogenes* (re)contamination, reveals major non-compliances and defines possibilities for improvement in the FSMS.

4.2 Materials and methods

4.2.1 Characterization of the hospital food service operation

A Belgian hospital food service operation with approximately 120 employees has been selected for the case study. This hospital is inspected and certified for its legally demanded self-checking system, based on good practices and HACCP, according to EU Regulation 852/2004 (Anonymous, 2004) and relevant Belgian legislation (Anonymous, 2003). Meals are prepared and cooked in the on-site hospital kitchen and distributed directly to the patients under responsibility of the nursing staff or are served in the hospital canteen which is accessible for patients, visitors and staff. The hospital food service operation is working with two production lines. One production line, referred to as “hot kitchen”, is used to produce hot meals from raw materials, ingredients or cooked half-fabricates. Another production line, referred to as “cold kitchen”, is used to produce cold meals such as salads and sandwiches. The hot kitchen, where food handlers start with processing raw materials (e.g. frying of the meat, cooking of vegetables), is the most important production line producing approximately 1 200 hot meals/day. As soon as the food is prepared, it is kept at temperatures of >65°C in hot water baths until lunch time. Just before serving time all food

handlers are involved in composing the meal on a plate, thus with risk of post-contamination, according to dietary needs of patients. Final composed meals are then collected in preheated trolleys to transport them to the patients. The cold kitchen processes (e.g. cutting of vegetables) and assembles raw vegetables and ready-to-eat products (e.g. smoked salmon) into salads or sandwiches which are mainly served in the hospital canteen. In total approximately 2600 meals are produced each day consisting of 600 patient breakfasts, 700 hot patient meals, 500 hot canteen meals, 700 patient dinners (mainly cold meals) and 100 cold canteen meals.

4.2.2 (Self-)assessment questionnaire

The objective of the questionnaire is to analyze and assess a selected number of major food safety management activities to get a broad and overall impression on the efforts taken to prevent, in this case study, *L. monocytogenes* (re)contamination. Therefore the context in which the hospital food service operates and which puts demands on the FSMS, the level of implemented core assurance and core control activities of the food safety management, and the microbiological system performance which is the output of a FSMS (Figure 4.1) were assessed with the (self-) assessment questionnaire which is composed of lists of indicators (Table 4.1) (Jacxsens et al., 2010). The (self-)assessment questionnaire was developed for food processing companies by Jacxsens et al. (2010) and Luning et al. (2008, 2009, 2011a) but have been slightly adapted for its use in (hospital) food service operations. The modified indicators for food service operations are indicated with an asterisk in Table 4.1. The situations/levels of the different indicators were assessed with an on-site visit and a 3 h face-to-face interview with the HACCP-coordinator of the hospital food service operation.

4.2.2.1 Structure of (self-)assessment questionnaire

The questionnaire is subdivided into a part with context indicators to assess the situation in which the hospital food service is operating, a part with activity indicators to assess the currently implemented core assurance and core control measures and a part with microbiological system performance indicators for assessing the output of the food safety management in place (Figure 4.1).

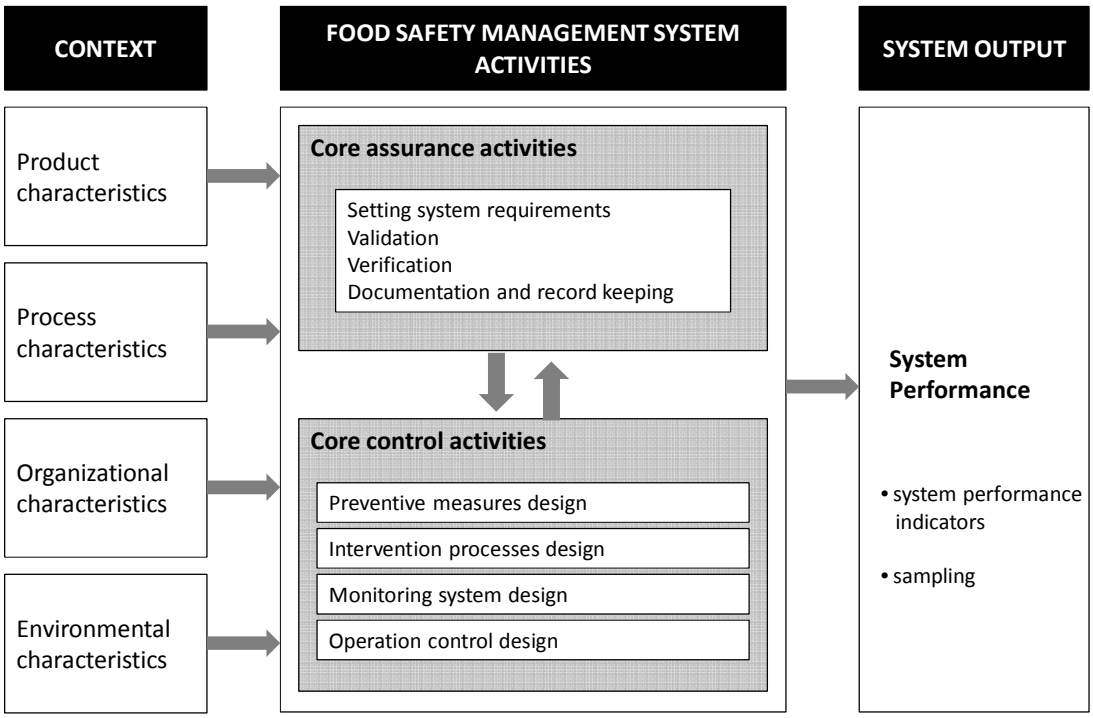


Figure 4.1. Structure and relationship between the groups of the (self-)assessment questionnaire

The context has been defined as a condition, characteristic or situation which is a given fact or cannot be easily changed on the short term, but which can influence the performance of the FSMS. Contextual factors include product, process, organizational and chain environmental characteristics. Core control and assurance activities form the actual FSMS where assurance activities such as setting system requirements, validation, verification,... have the aim to provide evidence and confidence to stakeholders. Control activities are activities that create circumstances to prevent entry and/or growth of pathogens in food production systems (preventive measures design), activities that inactivate or eliminate pathogens in order to reduce them to acceptable levels (intervention process design), activities that measure (critical) product or process parameters (monitoring system design) and activities that concerns the way the activities are operating in practice (operation control strategies) and have the aim of keeping product properties, production processes and human processes between certain acceptable tolerances. The system output was assessed in the questionnaire with system performance indicators to provide an indication of the current status of the functionality of the implemented FSMS, i.e. what is the expected

quality and safety of the meals produced in the hospital food service operation and how is their FSMS currently evaluated by third parties.

4.2.2.2 Indicators of the (self-)assessment questionnaire

The list of indicators used in the questionnaire is shown in Table 4.1. The questionnaire comprises 15 context indicators, 34 activity indicators and 7 system performance indicators. Each context indicator (Figure 4.2A) has a grid with descriptions of three contextual situations (low, moderate and high risk) to assess the risk type of the food service operation. A more risky context will put higher demands on the FSMS e.g. incoming (raw) materials with potential presence of pathogens will demand cooling conditions, more severe supplier selection and also a strict follow up during processing. Each activity indicator (Figure 4.2B) has a grid with descriptions of four different levels (not applicable, basic, generic and advanced) of performance where an activity level will be classified as basic when the activity is based on companies own information and history and when general working methodology is applied. A generic level is assigned when the activity is based on 'best practices' or 'best present technology' and is based on generic sector information. An advanced level will be assigned if the activity is based on scientific knowledge, adequate information and tailored to the specific situation of the food service operation. Each system performance indicator (Figure 4.2C) has a grid with descriptions of four different levels (absent, poor, moderate and good) of microbiological performance. The HACCP-coordinator had to assign for each indicator which level or situation was most representative for his food service operation.

4.2.2.3 Interpretation of the (self-)assessment questionnaire

The indicators of the assessment will create a profile of the FSMS. In case of context indicators, a more risky contextual situation is expected to result more easily in food safety problems, and therefore higher demands will be put on the FSMS. In case of activity indicators, a higher/more sophisticated level of control and assurance activities means that the food service operation has a more advanced FSMS in place, and can control their microbiological food safety output better. In case of the system performance indicators, a better system performance means that the likelihood of food safety problems is reduced.

A. Example of a context indicator

In which of the following situations would you place the risk of the meals in your kitchen?

Risk of meals **Assumption**: Meals which are susceptible to pathogen growth or toxin formation (due to the intrinsic product properties and or applied inactivation technique), increase the chance on lower food safety performance, and put higher demands on the FSMS by requiring advanced control and assurance activities.		
Situation 1 (low risk)	**Situation 2 (moderate risk)**	**Situation 3 (high risk)**
Major meals are microbiologically stable (a_w <0.6 or pH <4.2 or contains intrinsic antimicrobial agents). A complete inactivation of the flora takes place and post-contamination is not likely. The meals can be served as bought and do not require handling before service.	The meals have following characteristics: 0.98>a_w>0.6 or 4.2 <pH <6.5 or contains no antimicrobials). Contamination of meals is not likely to occur. The meals are cooked/reheated and then immediately served.	The meals have following characteristics: a_w >0.98 or pH 6.5-7.5 or contains no antimicrobials). Contamination of meals can occur (no inactivation of original flora or post-contamination). The meals are fresh-type meals or hot-held meals.

B. Example of an activity indicator.

At which level would you place the method regarding the hot-holding of the meals in your kitchen?

Hot-holding methods **Assumption**: Adequate hot holding methods better maintain strict temperature conditions to prevent growth of micro-organisms and pathogens, which will positively contribute to food safety.			
Level 0 (not applicable)	**Level 1 (basic)**	**Level 2 (generic)**	**Level 3 (advanced)**
Hot-holding of meals is not applied in the kitchen.	Hot-holding method is based on company knowledge/ experience and has not been tested on effectiveness for kitchens 'specific food production system.	Hot-holding method is based on sector guidelines, legislative requirements and/ or expert knowledge, but has not been tested on effectiveness for kitchens' specific food production system.	Hot-holding method is based on legislative requirements/ guidance documents but adapted for own production process and tested on effectiveness for kitchens' specific food production system. Actual product temperature is checked for different circumstances.

C. Example of a system performance indicator

At which level would you place the customers' complaints regarding microbiological food safety?

Food safety complaints Assumption: The presence of a good functioning system for complain registration and evaluation is an important aspect in the FSMS. Low number of complaints regarding microbiological food safety of final products and hygiene indicates a good system performance. When complaints can be dedicated to one specific aspect of the FSMS or one type of pathogen/hygiene indicator, a well performing FSMS and a good system performance can be expected.			
Level 0 (absent)	**Level 1 (poor)**	**Level 2 (moderate)**	**Level 3 (good)**
No indication of system performance because complaints are not registered.	Various complaints that can be traced back to several problems of the operation of the FSMS.	A limited number of complaints that can be traced back to one specific problem with the functioning of the FSMS	No complaints on the microbiological safety of the meals.

Figure 4.2. Example of an indicator for the context (A), an activity (B) and system performance (C) as used in the (self-) assessment questionnaire.

4.2.3 Risk-based sampling plan

As the goal of the sampling plan is to verify the HACCP-system (i.e. check if the HACCP is followed as it is described and if it is appropriate), but a multitude of products and processes is involved, it was decided to implement a risk-based sampling plan to focus on those products and locations that involve a risk towards the presence of *L. monocytogenes*,

an important hazard in hospital food service operations. As such, sampling of incoming high risk (raw) materials, meal components, final products and (non-) food contact surfaces was performed to verify supplier selection and implemented control measures toward *L. monocytogenes*. Microbiological analysis cannot assure food safety on its own, but can be used to evaluate whether a FSMS is providing the control it was designed to deliver. Sampling was executed three times in a three month period (January-March 2011). In total 145 environmental samples and 49 food samples were analyzed.

4.2.3.1 Identification of at risk foods and critical sampling locations

Because the range of incoming (raw) materials in the food service operation is broad and not all food products are a risk in terms of presence of *L. monocytogenes*, an identification of at risk products was elaborated to select those incoming (raw) materials of interest to be taken up in the sampling plan. Incoming (raw) materials were therefore classified into food types with similar microbiological ecology toward *L. monocytogenes* based upon the type of commodity and prior processing or preservation method applied (Lahou et al.,2012). These food types were then screened for i) the reported epidemiological association of the food type with listeriosis outbreaks, ii) the reported prevalence of *L. monocytogenes* in the food types, and iii) the potential of *L. monocytogenes* to grow or survive during storage and/or further processing to identify high risk products. This screening was performed with a literature study carried out in ICMSF books (ICMSF, 1986, 2002, 2005) complemented with the Community Summary Reports on Trends and Sources of zoonoses of the European Food Safety Authority (EFSA, 2006, 2007, 2009, 2010, 2011, 2012). To verify the general EU situation for the regional situation, the Annual Reports on Zoonotic agents in Belgium of the Federal Agency for the Safety of the Food Chain (FASFC) and the Belgian Report on Zoonoses and Zoonotic agents were consulted (Dierick and Botteldoorn, 2007; Dierick, et al., 2009; FAVV, 2004, 2006b, 2007, 2008b, 2009a,b). Critical sampling locations were identified by analyzing the flow chart of the production process. Possible sites of cross-contamination or post-contamination, such as utensils, slicing machines, hands of food handlers and plates were taken up in the sampling plan. Non-food contact surfaces, such as drains, ventilation, vans, trolleys, door handles, wheels and conveyer belts were selected on the basis of information from literature and reports about the distribution and transmission of *L.*

monocytogenes in food service operations and retail (Hoelzer et al., 2011; Lianou and Sofos, 2007; Lund and O'Brien, 2009; Rodriguez et al., 2011).

4.2.3.2 Elaboration of the risk based sampling plan

The attribution of a risk level to the defined incoming (raw) material food types and the identification of critical sampling locations was the basis for selection of incoming (raw) materials and environmental samples to be taken up in the sampling plan. The daily menus of the food service operation were obtained from the HACCP coordinator a week prior to the visit and were screened for high risk ingredients. A total of 49 food and 145 environmental samples were collected during three visits on three days at various critical sampling locations (CSL) in the process from raw material to final food product. For food products, 100 g was aseptically collected with a sterile spoon or forceps and transferred to a sterile sampling bag. Food contact surfaces, hands and gloves were swabbed in a deliminated area of 100 cm^2 using a sterile Quantiswab® (Biomérieux) premoistened in neutralizing solution. The food samples and the moistened swabs were transported in a cool box at 4°C to the laboratory where microbiological analyses to detect *L. monocytogenes* were performed within 6 h of sample collection. The moistened swabs were enriched with 225 ml demi-fraser for 24 h at 30°C, followed by transferring 0.1 ml enrichment to 10 ml fraser broth. After incubation for 24 h at 37°C, 0.5 ml was transferred to a VIDAS LMO2 strip (BioMérieux) which was analyzed for presence of *L. monocytogenes* (AFNOR n° BIO-12/11-03/04). Detection of *L. monocytogenes* was performed on 25 g food product also according Vidas LMO2 (BioMérieux), an AFNOR validated enzyme linked fluorescent assay (ELFA) (AFNOR n° BIO-12/11-03/04). When positive results were obtained, *L. monocytogenes* was enumerated from the food sample according to ISO 11290-2:1998/Amd 1:2004 (plating on ALOA and incubation of 48 h at 37°C).

4.3 Results

4.3.1 (Self-)assessment questionnaire

4.3.1.1 Context factors

Table 4.1 lists the results of the (self-)assessment questionnaire. Important contextual factors which influence the FSMS in this food service operation belong to product and production process characteristics. The high diversity of incoming (raw) materials used in the hospital food service operation (e.g. raw meat, raw fish, smoked fish, ready-to-eat vegetables and fruits, cooked meat) and the high microbial load of the products (e.g. 10^6 CFU/g on raw meat and poultry), which may also contain pathogens such as *Salmonella* spp. and *L. monocytogenes*, contribute to a high risk situation (Jacxsens et al., 2011; Luning et al., 2011a; Uyttendaele et al. 2010). The produced meals were classified as a high risk situation because they are sensitive to pathogen growth as a result of the intrinsic properties of the products and are prone to post-contamination. The organizational characteristics, which give insight in the ability to prevent safety problems, and the chain environment characteristics, which refer to the position of the food service operation in the food chain and its relationship with stakeholders such as suppliers and controlling bodies, are in this food service operation in general at lower risk for the food safety management performance. However, the information system wherein information, knowledge and data should be systematically recorded to support decisions on food safety and quality issues was not accurate to take food safety control decisions and was recorded manually which results in a high risk situation. As a hospital food service operation is situated in the last part of the food supply chain and is serving meals to a “susceptible” group, it has a critical position with respect to reduction and/or inactivation of pathogens. Therefore the indicator “safety contribution in chain position” has been classified as a high risk situation.

Table 4.1. Results of the (self-)assessment questionnaire.

	Indicator	Situation[a] level[b]	Motivation
Context factors	Risk of raw material	High risk	High diversity, main products (e.g. raw meat, fresh vegetables) may carry pathogens and have a high initial microbial load.
	Risk of meals*	High risk	Produced meals are sensitive to pathogen growth as a result of intrinsic properties of the products and are prone to post-contamination.
	Intervention steps	Moderate risk	The production processes contain intervention steps (e.g. cooking) to inactivate vegetative cells, but spores can still survive and recontamination can still occur after the lethal ntervention step (e.g. when assembling meals).
	Assortment of meals*	Moderate risk	Only a restricted number of recipes are prepared (Max. three"hot" daily menus) which allows in-between cleaning and disinfection interventions
	Rate of menu changes*	Moderate risk	Repeating menu cycle of 4 weeks allows less product and process modifications.
	Technological staff	Moderate risk	HACCP-team of six persons is available.
	Variability in workforce composition	Low risk	Low turnover of employees, no temporary operators.
	Operator competences	Low risk	Chefs have professional education level in cuisine, employees attend specific food safety training on recruitment.
	Management commitment	Low risk	Food service operation has detailed written vision statement on safety and has official quality team.
	Employee involvement	Low risk	Employees are involved in the design and modifications of the FSMS (e.g. notification of problems, ideas on improvement) Standard operational procedures and documentation are available for employees.
	Formalization	Low risk	Temperatures are recorded manually by staff, information is limited available and not accurate to take food safety control decisions.
	Information systems	High risk	
	Safety contribution in chain position	High risk	Direct serving of meals to "susceptible" group, thus it has a critical position with respect to reduction and/or inactivation of pathogens to acceptable evels.
	Power in supplier relationships	Low risk	Discussion on product specifications possible, perform audits at new suppliers. General legislative requirements on food safety, execute self-assessment.
	Strictness of stakeholders requirements	Low risk	
Core control activities	Hygienic design of equipment and facilities	Basic	Equipment and facilities are not well designed to prevent contamination and pathogens entrance (e.g. no strict separation of preparation rooms).
	Cooling facilities	Advanced	Presence of industrial cooling facilities adapted for food service food production which are automatically controlled (< 4°C).
	Sanitation programs	Advanced	Presence of complete full-step cleaning procedure with pre-cleaning, cleaning, disinfection and in-between rinsing with instructions and use of specific cleaning agents.
	Personnel hygiene requirements	Advanced	Specific requirements on clothing for all employees, personnel care and health and tailored facilities to support personnel hygiene.
	Raw material control	Advanced	Incoming materials are systematically checked based on actual data of suppliers and quality is visually assessed at entrance.
	Meal preservation*	Not applicable	Meals are not stored.
	Defrosting methods*	Advanced	Based on legislative requirement and guidance documents, method has been tested.
	Hot-holding methods*	Advanced	Based on legislative requirement and guidance documents, method is tested daily.
	Physical intervention equipment	Advanced	The present intervention equipment (steam ovens, cooking pots, frying pans) are adequate for the production process (different programs available) and capability is tested by monitoring of core temperatures (≥ 70°C).
	Maintenance and calibration program for equipment	Generic	Structural program is present with specific instructions about frequency and maintenance tasks but is not specifically designed for process.
	Effectiveness of intervention methods	Advanced	Intervention equipment is tested by measuring core temperatures of prepared food products.
	CCP analysis	Generic	Based on hygiene codes for sector according to official Codex guidelines, but are not tested.
	Standards and tolerances design	Advanced	Standards and tolerances are scientifically underpinned, comply with legislative requirements.
	Analytical methods to assess pathogens	Advanced	Analytical methods used by lab are internationally validated and accreditated methods.
	Measuring equipment to monitor process/product status	Generic	Standard available measurement equipment complying with ISO norms, on-line/in-line measurement (e.g. probes in steam ovens).
	Calibration program for measuring equipment	Advanced	Calibration program with tasks and frequencies which are in-house documented.
	Sampling design	Basic	No own samples are taken, in case of new supplier samples are taken from the raw materials, once a year external control by government.
	Corrective actions	Advanced	Presence of complete descriptions on what to do in case product and/or process parameters exceed tolerances or limits.
	Actual availability of procedures	Generic	General working instructions are available on the workplace but are paper-based.
	Actual compliance to procedures	Generic	Majority of employees are familiar with existence of procedures, tasks are executed based on habits.

	Actual hygienic performance of equipment/facilities	Advanced	Stable hygiene performance of equipment and facilities based on data of executed tests (two times/year external control with rodac and swabs, every three months ATP-measurement themselves).
	Actual cooling capacity	Advanced	Stable performance of cooling facilities (< 4°C), temperature is automatically monitored, alarm when temperature deviates.
	Actual hot-holding capacity*	Advanced	Stable performance of hot-holding facilities (> 80°C), temperature is systematically monitored.
	Actual process capability of intervention processes	Advanced	Stable process, core temperatures of the food are measured with probes and intervention process is adjusted.
	Actual performance of measuring equipment	Generic	Measuring equipment is sensitive for a few specific well known meal production changes.
Core assurance activities	Translation of stakeholder requirements	Generic	Systematic translation of stakeholder requirements into own FSMS.
	Systematic use of feedback information to modify system	Advanced	HACCP-team evaluates feedback information from validation and verification reports.
	Validation of preventive measures	Basic	Effectiveness of preventive measures is ad hoc judged by own HACCP-team.
	Validation of intervention systems	Basic	Effectiveness of intervention processes is ad hoc judged by own HACCP-team.
	Validation of monitoring systems	Basic	Effectiveness of monitoring system is ad hoc judged by own HACCP-team.
	Verification of people related performance	Basic	No internal audit is executed to check if the compliance to procedures are operating in practice.
	Verification of equipment and methods related performance	Generic	Analyzing records data loggers on a regular basis, but no confirmation by actual testing.
	Documentation	Generic	Structured kept-to-date documentation system , but only available for authorized persons.
	Record keeping system	Generic	Full registration of critical product and process data but only accessible for authorized persons.
System performance output	Evaluation of FSMS	Poor	No own evaluation of the system, rely on the yearly inspection of the national food safety agency.
	Severity of complaints	Good	No complaints or remarks from the national food safety agency.
	Food safety complaints	Good	No complaints concerning microbiological food safety.
	Hygiene complaints	Good	No complaints concerning microbiological hygiene indicators.
	Product sampling	Poor	Only ad hoc sampling of raw materials and end products.
	Assessment criteria	Moderate	Use only legal criteria and requirements, no own specifications defined.
	Non-conformities regarding food hygiene and pathogens	Moderate	Only a few non-conformities regarding one specific problem (e.g. hand hygiene).

* specific indicator for food service operations

[a] in case of context factors.

[b] in case of core control activities, core assurance activities and system performance output.

4.3.1.2 Core control activities

Control activities concern the ongoing process of evaluating performance of both technological and human processes and taking corrective actions when necessary. It is assumed for control activities that a better activity level is better able to keep product properties, production processes and human processes between certain acceptable tolerances (Luning et al., 2008). Core control activities are in general well implemented in the FSMS of the food service operation (Table 4.1). However, a basic level was assigned to the hygienic design of equipment and facilities, which means that equipment and facilities are not well designed to prevent (cross-)contamination and entrance of pathogens. More specific, there is no strict separation between the different preparation areas within the food service operation. Contamination of products or parts of the environment increases the risk to contaminate other products and other parts of the area. A basic level was also assigned to the sampling design because samples are only taken from incoming materials supplied by new suppliers and no information is available on the distribution of pathogens in the food service operation as they take no specific (environmental) samples.

4.3.1.3 Core assurance activities

Core assurance activities are activities that provide evidence and confidence to stakeholders that safety requirements will be met. It is assumed for assurance activities that a better activity level is better able to provide confidence that safety requirements will be met because better requirements are set on the system, its performance is better evaluated and changes are better organized (Luning et al., 2009). From Table 4.1 it can be derived that the core assurance activities are performing on a basic to a generic level. A basic level was assigned to the validation of preventive measures, intervention systems and monitoring systems because its effectiveness is only ad hoc judged by their own HACCP-team instead of being validated independently. The verification of people related performance, checking whether requirements on people related activities (i.e. compliance to procedures) are operating in practice as designed, was also assigned a basic level because no verification of the procedures is executed and compliance is based on checking their presence by dependent persons.

4.3.1.4 System performance indicators

The system performance indicators provide more information about the output of the FSMS. It is assumed that a better level is associated with a better system performance which means that the likelihood of food safety problems is reduced (Jacxsens et al., 2010). As noticed in Table 4.1, the evaluation of FSMS is poor because the food service operation performs no self-evaluation of the FSMS e.g. via internal auditing. Besides it only relies on data from the yearly inspection of the national food safety agency to judge their FSMS. Product sampling is also poor because there is only ad hoc sampling of incoming (raw) materials in case of a new supplier and ad hoc sampling of end products on demand of third parties, e.g. the government.

4.3.2 Risk based sampling plan

The sampled high risk incoming (raw) materials for *L. monocytogenes* in the food service operation and their results are presented in Table 4.2. These raw materials were also sampled at critical sampling locations along their production process where, besides the food product, samples were taken from the direct (food contact surfaces such as utensils, slicers, plates) and indirect (non-food contact surfaces such as ventilations, floor drains, door handlers, trolleys, conveyer belts) environment. *L. monocytogenes* could be detected (in 25 g) in 3.61% of the samples (n= 194). *L. monocytogenes* was detected in six incoming (raw) material samples (n= 19), namely salt-free cooked ham, raw meat sandwich spread, raw salmon, smoked salmon (2x) and raw poultry meat, and in one final product (n= 9), namely the Ardennes egg which consist out of salt-free cooked ham, lettuce, tomatoes, carrots, cooked egg and mayonnaise. However, enumeration of the positive samples revealed that the concentration of the pathogen was < 100 CFU/g. No *L. monocytogenes* was detected in any of the 145 environmental samples.

Table 4.2. Overview of analysed samples and number of positive (i.e. present in 25 g but < 100 CFU/g) *L. monocytogenes* samples.

	1e visit	2nd visit	3th visit	Total
Raw materials (raw meat, raw fish, smoked fish, sandwich spread, ready-to-eat vegetables and fruits, cooked meat)	3/8	1/6	2/5	6/19
Meal components (fried meat, cooked fish, milkshake, sliced vegetables, tuna spread)	0/6	0/9	0/6	0/21
Composed meal (final product) (Ardennes egg, Veal stew with hot vegetables and potatoes, Meat escalope with hot vegetables and rice, fresh mixed fruit meal (3x), tuna salad, chicken salad, meat loaf with hot vegetables and potatoes, tomato salsa)	1/3	0/3	0/3	1/9
Environment				
Direct (food containers, utensils, slicing machine, plates, cooking kettle, food handlers hands)	0/33	0/30	0/24	0/87
Indirect (ventilation refrigerator, floor drains, trolley, door handle, walls, conveyer belt, wheels, hood, dishwasher belt, plateau)	0/18	0/20	0/20	0/58
Total	4/68	1/68	2/58	7/194

4.4 Discussion

The principle behind the (self-)assessment questionnaire is that a food service operation operating in a more vulnerable (to safety problems), uncertain (due to the lack of information), ambiguous (due to the lack of insight in underlying mechanisms) and unpredictable situation, which can be seen as a high-risk context, requires control and assurance activities at a more advanced level (Luning et al., 2011a). In this case study, a high risk context was posed by the high diversity of (raw) ingredients entering the food service operation. Therefore, well controlled storage conditions, proper supplier selection and a good follow up during further interventions processes are required to deal with this microbiological risk and thus higher demands are posed on the FSMS (Jacxsens et al., 2011; Luning et al., 2011a). A high risk context was also posed by the variability of the produced meals and the high risk position of the food service operation due to serving meals directly to the patient/consumer. These risky situations were largely compensated by the requirement of specific competences for employees and by low personnel turn-over and the absence of part-time workers, which decrease the chance of poor execution of tasks. However, food service operations commonly have a relatively high turn-over of personnel or

temporary staff, which may complicate the development of a regular training program and cause problems with poor handling practices, or would require stronger management to ensure that staff adhere to food safety controls (Jones and Angulo, 2006; Jones et al., 2008; Worsfold, 2001). Besides, a higher staff turnover makes it more difficult to create a food safety culture, which is built on a set of shared values that operators and their staff follow to produce and provide food in the safest manner (Powell et al, 2011). In the current food service operation, employees are also involved in the design and modifications of the food safety management (e.g. notification of problems, ideas on improvement) which results in a higher commitment and motivation of the staff, lowering the risk context. Besides, standard operational procedures and documentation (assessed by the formalization indicator) were present which results in the absence of higher demands on the FSMS (Luning et al., 2011). Moreover, the food service operation has the ability to discuss microbiological specifications with their suppliers and to select the supplier with the best specifications. This ability of the food service operation to influence the quality and handling practices of the foods before they enter the hospital kitchen and thereby ensuring that supplies are obtained from high-quality suppliers and thereby reducing the likelihood of contaminated products entering the food service operation, contributes to a lower risk context (Jones et al., 2006; Lianou and Sofos, 2007). Core assurance activities were in general implemented on a basic level, which is often noticed in food service operations. The verification of people related performance, i.e. checking whether requirements on compliance to procedures are operating in practice as designed, is one of these activities which is executed on a basic level. This means that they only check if procedures are present, but no internal audits are performed to check the actual behavior of the food handlers and to assure that they work in compliance with procedures. However, researchers have suggested that the observation of food preparation practices and the assessment of food-handling practices of the employees through internal observations, external evaluations and inspections, contribute to a food safety culture which is one of the most effective measures to reduce rates of foodborne illness (Powell et al., 2011). Studies have shown that improper food handler practices (e.g. inadequate handwashing, wearing the same gloves for extended periods of time, handling unwrapped RTE meats after handling raw meats without washing hands) may result in cross-contamination of RTE foods and account for approximately 97 % of foodborne illnesses (Green et al., 2006; Lianou and Sofos, 2007; Neal et al. 2012). Therefore, performing

observations on the behavior of food handlers and changing incorrect behavior can improve the level of this indicator leading to a more advanced FSMS but will also contribute to the developing of a food safety culture (Luning et al., 2009). Nowadays, education and training are the focus of many food-handling behavior interventions, however these programs are often inconsistent and their knowledge evaluation is a poor indicator of changes in practices (Powell et al., 2011). Other core assurance activities, such as validation of preventive measures, validation of intervention processes and validation of the monitoring system, which are now performed on a basic level will become more advanced if the validation is based on scientific evidence and if it is systematic and independently performed (Luning et al., 2011a). However, the validation, to assure that they work well, is currently based on historical knowledge judged by own people and only ad-hoc performed. Core control activities, on the other hand, were implemented on a more advanced level. Especially their activities that create circumstances to prevent growth of pathogens in food production systems (preventive measures design), such as the adequacy of their cooling facilities, and their activities that inactivate or eliminate pathogens in order to reduce them to acceptable levels (intervention process design), such as the effectiveness of the intervention equipment, are well established. However, major improvements can be made on the level of the hygienic design of equipment and facilities and on the level of a sampling design. The facilities had no strict separation of preparation rooms which may facilitate cross-contamination because there is no forward flow with returns and crossing among raw materials, ready-to-eat meals and trash. The design of this food service operation was drawn in 1977 and it is difficult to change in the short-term. However, a shift in location of the food service operation took place in 2012. In this new production area, the hygienic design of the facility and equipment was adjusted to meet more modern standards regarding space efficiency, flexibility, product flow, food safety and ergonomics, which will improve the level of this indicator. Food service operations, however, are frequently of unhygienic design and crowded with staff and equipment to satisfy occasional workloads which makes it difficult to control basic sanitary standards resulting in an increased number of critical control points to prevent the risk of cross-contamination and recontamination of food (Panisello and Quantick, 2001). In 2008, the national agency for the safety of the food chain performed 12,492 inspections regarding the infrastructure, design and hygiene in food service operations. These inspections revealed that only 56 % of the food service operations

were in accordance with the criteria regarding the infrastructure, design and hygiene in food service operations (FAVV, 2009b). The adequacy of the sampling plan could also be improved to obtain a more advanced level. However, a sampling plan is not provided in the self-checking guide for the sector of food service operations and health care institutions (FAVV, 2006a, 2008a). Therefore the food service operation is not obliged to take own samples of final products. For these results the food service operation relies currently on the sampling of the national agency for the safety of the food chain which is performed in the frame of inspection, but this sampling is not frequently performed (once a year) and is rather limited. On the other hand, samples are taken from incoming (raw) materials in case of new suppliers to verify their specifications. Therefore, raw material control is performing on a more advanced level (Luning et al., 2008). Besides, selection of credible suppliers is based on raw material specifications and supplier audits, which reduces the likelihood of contaminated products entering the food service operation (Lianou and Sofos, 2007; Luning et al., 2008). However, testing should be used to verify that risk-reduction measures are working as intended and therefore a sampling plan should be designed (Dufour, 2011; Powell et al., 2011). This would also increase the level of the core assurance activities by increasing the level of the indicator "verification of equipment and methods related performance". In this case-study a risk based sampling plan was developed and elaborated to verify the control measures toward *L. monocytogenes*. Samples have been taken from incoming (raw) materials, meal components and final products as well from the environment, because environmental sources may, under conditions of poor cleaning and sanitation, harbor pathogenic micro-organisms such as *L. monocytogenes* or serve as vehicles for cross-contamination (Lianou and Sofos, 2007). Especially, non-food contact surfaces, such as floor drains and cold floors, have a higher prevalence of *L. monocytogenes* in comparison with food contact surfaces such as slicers and utensils (Carpentier and Cerf, 2011; Dimitrijevíc et al., 2011; Hoelzer et al., 2011). In this case study, the pathogen was not found in the environmental samples. However, a study of Hoelzer et al. (2011) revealed that in 58 % (n= 241) of retail deli establishments, *L. monocytogenes* isolates were found in the environment of the establishment. Therefore, cleaning and disinfection applied in this food service operation is efficient to prevent an accumulation of high numbers of *L. monocytogenes* in the food production environment. However, recovery rates of swabs are low and therefore small amounts of the pathogen may be present in the food production

environment but could not be detected (Hedin et al., 2010; Moore and Griffith, 2007). Incoming raw materials, namely raw salmon and raw poultry meat, and RTE products, such as smoked salmon, raw meat sandwich spread and cooked ham, have been testing positive (in 25 g) for the presence of *L. monocytogenes*. A study of Uyttendaele et al. (2009) shows that the prevalence of *L. monocytogenes* for cooked meat is approximately 1.1 % (n= 639) and for smoked fish is approximately 27.8% (n= 90). Van Coillie et al. (2004) detected a prevalence for *L. monocytogenes* in smoked salmon of 19 % (n= 42). This high prevalence of *L. monocytogenes* in fish products, may explain the finding of *Listeria* positive raw materials although a small number of samples has been taken. The cooked meat was salt-free because it was adapted to the dietary needs of the patients. However, the reduction of salt increases the survival of *L. monocytogenes* in this food product when post-contamination occurs (Stollewerk et al. 2012). Thus, incoming products (including the exterior of their packages) that have been contaminated at food processing facilities pose a risk for cross-contamination of foods in the (hospital) food service operation when these incoming foods are opened and/or handled in the food service operation (Lianou and Sofos, 2007). Therefore higher demands, such as well controlled storage conditions, well separated storage facilities and a forward flow allowing no crossing among raw materials, RTE meals and trash, are posed on the FSMS in the food service operation. It can be noticed that the raw materials such as the raw salmon and the poultry meat, which were tested positive for *L. monocytogenes*, were not contaminated anymore after heat processing. *L. monocytogenes* is considered to be intolerant to the temperatures achieved during food processing, such as cooking and pasteurization (Kells and Gilmour, 2004). Thus, the intervention processes applied in the food service operation are effective to inactivate the initial contamination. Therefore contamination of RTE foods (including hospital meals) with *L. monocytogenes* is almost exclusively due to post-processing contamination at the producers company and these foods, together with non-processed foods, are therefore more likely to be associated with listeriosis outbreaks than others (Hoelzer et al., 2011; Lianou and Sofos, 2007). One hospital meal has been found positive for *L. monocytogenes*. This meal, called Ardennes egg, contained salt-free cooked ham, which was already contaminated as incoming RTE food product. Contamination, which had taken place at the manufacturer operation, could not be reduced because no heating step in the food service operation could be applied for this meal type. The positive incoming (raw) materials, raised

the awareness that cross-contamination is possible and extra attention should be paid to the slicing machine to cut slices from the cooked meat. The same slicing equipment is used for normal cooked ham and salt-free cooked ham and no cleaning and disinfection step is currently present between the use of these different types of cooked meat, increasing the risk of cross-contamination (Lianou and Sofos, 2007). It also suggests that incoming (raw) material control of suppliers, including the usual suppliers, should be performed on a regular base. From this case study it became also apparent that high-risk foods are still served in hospital food service operations, even though these high risk foods are not directly served to the patients but rather indirectly by offering them in the hospital canteen. However, the types of food served should be selected to minimize the risk of foodborne disease in patients (Lund and O'Brien, 2009; Rodriguez et al., 2011). This means that high risk foods should not be served in the canteen. An operator willing to take such decisions and providing guidelines for a menu builder to reduce the risk of *L. monocytogenes*, would also contribute to a positive food safety culture (Powell et al., 2011). No complaints concerning food safety or hygiene and having no non-conformities with regard to food hygiene and food pathogens cannot guarantee that foodborne illness will be prevented. Especially if the food service operation relies only on guidance or oversight by government or auditors to ensure consumers receive safe food products. A food service operation should be more proactive by evaluating their FSMS on a regular basis and performing product analyses, because the risk of a food service operation to cause foodborne illnesses is to a large extent, a consequence of its own activities (Powell et al., 2011). Therefore, effective food safety systems and practices need to be shared by all levels of the organization, not just management, and communication should be an integral part (Neal et al., 2012). Moreover, by analyzing the risks associated with their products and to know how to manage these risks, a more positive food safety culture can be established. Powell et al. (2011) concluded that the best food producers should go above and beyond minimal government and auditor standards.

4.5 Conclusion

The use of a (self-)assessment questionnaire to evaluate the current FSMS performance and the implementation of a risk based sampling plan to verify the implemented control measures toward the presence of *L. monocytogenes* in a hospital service setting, are useful tools for a food service operation to gain more insight into and to adjust their own food management system. The risk based sampling plan helps to set priorities in selecting incoming materials and defining critical sampling locations to detect *L. monocytogenes* in the environment. By the application of both tools, an overview is obtained on the performance levels of the current implemented control and assurance activities and the results can be used as an internal audit to improve their system. From this case study, it becomes clear that incoming (raw) materials, produced final products and their immediate supply to patients/consumers are high risk situations. This was demonstrated by the presence of *L. monocytogenes* in incoming (raw) materials and final product. These risky situations are in need to be mitigated by the implementation of proper control measures, e.g. intensified supplier control, low storage temperatures, cleaning and disinfection to control cross-contamination. However, in terms of assurance activities, such as setting up a sampling plan, validation and verification of their FSMS, only a basic level was obtained. Therefore, the food service operation does not know how well they are performing and is not capable of self-evaluation. Besides, being more pro-active a food service operation should also work to a good food safety culture, with all levels of the organization involved.

CHAPTER 5

EFFECTIVENESS OF INACTIVATION OF FOODBORNE PATHOGENS DURING SIMULATED HOME PAN FRYING OF STEAK, HAMBURGER OR MEAT STRIPS

Published as:

Lahou, E., Wang, X., De Boeck, E., Verguldt, E., Geeraerd, A., Devlieghere, F. and Uyttendaele, M. (2015). Effectiveness of inactivation of foodborne pathogens during simulated home pan frying of steak, hamburger or meat strips. International Journal of Food Microbiology 206, 115-129.

Abstract

In order to evaluate the effect of simulated home pan frying of raw meat and meat preparations of different animal species on the thermal inactivation of pathogens, the heat resistance (D-value) of three strains of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and two strains of generic *E. coli* was validated in BHI and adjusted BHI (i.e. pH 5.6 and 1.5 % NaCl) at 60°C. The D-values were obtained of the linear phase of the survivor curves created in GInaFiT, a freeware tool to fit models to experimental data. The obtained D-values corresponded to those previously published in literature and confirmed *L. monocytogenes* to be the most heat resistant pathogen among them. Heat treatment in adjusted BHI significantly increased heat resistance of *E. coli* O157:H7 and generic *E. coli*. Subsequently, the thermal inactivation of *L. monocytogenes*, *Salmonella* spp., C. jejuni and *E. coli* O157:H7 was evaluated using a standardized procedure simulating commonly used home pan frying of various types of meat including steaks or filets, hamburgers and meat strips from various animal species such as pork, beef, chicken, lamb and some turkey, horse, kangaroo and crocodile meat. The corresponding process lethality was calculated based upon measured core time/temperature profiles. It was noted that a core temperature of 70°C was not always achieved and, moreover, a heat treatment equivalent to 2 min at 70°C was also not always obtained. This was in particular noted in hamburgers although the meat was visually judged well done. On several occasions, residual survivors of the initial inoculated (4 log CFU/g) foodborne pathogens could be recovered either by enumeration (detection limit of 1 log CFU/g) or by the presence/absence testing per 25 g. Pan frying of hamburgers yielded the highest number of surviving pathogenic bacteria (46 %), followed by well-done filets and steaks (13 %) and meat strips (12 %). Taking only steaks (beef, horse, kangaroo, crocodile and turkey) into account, residual detection of pathogens occurred for all levels of doneness: 18 % for well-done, 71 % for medium and even 90 % for rare steaks. Numbers of *L. monocytogenes* recovered after heat treatment ranged from < 1 log CFU/g to 2.6 log CFU/g. Although, the prevalence of pathogens in meat might be low, and the numbers present in case of natural contamination are probably lower than the current used inoculum of 4 log CFU/g, consumers could still be exposed to surviving foodborne pathogens in case of these commonly used pan frying of raw meat and meat preparations at consumer's home.

5.1 Introduction

Despite preventive measures during slaughter and good hygiene and good manufacturing practices during further processing, raw meat and meat preparations are still occasionally contaminated with pathogenic bacteria such as Listeria monocytogenes, *Salmonella* spp., *Campylobacter* spp. and pathogenic verotoxin-producing *E. coli* (VTEC) (Frank et al., 2011; Hendriksen et al., 2011; Kirkpatrick and Tribble, 2011; Milillo et al., 2012; Scallan et al., 2011; Söderström et al., 2008; Taylor et al., 2012). In 2012, the Belgian government analyzed 2401 samples of meat and 3028 samples of meat preparations. From these analyses it was concluded that *Campylobacter* was present in 6.3 % and *Salmonella* in 4.1 % of the meat samples. *Salmonella* was also present in 0.5 % of the meat preparations, while *L. monocytogenes* and *E. coli* O157 were present in 0.2 % of meat preparations samples (FASFC, 2013). The presence of pathogens in (undercooked) meat can present a serious food safety threat and result in a food-borne outbreak (Takhar et al., 2009). European strong-evidence food-borne outbreaks (i.e. those outbreaks where the evidence implicating a particular food vehicle is strong) are summarized by EFSA and ECDC in their annual EU summary report on zoonoses, zoonotic agents and food-borne outbreaks. In 2012, *Salmonella* spp. were the most frequently reported cause of food-borne outbreaks (28.6 %) in the EU, with pig meat, broiler meat, bovine meat and their derived products responsible for respectively 5.8 %, 3.7 % and 2.0 % of the *Salmonella* strong-evidence foodborne outbreaks. *Campylobacter* spp. were responsible for 9.3 % of the foodborne outbreaks in 2012, with broiler meat and derived products as the most commonly reported cause of strong-evidence outbreaks (44 %). Although verotoxigenic *E. coli* was responsible for only 0.8 % of the total number of reported food-borne outbreaks, the main food vehicle in strong-evidence outbreaks was bovine meat and products thereof (50 %), followed by pig meat (16.7 %) (EFSA and ECDC, 2014). The main settings where strong-evidence food-borne outbreaks have occurred are households/domestic kitchens of consumers (39.7 %) and restaurants, cafés, pubs, bar and hotels (23.9 %) (EFSA, 2014b). A large part of these food-borne outbreaks and most of the separate cases of foodborne infections or poisonings can be attributed to careless actions by consumers during the preparation of the food (FASFC, 2012; Sampers et al., 2012). In a study of Fischer et al. (2007) participants claimed to prefer convenience and taste over food safety and effort. Among the regular occurrence of cross-

contamination events, another inadvertence by consumers during the preparation of food is undercooking (Sampers et al., 2012). The heat treatment of raw meat in consumer domestic kitchens or food service operations is of great importance to provide sufficient inactivation of possible pathogenic bacteria present (Murphy et al., 2004). However, preventing cross-contamination and respecting the cold chain is important as well to avoid a higher initial load because if the initial load is too high, it might not be possible to sufficient inactivate the micro-organisms. It is generally accepted that when meat (including hamburgers or any other comminuted meat) is subjected to a core temperature of 70°C for 2 min or was subjected to a heat treatment equivalent to 2 min at 70°C, it will accomplish a substantial inactivation (6 log reduction) of pathogens and therefore renders the meat to be safe (ACMSF, 2007). In the case of steak or filet it is assumed that the meat is internally sterile and that high temperatures on the surface during pan frying are sufficient to inactivate any pathogens present. However, it is not always clear which temperatures are actually reached during home pan frying of raw meat. Although measuring the internal temperature of meat is a useful method to assess readiness for consumption, the use of a thermometer to assess the doneness of food is currently uncommon in European households (Bearth et al., 2014). Besides, thermal inactivation of pathogens and presence of residual survivors in meat may also vary depending upon the exact nutritional composition (e.g. fat content), texture (e.g. fiber structure) and the initial number of micro-organisms present (Jay, 2000; Tuntivanich et al., 2008). The effectiveness of thermal inactivation processes during home cooking procedures should gain more attention as 36.6 % of the total fresh meat bought on the Belgian market are mixed meat preparations (sausages, mixed minced meat, hamburgers) (VLAM, 2014). These meat preparations have more opportunities for introducing pathogens in the meat, but also have an increased risk to contain pathogens in the core of the food product because they are more extensively handled and undergo extensive manipulations. This increases the risk of survival and cross-contamination of pathogenic micro-organisms in undercooked meat (Sampers et al., 2012). Besides, Bergsma et al. (2007) and de Jong et al. (2012) showed in their studies unsuspected survival of pathogens during consumer style cooking techniques. The objective of the present study is to evaluate the effect of simulated home pan frying of raw meat and meat preparations of different animal species on the thermal inactivation of pathogens.

5.2 Materials and methods

5.2.1 Selection of bacterial strains and culture conditions

In this study, 3 strains of *L. monocytogenes*, *Salmonella* spp., *Campylobacter* jejuni, (nalidixic acid resistant) *E. coli* O157:H7 and 2 strains of generic *E. coli* were used (Table 5.1). The strains were obtained from the culture collection of the Laboratory of Food Microbiology and Food Preservation (LFMFP) of Ghent University (Ghent, Belgium) and from the culture collection of the Belgian Veterinary and Agrochemical Research Centre (CODA, Brussels, Belgium). Stock cultures of *L. monocytogenes*, *Salmonella* spp., *E. coli* O157:H7 and generic *E. coli* strains were kept at −75°C in Tryptone Soy Broth (TSB, Oxoid, Bastingstoke, UK), supplemented with 0.6 % yeast extract (YE, Oxoid) and 15 % glycerol (Prolabo, Heverlee, Belgium). Working stocks were stored refrigerated at 4°C on Tryptone Soy Agar (TSA, Oxoid) slants (supplemented with 50 µg/ml nalidixic acid for *E. coli* O157:H7) and were renewed monthly. Working cultures were activated by transferring a loop culture from slants into 10 ml of Brain Heart Infusion broth (BHI, Oxoid) (supplemented with 50 µg/ml nalidixic acid for *E. coli* O157:H7) and incubation at 37°C for 24 h. A reference stock culture of *C. jejuni* strains was kept at −75 °C in full-horse blood (E&O Laboratories, Bonnybridge, England). A swab of each strain was transferred into 10 ml of selective Bolton broth (Oxoid) and incubated at 41.5°C for 48 h under microaerobic conditions provided by Campygen packs (Oxoid) in closed jars. Working stocks were stored at 4°C under microaerobic conditions, and were renewed monthly. The working cultures were prepared by transferring 0.1 ml of each stock culture into 10 ml of fresh Bolton broth and incubation under microaerobic conditions at 41.5°C for 48 h to stationary phase. Purity and verification of all the cultures concentration (8 – 9 log CFU/ml) were confirmed by 10-fold serial dilutions from working cultures into Peptone Physiological Salt solution (PPS, containing 1 g/l neutralized bacteriological peptone and 8.5 g/l NaCl) and spread plating 0.1 ml from selected dilutions onto duplicates of TSA plates.

Table 5.1. Bacterial strains used for thermal inactivation experiments.

Strain	Strain number	Origin	Comment
Listeria monocytogenes 4b	392	Liver pate	
Listeria monocytogenes 4b	421	Human isolate	
Listeria monocytogenes	491	Tuna deli-salad	
Salmonella Derby	872	Pig	CODA strain 2011/01431
Salmonella Enteritidis	875	Poultry	CODA strain 2011/00166
Salmonella Typhimurium	877	Poultry	CODA strain 2011/01081.9
Campylobacter jejuni	595	Poultry	
Campylobacter jejuni	866	Poultry	
Campylobacter jejuni	867	Human faeces	
Escherichia coli O157:H7	846	Beef carpacio	Nalidixic acid resistant strain
Escherichia coli O157:H7	847	Human faeces	Nalidixic acid resistant strain
Escherichia coli O157:H7	849	Bovine faeces	Nalidixic acid resistant strain
Escherichia coli	063	Human faeces	
Escherichia coli	168	Human faeces	

5.2.2 Determination of D-values of bacterial strains

5.2.2.1 Heat challenge

A stationary phase culture of each tested bacterial strain was diluted in BHI or Bolton broth, in case of *C. jejuni* strains, to around 6 log CFU/ml. At set time points (i.e. 0, 2, 4, 6, 7, 8 and 9 min), 1 ml of the diluted culture was used to inoculate 9 ml of pre-heated heat challenge medium to establish heat inactivation curves. The heat challenge medium BHI (or Bolton broth in case of *C. jejuni* strains) was dispensed in test tubes, submerged in a water bath (Memmert,WNB 10, Schwabach,Germany) and preheated to the target inactivation temperature of 60°C before being inoculated. The temperature of the medium was monitored using a Testo 177-T4 temperature data logger (Testo AG, Lenzkirch, Germany) in a test tube with non-inoculated BHI or Bolton broth throughout the duration of the heat treatment (i.e. 10 min). Ten minutes after the first inoculation all inoculated test tubes were taken together from the hot water bath and placed in an iced water bath to cool down before enumeration. The heat treatment was performed in triplicate for each strain. In addition, the heat resistance of all strains was also determined in a heat challenge medium (BHI or Bolton broth) adjusted towards pH 5.6 and 1.5 % NaCl (w/w); both values mimicking those measured in ground pork meat. Lactic acid (10 mol/l) (Roland Chemicalien, Brussels,

Belgium) was used to adjust the medium to pH 5.6 (after autoclaving). The added volume of lactic acid did not significantly affect the volume of the challenge medium. The pH and a_w values of the adjusted broth were confirmed with a digital pH-meter (pH flash seven easy, Mettler-Toledo, Zaventem, Belgium) and an a_w-cryometer (NAGY AWK-30, NAGY Messysteme, Gaufelden, Germany).

5.2.2.2 Enumeration of surviving organisms

The number of surviving organisms was determined by tenfold dilutions of the inoculated heat medium in PPS, followed by plating on appropriate selective isolation media. Enumeration of *L. monocytogenes* was performed by spread plating 0.1 ml on Agar Listeria Ottaviani & Agosti (ALOA) (Biolife,Milano, Italy),while *Salmonella* was enumerated on xylose lysine deoxycholate agar (XLD, Oxoid), *C. jejuni* on CampyFood agar (CFA, bioMérieux, Marcy l'Etoile, France), *E. coli* O157:H7 on Chromocult® Coliform Agar (Merck, Darmstadt, Germany) supplemented with 50 µg/ml nalidixic acid and generic *E. coli* on RAPID'*E. coli* 2 Medium (REC, Bio-Rad, CA, USA). When increased sensitivity was required, 1.0 ml of the undiluted suspension was spread plated on three plates (0.3, 0.3 and 0.4 ml). Incubation took place for 24 h at 37°C for *Salmonella*, *E. coli* O157:H7 and generic *E. coli*, and 48 h at 37°C for *L. monocytogenes*. The CFA plates were incubated microaerobically for 48 h at 41.5°C.

5.2.2.3 Data analysis

The inactivation data were analyzed by linear and non-linear models by the software GInaFiT (version 1.7, under preparation) (Geeraerd et al., 2005). The goodness of fit of the models was assessed using the automatically reported regression coefficient (R2) and root mean square error (RMSE). The Akaike information criterion (AIC) was calculated to detect model overfitting (Akaike, 1973). The kinetic parameters and the maximum specific inactivation rate (kmax) were obtained from the best fitted model. The D-values (time to inactivate 90 % of the viable cells) was calculated as ln10/kmax for each strain. Statistical interpretation of differences between different organisms and broths were determined using one-way ANOVA (the Tukey HSD test was used as a post hoc test) with SPSS statistical

software (version 21.0, SPSS Inc., Chicago, USA). Differences were considered significant when the P-value was less than 0.05.

5.2.3 Simulation of home pan frying of meat and meat preparations

5.2.3.1 Description of the meat and meat preparations

Simulation of commonly used pan frying of meat and meat preparations was performed using pork, chicken, beef and lamb. For each animal species, three different meat types were chosen, namely (i) a meat preparation based on minced meat (i.e. hamburger), (ii) intact fresh meat (e.g. steak or filet) and (iii) a meat preparation of intact meat (i.e. pitta meat being marinated meat strips). In addition, pan frying of steak was also performed on horse, kangaroo, crocodile and turkey to assess the effect of meat structure and origin on the survival of pathogenic bacteria. Realistic consumer meat portions were used, which is for intact fresh meat a piece of 150 g (thickness 1.5 cm), for meat preparations of intact meat a portion of 300 g meat strips, and for meat preparations based on minced meat a hamburger of 120 g (diameter 8 cm, thickness 1.5 cm). All experiments were performed in triplicate starting from three different batches of meat which were ordered in the butchery department of a local retailer. The characteristics of the selected meat and meat preparations are shown in Table 5.2. The pH was measured by a pH-electrode (InLab® 427, Mettler Toledo GmbH, Schwerzenbach, Switzerland) connected with a pH-meter (SevenEasy™, Mettler Toledo GmbH). The water activity (a_w) was measured with an automated a_w cryometer (AWK-20, NAGY Messysteme GmbH, Gäufelden, Germany). The chloride content (indicator of the NaCl content) was determined according to the Mohr method (ISO 9297:1989). The chloride was extracted from the meat samples by cooking the meat for 10 min in distilled water. The concentration of chloride ions was determined by means of a silver nitrate (Merck, Darmstadt, Germany) titration with a 5 % (w/v) chromate indicator (Merck, Darmstadt, Germany). The dry content was determined gravimetrically after drying an aliquot of the homogenate at 105°C. The homogenate was heated in aluminum dishes containing sea sand to avoid splashing. The fat content of the meat was assessed using the Weibull Analysis (Egan et al., 1981).

5.2.3.2 Assessment of the initial quality and safety of the meat and meat preparations

The total aerobic psychrotrophic count, the number of generic *E. coli* and the presence/absence of *Salmonella* spp., *L. monocytogenes*, *E. coli* O157 and *C. jejuni* were assessed for the purchased meat and meat preparations. For enumeration, 10 g of each food sample was homogenized for 1 min in 90 ml of sterile peptone water, followed by tenfold serial dilutions in PPS to assess the number of micro-organisms. A modified ISO 4833:2003 method (plating on Plate Count Agar (PCA, Oxoid) and 120 h incubation at 22 °C) was used for the enumeration of the total aerobic psychrotrophic count. An AFNOR validated method (no BRD 07/1-12/04) was used for the enumeration of generic *E. coli* (plating on RAPID'E.coli 2 (Bio-Rad) and 24 h incubation at 37°C). For detection of *Salmonella*, *L. monocytogenes*, *E. coli* O157 and *C. jejuni*, a 25 g subsample was weighed in a stomacher bag and homogenized for 2 min in 225 ml of the respective (semi)selective medium, i.e. buffered peptone water for the detection of *Salmonella* and *E. coli* O157, demi-Fraser for the detection of *L. monocytogenes* and Bolton broth for detection of *C. jejuni*. The detection of *L. monocytogenes* was performed using VidasLMO2 (Biomérieux), an AFNOR validated Enzyme-Linked Fluorescent assay (ELFA-) method (no BIO-12/11-03/04). Confirmation of presumptive positive results was performed by plating out suspected samples on ALOA. The detection of *C. jejuni* was performed using CampyFood Agar (Biomérieux) and micro-aerophilic incubation for 24 – 48 h at 41.5°C. The detection of *Salmonella* and *E. coli* O157 was performed using real-time PCR according to the GeneDisc principle (Pall, NY, USA) which allows simultaneous detection of genes encoding Shiga toxins 1 and 2 (stx1 and stx2), intimin (eae), the *E. coli* O157 antigen (rfbE), and *Salmonella* spp.-specific genes. Confirmation of presumptive positive results obtained by the Genedisc for *Salmonella* and *E. coli* O157 was performed by plating out suspected samples on selective media, i.e. XLD for confirmation of *Salmonella* (after 24 h enrichment in RVS broth) and Chromocult® Coliform Agar (Merck) with 50 µg/ml nalidixic acid for confirmation of *E. coli* O157 (after concentration with Dynabeads® *E. coli* anti-O157 (Thermo Fisher Scientific Inc., MA, USA)). Further confirmation of the isolates was then performed using Crystal E/NF ID (BD Benelux N.V, Erembodegem, Belgium) for *Salmonella* and *Escherichia coli* O157 Latex Test for *E. coli* O157 (Thermo Fisher Scientific Inc.).

5.2.3.3 Inoculation of the meat and meat preparations

The stationary phase cultures of the different strains of each microorganism were mixed in equal quantity to obtain a mixed culture. Each mixed culture was diluted in PPS to achieve an inoculation level of ca. 4 log CFU/g on the purchased meat and meat preparations. The inoculation procedure is based on the procedure described in the "Technical guidance document on shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods" (EU CRL, 2008). The steak or filet was inoculated at the surfaces and edges to mimic contamination during processing (e.g. meat cutting). The inocula were distributed across the surface using a sterile L-shaped plastic spreader. The purchased ground meat was inoculated in depth, blended and shaped into hamburgers to obtain homogeneous contaminated hamburgers. The meat strips were mixed with the inoculum to achieve a distribution of the micro-organisms. All inoculated meat and meat preparations were stored for 18 h at 7 °C. The concentration of the micro-organisms was determined immediately after inoculation and after refrigerated storage using serial dilutions in PPS, followed by plating on selective media as described in Section 2.2.2.

Table 5.2. Characteristics of the meat and meat preparations subjected to the treatments.

Animal species	Meat type	pH	a_w	% NaCl	% dry content	% fat
Pork	Hamburger	5.59	0.9846	1.545	35.61	8.10
	Filet	5.55	0.9946	0.060	25.69	1.33
	Meat strips	5.93	0.9831	1.265	34.47	12.07
Chicken	Hamburger	6.32	0.9790	0.857	27.20	7.76
	Filet	6.27	0.9892	0.053	24.07	1.44
	Meat strips	6.28	0.9808	0.730	30.60	9.30
Beef	Hamburger	5.72	0.9894	0.227	24.01	1.14
	Filet	5.62	0.9891	0.070	23.63	0.34
	Meat strips	5.71	0.9798	1.320	29.75	4.54
Lamb	Hamburger	5.74	0.9761	0.993	37.51	15.50
	Filet	5.93	0.9896	0.053	27.43	6.2
	Meat strips	5.83	0.9815	0.987	27.80	8.33
Horse	Steak	5.83	0.9892	0.137	25.92	2.61
Kangaroo	Steak	6.05	0.9888	0.093	24.14	1.23
Crocodile	Steak	6.63	0.9922	0.057	23.94	4.25
Turkey	Steak	5.78	0.9884	0.077	25.77	2.08

5.2.3.4 Pan-frying of the meat and meat preparations and temperature measurement

The steaks, filets and hamburgers were heat treated for several minutes on each side, depending on the meat type, in a frying pan in hot butter at high temperatures in a standardized manner (Table 5.3) to simulate commonly used cooking practices of this type of meat and meat preparations in Belgium. After these cooking practices the meat and meat preparations were allowed to rest for 3 – 5 min on a serving plate at room temperature before analysis took place. As it is currently uncommon in Belgian households to use a thermometer to assess the doneness of food, standardization of pan frying was driven by operational practices of preparation (state of electrical fire and time of heating) in order to obtain a visually assessed level of doneness of the meat and meat preparations, rather than the achievement of a core temperature. The standardized heat treatments were set as those to be commonly used and to provide visually well-done meat as assessed by a team of 5 lab collaborators. As steak is commonly prepared and consumed in Belgium as rare, medium or well-done, steaks from different animal species (such as beef, horse, kangaroo and crocodile) have also been subjected to milder heat treatments in a frying pan in hot butter using a standardized time for each side to achieve the steak to be assessed as either rare (2’), medium (4’) or well-done (6.5’) (Table 5.3). Stir frying was preferred for the marinated meat strips. During the heat treatment and the resting time on the serving plate, core temperature and temperatures of the sub-surfaces of the filets, steaks or hamburgers were monitored every 5 s in parallel on non-inoculated meat and meat preparations. To measure the core temperature, a wireless temperature probe (DataTrace T-logger,Mesa Laboratories Inc., Colorado, USA) was placed into the core of the meat. The edge temperatures were measured on top and on the bottom with a data logger (Testo 177-T4, Testo NV/SA, Ternat, Belgium). To measure the temperature during stir frying, the wireless temperature probe was co-stir fried with the 300 g portion of this meat preparation.

5.2.3.5 Detection and enumeration of surviving bacteria

After performing the pan frying or stir frying, enumeration of the residual pathogens on artificially contaminated meat was performed using appropriate selective media described in Section 5.2.2.2. For enumeration, a cross section of 10 g of each food sample was

Table 5.3. Standardized commonly used cooking practices for intact meat and meat preparations.

Action	Meat preparations of ground meat		Intact meat				Meat preparations of intact meat	State of electrical fir (from 1 (low) to 7 (high)
	Pork, Beef	Lamb, Chicken	Pork	Lamb	Chicken	Beef,Horse, Turkey, Kangaroo, Crocodile	Pork, Chicken, Beef, Lamb	
Preheating Pan	2'	2'	2'	2'	2'	2'	2'	7
Melting butter	2'	2'	2'	2'	2'	2'		7
Side 1	5'	6'	5'	6'	10'	2' or 4' or 6'30"		5
Side 2	5'	6'	5'	6'	10'	2' or 4' or 6'30"		5
Stir frying							7	7

': minutes ":seconds

homogenized for 1 minute in 90 ml of sterile peptone water. Because low numbers were expected for the residual pathogens, 1 ml of the inoculum was spread on three selective plates (detection limit of 1 log CFU/g). Besides enumeration, the presence or absence was assessed using enrichment methods described in Section 5.2.3.2. Depending on the amount of available meat, the detection test in a cross section of 25 g of each food sample was performed either in singular (hamburgers), in duplicate (filets or steaks) or in triplicate (meat strips).

5.2.3.6 Data analysis

Data were analyzed using Microsoft Excel (version 2010). Means and standard deviations of the bacteria on the meat and meat preparations were determined over the three replicates (each using a different batch of meat or meat preparation). As the heating process is non-isothermal the inactivation (i.e. process lethality *P*) can be approximated by summating the lethal rates (*L*) between the measuring intervals (Gaze, 2006). The lethal rate for every interval is calculated with following formula:

$$L = \int_0^t 10^{\frac{(Tt-Tref)}{z}} dt$$

where *Tt* is the temperature at the core of the meat at time *t*, and *Tref* is the reference temperature. In this study *Tref* was 70°C and the z-value was 7°C as recommended for *L.*

monocytogenes (van Asselt and Zwietering, 2006). Because the lethal effect below 55°C is negligible, only *Tt* ≥ 55.0°C were used to calculate the process lethality *P* (Claeys et al., 1998).

5.3 Results

5.3.1 Determination of D-values of bacterial strains

Among the linear and non-linear inactivation models included in the GInaFiT software, the biphasic model (with or without shoulder) provided the best fit to data with high R2-values, relatively low RMSE values and smaller AIC values (data not shown). Both in normal (data not shown) and adjusted broth (Figure 5.1), shoulders (lag phase) were observed for *L. monocytogenes* and *E. coli* O157:H7. The *kmax* was derived from the first phase of the biphasic model, therefore the obtained D-value for each strain in normal and adjusted broth represents the first log reduction. These D-values are listed in Table 5.4. A full comparison of the D-values of different organisms indicated that *C. jejuni* was less heat resistant than other organisms in both challenge media. In normal broth, *L. monocytogenes* was the most heat resistant pathogen. All D-values of the organisms treated in adjusted broth were greater than those in normal broth. However, the difference was only significant for *E. coli* 0157:H7 and generic *E. coli*. Average D-values of the pathogens in broth or buffers reported from the Combase database and literature (Clavero et al., 1994; Nguyen et al., 2006) where experimental conditions laid down in normal broth (pH of 7 – 7.5, a_w of 0.99 – 1.00) were also listed and compared to the ones estimated in this study. The D-values obtained in our study were in the range of reported values ($P < 0.05$).

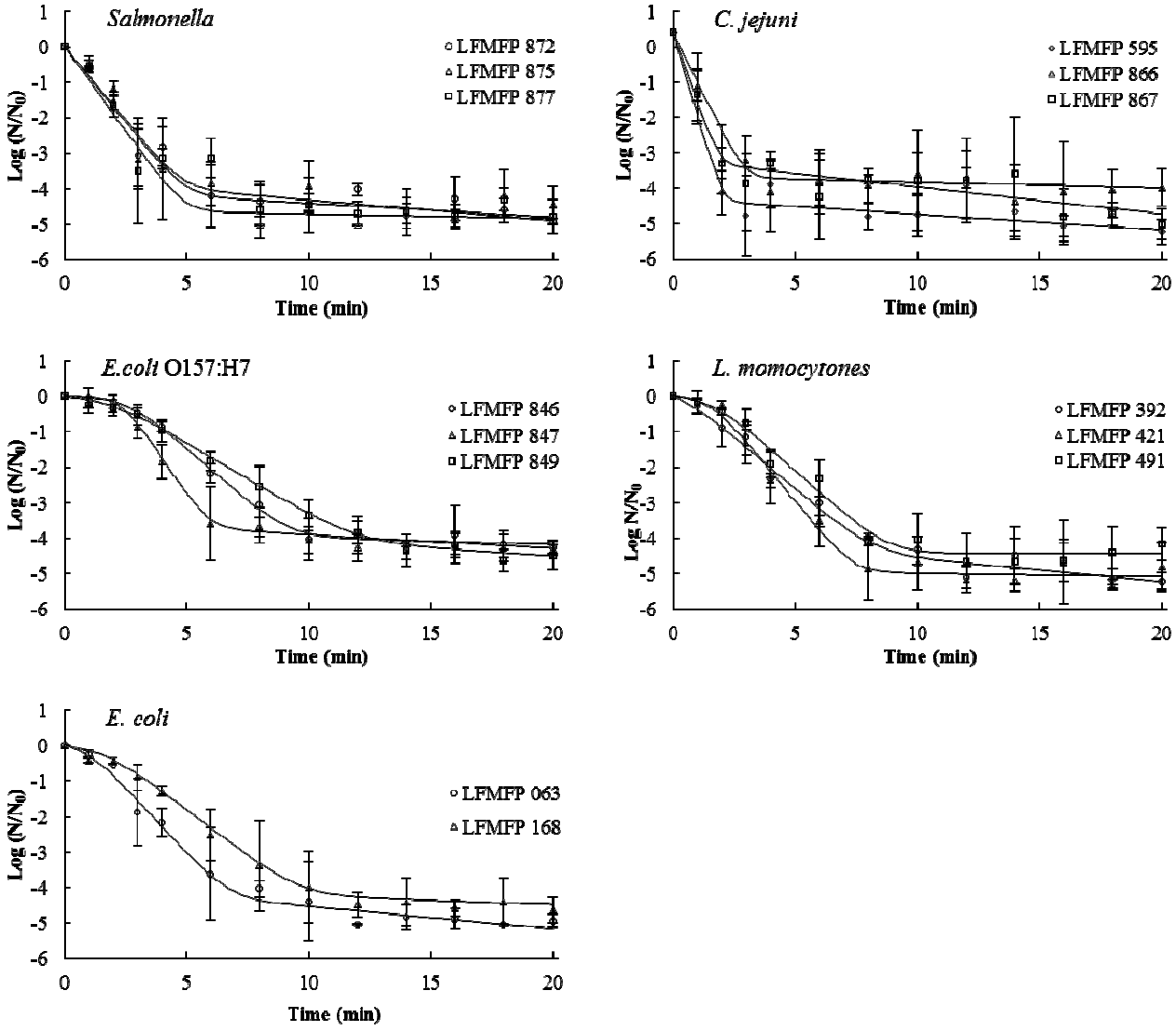


Figure 5.1 Thermal inactivation curves of foodborne pathogens at 60 oC in adjusted broth (pH 5.6 and 1.5% NaCl). Each symbol represents an individual strain of pathogen. Solid lines are regression lines fitted with GInaFiT.

Table 5.4. Estimated D-values at 60°C in normal and adjusted broth in this study compared with D-values reported in previous studies.

Bacterial strain		D-values from normal broth[a] (min)	Mean D-values from published data (min)	D-values from adjusted broth[a] (min)
Salmonella	872	0.59 ± 0.13	0.75 ± 0.74 (n = 62 [d]) AX	0.72 ± 0.39
	875	0.64 ± 0.02		0.95 ± 0.59
	877	0.62 ± 0.08		1.42 ± 0.44
	Mean[b]	0.61 ± 0.08 ABX [c]		1.03 ± 0.52 BX
C. jejuni	595	0.36 ± 0.00	0.31 ± 0.26 (n = 12) AX	0.43 ± 0.07
	866	0.30 ± 0.04		0.58 ± 0.15
	867	0.54 ± 0.18		0.46 ± 0.07
	Mean[b]	0.40 ± 0.14 AX		0.49 ± 0.11 AX
E. coli O157:H7	846	0.73 ± 0.10	1.35 ± 0.72 (n = 37) BXY	1.66 ± 0.49
	847	0.88 ± 0.14		1.06 ± 0.33
	849	0.89 ± 0.11		2.17 ± 0.03
	Mean[b]	0.84 ± 0.13 BX		1.63 ± 0.56 CY
L. monocytogenes	392	1.37 ± 0.46	1.74 ± 1.12 (n = 33) BX	1.26 ± 0.25
	421	1.05 ± 0.10		1.07 ± 0.31
	491	1.42 ± 0.40		1.90 ± 0.50
	Mean[b]	1.28 ± 0.36 CX		1.41 ± 0.50 BCX
E. coli	063	0.69 ± 0.07	N.D.[e]	1.26 ± 0.38
	168	1.01 ± 0.13		1.47 ± 0.29
	Mean[b]	0.85 ± 0.20 BX		1.37 ± 0.32 BCY

[a] Values are expressed in mean standard deviations from three replicates.
[b] Mean D-values of three or two strains of each organism.
[c] Means in the same column with a different letter (A through C) are significantly different ($P < 0.05$). Means in the same row with different letters (X through Y) are significantly different ($P < 0.05$).
[d] n, Number of D-values reported from ComBase database and literature .
[e] N.D. no data.

5.3.2 Assessment of initial quality and safety of the meat and meat preparations

An overview of the initial quality of the meat and meat preparations prior to the heat treatments is shown in Table 5.5. The meat and meat preparations had a variable microbiological quality, as can be deduced from the results. Pork filet, marinated pork strips, beef steak, marinated beef strips, crocodile and turkey steak had aerobic psychrotrophic counts (APC) of 3 – 4 log CFU/g, indicating that those products had a very good initial microbial quality. The hamburgers in general as well as the chicken filet and marinated chicken strips had APC of ca. 5 log CFU/g. Horse steak showed the highest APC exceeding 6 log CFU/ g. This indicated less good initial quality, however, none of the products showed off-odors and they were all visually acceptable for consumption. Generic *E. coli*, a fecal hygiene indicator, was used to assess the hygienic conditions of the meat samples. The presence of enumerable generic *E. coli* (1 – 2 log CFU/g) was observed occasionally, in particular in all types of raw chicken meat, as well as in beef hamburgers and horse,

kangaroo, and turkey steak. *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157 were not detected in any of the meat samples. *L. monocytogenes* was detected two out of three times in chicken and lamb burgers and was found once in a batch of marinated chicken strips and horse steak. The pathogen numbers were all below the level of standard enumeration i.e. < 1 log CFU/g, but these results show the potential presence of low numbers of pathogenic bacteria in raw meat and meat preparations for sale in Belgium.

Table 5.5. Microbial quality (in log CFU/g) of the meat and meat preparations prior to heat treatments;

Animal species	Meat type	Total aerobic psychotropic count	*E. coli*
Pork	Hamburger	4.9 ± 0.6	< 1.0
	Fillet	3.8 ± 0.4	< 1.0
	Marinated strips	4.0 ± 0.4	< 1.0
Chicken	Hamburger	5.0 ± 0.2	1.8 ± 0.8
	Fillet	5.3 ± 0.6	1.3 ± 0.3
	Marinated strips	5.0 ± 0.4	1.6 ± 0.6
Beef	Hamburger	5.5 ± 0.4	1.4 ± 0.6
	Steak	3.4 ± 0.3	< 1.0
	Marinated strips	3.8 ± 1.4	< 1.0
Lamb	Hamburger	5.3 ± 0.5	< 1.0
	Fillet	4.7 ± 0.4	< 1.0
	Marinated strips	4.6 ± 0.8	< 1.0
Horse	Steak	6.1	1.9
Kangaroo	Steak	4.8	1.3
Crocodile	Steak	4.2	< 1.0
Turkey	Steak	3.7	1.0

5.3.3 Temperature profile during pan-frying of meat and meat preparations

During pan-frying of the inoculated raw meat and meat preparations, the core temperature and temperatures at sub-surface were measured in parallel on non-inoculated meat and meat preparations. The core temperature profiles of the simulated home cooking practices (2-sided pan-frying or stir-frying) for the various types of animal meat species are shown in Figure 5.2. From these results it can be noted that core temperatures of 70°C were only achieved in 24 out of 36 occasions. In another 3 experiments, core temperatures of 70°C were only achieved after the meat was taken out of the pan and allowed to rest for 3 – 5min on the serving plate. In 5 out of the 12 experiments of pan-frying of hamburgers, core temperatures of 70°C were not achieved, even not after the 3 – 5 min resting time on the serving plate. During stir frying of marinated meat strips, temperatures of more than 80°C can be measured (Figure 5.2). However, as it was not feasible to insert the temperature probe in the actual small meat strips, the temperature probe was added to the frying pan

with the meat portion and treated as a piece of meat strip, i.e. stir fried, thus it is uncertain how representative this measured temperature actually is for the meat strips' surface temperature. Still, meat strips are small meat parts for which heat transfer and heating up may easily occur during heat treatment in particular if the meat is frequently stirred. The process lethality (*L*) and the equivalent time of each simulated cooking practice was calculated for the frying time (thus excluding the resting time on the serving plate). These results are summarized in Tables 5.6a and 5.6b. It should be mentioned that the calculated *L*-value was based on the temperature profile obtained during pan-frying of non-inoculated meat and meat preparations. For a sufficient inactivation of *L. monocytogenes* (6 log reduction) a heat treatment equivalent to 2 min at 70 °C, thus a *P*-value of 2, is usually recommended (ACMSF, 2007; ILSI, 2012; Lund, 2014). From the results it can be concluded that high *P*-values are obtained for marinated meat strips. However, for the other meat types the *P*-value may be variable even among independent repetitions of pan-frying, although the pan-frying process was standardized and at all times the meat was judged as well-done after preparation by the executing lab team members. When a sufficient inactivation was not obtained during the heat treatment, *P*-valueswere recalculated (data not shown) to include the temperature data of the meat on the serving plate. In 5 out of 36 times the *P*-value of 2 was only achieved during the 3–5 minute time that the pan-fried meat was kept on a serving plate (Tables 5.6a and 5.6b). It was indeed noted that the core temperature of the meat still slightly increased after taking the meat out of the pan and putting it on the serving plate at room temperature. On a few occasions, i.e. in pan frying of pork filet, chicken filet, beef steak and beef and lamb hamburgers, the heat process of 70 °C for 2 min was not achieved throughout the experiments' time duration (thus including 3–5 min resting time) (Table 5.6).

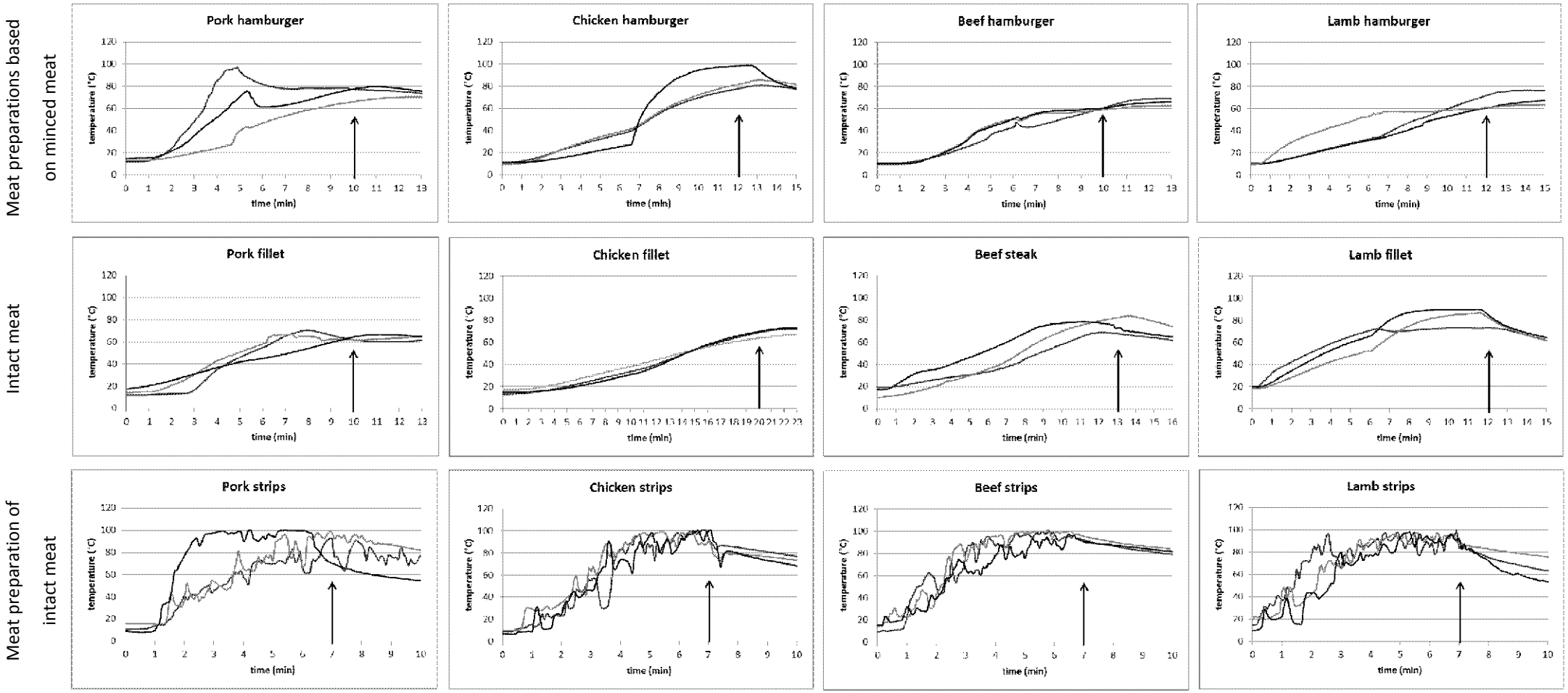


Figure 5.2 Core temperature profiles measured on non-inoculated meat and meat preparations. The different lines are different repetitions. The arrow illustrates the time that the meat was taken out of the pan.

Table 5.6. Process lethality *P* calculated with a z-value of 7°C (Table A) or a z-value of 5°C (Table B) and core temperatures (≥ 55°C) measured during the heat treatment of the different meat and meat preparations.

A

Animal species	Meat type	Frying Time	Repetition 1 *P*-value	Equivalent time[a] needed to obtain P=2	Repetition 2 *P*-value	Equivalent time[a] needed to obtain P=2	Repetition 3 *P*-value	Equivalent time[a] needed to obtain P=2
Pork	Hamburger	10'	3 547	3'55"	0.24	*12'25"*	10.8	5'30"
	Filet	10'	1.55	Not achieved[b]	0.65	Not achieved[b]	0.13	Not achieved[b]
	Marinated strips	7'	403	5'30"	5 954	3'50"	38 192	2'05"
Chicken	Hamburger	12'	10.9	10'50"	35.2	10'20"	17 230	8'10"
	Filet	20'	0.80	*21'10"*	0.21	Not achieved[b]	1.15	*20'45"*
	Marinated strips	7'	14 128	3'55"	13 369	3'25"	9 663	3'35"
Beef	Hamburger	10'	0.03	*12'45"*	0.06	Not achieved[b]	0.12	Not achieved[b]
	Steak	13'	1.16	Not achieved[b]	31.3	11'00"	28.4	9'10"
	Marinated strips	7'	17 082	3'00"	37 622	2'35"	3 785	4'00"
Lamb	Hamburger	12'	0.91	*12'25"*	0.03	Not achieved[b]	0.11	Not achieved[b]
	Filet	12'	12.2	6'45"	418	8'05"	2 317	6'45"
	Marinated strips	7'	7 533	2'00"	7 711	3'15"	5 348	3'00"

B

Animal species	Meat type	Frying time	Repetition 1 *P*-value	Equivalent time[a] needed to obtain P=2	Repetition 2 *P*-value	Equivalent time[a] needed to obtain P=2	Repetition 3 *P*-value	Equivalent time[a] needed to obtain P=2
Pork	Hamburger	10'	92 832	3'50"	0.11	*12'40"*	21.2	5'20"
	Filet	10'	1.32	Not achieved[b]	0.34	Not achieved[b]	0.05	Not achieved[b]
	Marinated strips	7'	6 875	5'20"	198 401	3'50"	1 685 937	2'00"
Chicken	Hamburger	12'	23.1	10'45"	125	10'15"	610 683	8'05"
	Filet	20'	0.52	*21'20"*	0.07	Not achieved[b]	0.84	*20'55"*
	Marinated strips	7'	597 302	3'55"	471 671	3'25"	377 241	3'35"
Beef	Hamburger	10'	0.01	*13'20"*	0.01	Not achieved[b]	0.03	Not achieved[b]
	Steak	13'	0.90	Not achieved[b]	118	10'50"	74.9	9'00"
	Marinated strips	7'	618 074	3'00"	1 724 591	2'25"	99 597	3'55"
Lamb	Hamburger	12'	0.82	*12'25"*	0.01	Not achieved[b]	0.03	Not achieved[b]
	Filet	12'	16	6'40"	3 012	8'00"	285	6'40"
	Marinated strips	7'	236 786	1'55"	204 414	3'10"	150 769	3'00"

‘ minutes “seconds

[a] The safety barrier of 70°C for 2 minutes is used as this time/temperature combination is currently applied to a wide range of foods for a wide range of pathogens. The equivalent time may exceed the total heating time (shown in italics), however the meat was allowed to rest for 3-5 min before the temperature measurement was cancelled and analyses was performed.

[b] The heat process of 70°C for 2 minutes is not achieved during the total heating time and resting time (max. 5 min) of the meat and meat preparations. Therefore the theoretical assumed 6 log reduction could not take place.

5.3.4 Detection and enumeration of surviving bacteria

The residual recovery of the pathogenic bacteria on the inoculated meat and meat preparations after simulated home-cooking practices is summarized in Table 5.7. From these results it can be concluded that hamburgers yielded the highest quantifiable pathogen recovery with pork meat burgers (40 %) yielding the most, followed by lamb meat (20 %), beef meat (13 %) and chicken meat (0 %). Levels of *L. monocytogenes* on hamburgers after the heat treatment ranged from < 1 log CFU/g to 2.6 log CFU/g. Levels of *Salmonella* spp., *E. coli* O157 and *Campylobacter* on these hamburgers ranged from <1 log CFU/g to 1.0 log CFU/g. Levels of generic *E. coli* on hamburgers were also situated on the level of the detection limit, i.e. 1.0 – 1.9 log CFU/g. In addition, also in pork meat strips in 1 out of 3 times *L. monocytogenes* cells could be counted (i.e. 1.0 log CFU/g) after heat treatment. All other meat strips samples did not show any quantifiable pathogen results. Intact meat such as a steak can be prepared with different graduations of pan frying: rare, medium or well-done. Residual detection of pathogens occurred for all levels of doneness: 18 % for well-done, 71 % for medium and even 90 % for rare steaks. As presented in Table 5.7, quantifiable recovery of pathogens was shown for “well-done” (i.e. 6.5 min each side) horse steak with levels ranging from 1.0 log CFU/g to 1.7 log CFU/g. *L. monocytogenes* was detected after enrichment on “well-done” meat 7 out of 30 times (23 %), *Salmonella* spp. 3 out of 29 times (10 %), *Campylobacter* spp. 8 out of 30 times (26.7 %) and *E. coli* O157 2 out of 29 times (7 %). This means that in most cases a 6 log reduction was not obtained using a standard consumer cooking technique to obtain well-done meat. However, when compared with steak baked “medium” (Table 5.8) a substantial reduction in detection is obtained for the “well done” steaks. In the case of “medium” pan fried steaks *L. monocytogenes* was detected 28 out of 30 times (93 %), *Salmonella* spp. 16 out of 30 times (53 %), *Campylobacter* spp. 25 out of times (83 %) and *E. coli* O157 16 out of 30 times (53 %). Pan-frying to obtain “medium” fried meat, i.e. 4 min each side, resulted into enumeration levels ranging from 1.4 ± 0.5 log CFU/g to 1.5 ± 0.8 log CFU/g over the different pathogens. When the steak was just fried on the outside (“rare”), almost all enrichments tested positive and quantifiable recovery ranged from 1.5 ± 0.4 log CFU/g to 2.1 ± 0.8 log CFU/g over the different pathogens included. These results demonstrate that a residual threat to consumers remains, even if the steak is pan fried “well-done”. However, all pathogens have

been frequently recovered at low levels (detected with enrichment methods) in the different types of meat and meat preparations after the simulated home-cooking practices. Taking all results presented in Table 5.7, *L. monocytogenes* was recovered by enrichment in 25 out of 96 experiments (26 %) with the highest number of detectable results in beef products. *Salmonella* spp. was detected in 17 out of 95 experiments (18 %) with the highest number of detectable results in beef products. *Campylobacter* spp. was detected in 12 out of 96 experiments (13 %) and *E. coli* O157 in 10 out of 95 experiments (11 %). As can be deduced from the results in the current study with artificially inoculated meat and meat preparations (inoculum of 4 log CFU/g), hamburgers, which were also internally contaminated, yielded the highest number of surviving pathogenic bacteria (46 %), followed by well-done intact meat (filet and steaks) (13 %) and marinated cut meat preparations (meat strips) subjected to stir-frying (12 %).

Table 5.7. Residual recovery of pathogenic bacteria on inoculated meat and meat preparations after simulated home-cooking practices. Results are presented in log CFU/g.

Inoculation level (log CFU/g)	After baking	Pork			Chicken			Beef			Lamb			Horse Steak[c]	Kangaroo Steak[c]	Crocodile Steak[c]	Turkey Steak[c]
		Hamburger	Filet	Marinated Strips	Hamburger	Filet	Marinated Strips	Hamburger	Steak[c]	Marinated Strips	Hamburger	Filet	Marinated Strips				
L. monocytogenes (4.2 ± 0.3)	Enumeration[a]	3/3	0/3	1/3	0/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3	0/3	0/3
	Mean	1.8 ± 0.8	<1.0	1.0	<1.0	<1.0	<1.0	1.3	<1.0	<1.0	2.6	<1.0	<1.0	1.0	<1.0	<1.0	<1.0
	Maximum	2.5	–	1.0	–	–	–	1.3	–	–	2.6	–	–	1.0	–	–	–
	Enrichment[b]	3/3	0/6	2/9	0/3	0/6	2/9	3/3	2/6	3/9	3/3	2/6	0/9	2/6	1/6	1/6	1/6
Salmonella spp. (4.0 ± 0.3)	Enumeration[a]	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	Mean	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
	Maximum	–	–	–	–	–	–	–	–	–	1.0	–	–	–	–	–	–
	Enrichment[b]	2/3	1/6	0/9	1/3	0/6	0/9	2/3	0/5	5/9	1/3	1/6	1/9	2/6	0/6	0/6	1/6
Campylobacter spp. (3.7 ± 0.5)	Enumeration[a]	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
	Mean	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.7	<1.0	<1.0	<1.0
	Maximum	1.0	–	–	–	–	–	–	–	–	–	–	–	1.7	–	–	–
	Enrichment[b]	3/3	0/6	0/9	0/3	0/6	0/9	0/3	0/6	1/9	0/3	0/6	0/9	4/6	0/6	2/6	2/6
E. coli O157 (3.6 ± 0.4)	Enumeration[a]	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
	Mean	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.3	<1.0	<1.0	<1.0
	Maximum	1.0	–	–	–	–	–	–	–	–	–	–	–	1.3	–	–	–
	Enrichment[b]	2/3	0/6	1/9	1/3	0/6	0/9	0/3	0/5	2/9	1/3	1/6	0/9	2/6	0/6	0/6	0/6
E. coli (3.9 ± 0.3)	Enumeration[a]	1/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3	0/3	1/3	0/3	0/3	0/3
	Mean	1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.0	<1.0	<1.0	1.9	<1.0	<1.0	1.0	<1.0	<1.0	<1.0
	Maximum	1.0	–	–	–	–	–	1.0	–	–	1.9	–	–	1.0	–	–	–

[a] These results present the number of times enumeration was possible for the different repeats

[b] These results present the number of times the pathogen was detected after enrichment in 25 gram food product

[c] These results present the inactivation of “well-done” heat inactivation (6’30”)

Table 5.8. Residual recovery of pathogenic bacteria on inoculated steaks (beef, horse, kangaroo, crocodile and turkey) after simulated home-cooking practices with different gradients of frying (i.e. rare, medium, well done). Results are presented in log CFU/g.

Inoculation level (log CFU/g)		*Listeria monocytogenes* 4.3 ± 0.3	*Salmonella* spp. 4.2 ± 0.3	*Campylobacter* 3.7 ± 0.7	*E. coli* O157 3.6 ± 0.3	*E. coli* 4.1 ± 0.4
Baking time for each side (min)						
2′ (=rare)	Enumeration[a]	11/15	9/15	7/15	10/15	9/15
	Mean	1.9 ± 0.5	1.9 ± 0.4	2.1 ± 0.8	1.5 ± 0.4	1.7 ± 0.5
	Maximum	2.5	2.4	3.1	2.1	2.3
	Reduction	2.5 ± 0.6	2.3 ± 0.4	2.0 ± 0.9	1.9 ± 0.5	2.3 ± 0.4
	Enrichment[b]	30/30	24/30	30/30	25/30	NA
4′ (=medium)	Enumeration[a]	6/15	4/15	2/15	3/15	3/15
	Mean	1.5 ± 0.5	1.4 ± 0.5	1.5 ± 0.8	1.4 ± 0.6	1.4 ± 0.6
	Maximum	2.3	1.9	2.1	2.1	2.1
	Reduction	2.8 ± 0.6	2.9 ± 0.5	2.5 ± 0.6	2.2 ± 0.8	2.6 ± 0.7
	Enrichment[b]	28/30	16/30	25/30	16/30	NA
6.5′ (=well done)	Enumeration[a]	1/15	0/15	1/15	1/15	1/15
	Mean	1.0	<1.0	1.7	1.3	1.0
	Maximum	1.0	<1.0	1.7	1.3	1.0
	Reduction	3.3	>4.2	2.0	2.3	3.1
	Enrichment[b]	7/30	3/29	7/30	2/29	NA

‘ minutes

[a] These results present the number of times enumeration was possible for the different repeats

[b] These results present the number of times the pathogen was detected after enrichment in 25 gram food product

5.4 Discussion

The study focused on the heat inactivation of pathogenic bacteria such as *L. monocytogenes*, *Salmonella* spp., C. jejuni and *E. coli* O157:H7 in artificially contaminated meat and meat preparations of different animal species, such as pork, beef, chicken and lamb, that were pan fried according to a standardized procedure to simulate commonly used home-cooking practices. From the obtained D-values (time needed to obtain 1 log reduction measured in broth at 60°C) of the bacterial strains used, it was confirmed that the pathogens used for the heat inactivation trials did not have unusual heat resistance. *L. monocytogenes* showed greater D-values than the other pathogens tested, which is consistent with previous studies that also show that Gram-positive bacteria exhibit more heat resistance than most non-sporeforming Gram-negative pathogens (Doyle et al., 2001). *L. monocytogenes* is therefore considered to be the most heat-resistant vegetative pathogenic bacterium in high water activity foods and, as such, is regarded as the target organism in setting performance objectives in thermal processing (ILSI, 2012). As the main variable for inactivation is temperature, the process lethality (*P*) is calculated to control if a sufficient inactivation is achieved. This process lethality uses a kinetic value (z-value) which is a measure of the relative "killing power" of the heating temperature. The determination of a z-value is classically performed based on survival studies in laboratory media. Therefore, one must be cautious when extrapolating the obtained secondary models to practical applications in real food products. Food properties such as fat and carbohydrate content also play an important role (van Asselt and Zwietering, 2006; Tamplin, 2002). As *L. monocytogenes* is mostly targeted in thermal processing, it was preferred to calculate the process lethality with a z-value of 7°C which is recommended for *L. monocytogenes* in the study of Van Asselt and Zwietering (2006) instead of a z-value of 5°C used for vegetative cells (Mossel et al., 1995). Thus, it can be seen as a worst case scenario, if the thermal process would be sufficient to inactivate expected levels of *L. monocytogenes*, it would be sufficient to inactivate other pathogens as well. However, calculating the process lethality (*P*) with a z-value of 7°C might give a failsafe effect if temperatures remain low during processing as can be deduced from Tables 5.6a and 5.6b where *P* -values are calculated with a z-value of 7°C and 5°C respectively. In the case of chicken filet a *P*-value of 0.80 is obtained using a z-value of 7°C. When the same temperature profile during cooking is used for

calculating the *P*-value with a z-value of 5°C, a *P*-value of 0.52 is obtained. The decreased *P*-value results in an increased time (i.e. 10 s in the case of chicken filet) needed to exceed the "safe harbor" process criterion of 70°C for 2 min. As such, using a z-value of 7°C results in an overestimation of the process lethality (*P*) if the temperature during processing remains low which should be taken in mind when calculating the process lethality. The difference in *P*-values among the independent repetitions may be huge although the pan-frying process was standardized and at all times the meat was judged as well-done after preparation. From the individual temperature profiles (Figure 5.2) it can be deduced that heating of meat and meat preparations may vary a lot. As such, a small increase or a faster increase in temperature resulting in a higher process lethality. Besides, the position of the temperature probe may influence the temperature profile and thus the process lethality as well. It is generally believed that when meat is heat treated to achieve the "safe harbor" process criterion of 70°C for 2 min (or equivalent time/temperature combination), a 6 log reduction of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* in meat products is obtained and that the meat will be free of pathogens and thus safe to eat (ACMSF, 2007; Bunning et al., 1990; Lund, 2014). However, the simulation of home-cooking practices showed the occasional presence of low numbers of *L. monocytogenes*, *Salmonella* spp., *C. jejuni* and *E. coli* O157:H7 in 25 g of heat treated meat. Core temperatures measured in filet, steak and hamburgers upon pan-frying did not necessarily achieve 70°C and a time/temperature combination equivalent to 2 min at 70°C (i.e. *P*-value of 2) was not always obtained during the simulated homecooking practices although the meat was visually judged as being cooked thoroughly. As demonstrated as well by Bergsma et al. (2007) on chicken filets and Van Laack et al. (1996) on beef, checking doneness of meat by means of visually inspecting the inside color is not a fully reliable method to verify microbiological safety of the meat. Therefore, thermometers are advised to be used as core temperatures assumed to be reached during heat inactivation processes might be overestimated and consumers may fail to reach the safety barrier of 2 min at 70°C during the heat treatment of meat and meat preparations (Bergsma et al., 2007; USDA, 2003; Whittington and Waldron, 2010). On the other hand, also in case the *P*-value of 2 was actually achieved, e.g. during preparation of marinated meat strips, occasional survival of pathogens was still noted. Besides, marinated meat strips, steaks and filets are contaminated on the outside and the inner meat is supposed to be sterile. Although core temperatures for steaks and filet may have failed to

reach 70°C, the temperature on the meat surface in contact with the frying pan reached easily up to 115 – 120°C. Therefore, the occasional detection of *L. monocytogenes*, *Salmonella* spp., C. jejuni and *E. coli* O157:H7 indicated that residual low numbers of the initial high inoculum level of 4 log CFU/g remained and thus the 6 log reduction performance criterion for pathogens in the meat, usually aimed for during a heat treatment equivalent to 70°C for 2 min, was not consistently achieved. It should be mentioned that the small difference in inoculation level is hampering good comparison as the more heat resistant *L. monocytogenes* has comparable recovery (absolute numbers) as *E. coli* O157 despite *L. monocytogenes* starts with 4.3 log CFU/g and *E. coli* O157 only with 3.6 log CFU/g. However, absolute numbers are only shown to demonstrate that a 6 log reduction is not obtained, not to compare the reduction of the different pathogens as for this the number of quantifiable results is too low. Adding an MPN-method or an extra enrichment (e.g. 1 g or 2.5 g) between the lowest enumerable level (1 log CFU/g) and the enrichment used (−1.4 log CFU/g) could have been useful to gain more absolute recovery numbers. In the study an inoculation level of approximately 4 log CFU/g was targeted, as in this case a 6 log reduction would result in non-detectable results after enrichment of 25 g. If a lower reduction would take place, detection would be possible and in worst cases (≤ 3 log reduction) enumeration would be possible. Therefore, the study gives an idea about the magnitude of reduction obtained during simulated home-cooking practices. However, it is demonstrated that the assumed inactivation of pathogenic bacteria in meat and meat preparations might be overestimated. This phenomenon was also shown by other studies (Bergsma et al., 2007; de Jong et al., 2012, Roccato et al., 2015). Although many data on heat resistance in buffer systems are available which show that heat resistance of microorganisms can vary depending on the species, strain and physiological state of microbiological cells, data in actual foods upon consumer-based cooking processes are comparatively scarce (Doyle and Mazzotta, 2000; Juneja et al., 2001; Lianou et al., 2006; Lianou and Koutsoumanis, 2013; Nguyen et al., 2006; Smelt and Brul, 2014). Thermal inactivation processes in food products are more complex than those in buffer systems. The heat inactivation of pathogenic bacteria in meat and meat preparations is also partly dependent on the exact nutritional composition (e.g. fat content), texture (e.g. fiber structure) and contamination profile (Hansen and Riemann, 1963; Jay, 2000; Juneja et al., 2001; Tuntivanich et al., 2008). Moreover, thermal inactivation studies in buffer systems are usually performed at an

isothermal condition. However, consumer style cooking techniques are normally non-isothermal. It is known that heating rate has an effect on microbiological survival. In slow heating, vegetative cells can adapt to stress conditions resulting in a lower inactivation rate (Smelt and Brul, 2014). Also cells grown at higher temperatures or exposed to sub-lethal heat shock, and those growing in a minimal, or fat-rich medium, are more heat resistant (Juneja et al., 2001; Wiegand et al., 2009). Cells attached to meat surfaces are more heat resistant than those that are unattached and dispersed throughout in foods (Juneja et al., 2001). Also changes in composition due to heating can cause changes in the thermal resistance of microorganisms (Smelt and Brul, 2014). Therefore, the unexpected survival of pathogenic bacteria in the heat treated meat in the present study may plausibly be due to the heating rate, the protective effect of some ingredients/texture or adaptation to stress conditions. If pathogens are present in raw meat and raw meat preparations, the initial contamination level of pathogenic bacteria is low (< 1 – 2 log CFU/g) as they could be detected in 25 g but not enumerated (Duffy et al., 2010; Uyttendaele et al., 1999). Quantification of *Salmonella* spp. on poultry meat samples has been performed in different studies and resulted in levels from −0.52 log MPN/g up to 1.53 log MPN/g (Cook et al., 2012; Roccato et al., 2015) and in a contamination curve with a mean of −2.79 log MPN/filet and standard deviation of 2.39 log MPN/filet (Straver et al., 2007). *Campylobacter* quantification on chicken meat resulted in levels of −0.52 log MPN/g to 1 log MPN/g (Cook et al., 2012). Habib et al. (2008) reported an average *Campylobacter* concentration of 1.68 log CFU/g with a standard deviation of ±0.64 (n= 656). Samples of minced pork meat positive to *L. monocytogenes* showed levels of ca. 14 – 17 CFU/kg (Andritsos et al., 2013) and from −0.52 log MPN/g up to 3 log MPN/g (Cook et al.,2012). Franco et al. (2009) showed concentrations of VTEC O157 ranging from < 2 to 5.78 log CFU/g in feces of sheep at slaughter illustrating that adult sheep represent a relevant source of environmental contamination from virulent VTEC O157, as well as a source of VTEC O157 contamination for food of ovine origin. However, in the present research study, the meat and meat preparations were challenged with higher numbers of pathogens (3 – 4 log CFU/g). If the cold chain is well respected (≤ 4–7°C) throughout distribution and refrigerated storage is restricted to a few days (as is usual the case for fresh meat and meat preparations), it is believed that the initial low contamination (< 1 – 2 log CFU/g) will not grow out to high(er) numbers. Thus, if meat is heated thoroughly and pan-frying is restricted to a heat inactivation of only 3 – 4 log as

demonstrated in this study, there would be no residual surviving pathogenic cells expected. Indeed, this deterministic calculation shows that there is an interplay between initial contamination level (determined by good hygiene and practices during slaughter and processing), storage conditions (potentially enabling growth) and heat treatment (causing inactivation). All these factors together determine the actual numbers of pathogenic cells that consumers are exposed to upon consumption of the prepared meat. In assessing the actual risk for consumers in consumption of pan-fried meat the overall process will need to be taken into account (Cassin et al., 1998; Guillier et al., 2013; Smith et al., 2013). Overall, it has been shown in the present study that if the meat is insufficiently heated there might be a residual threat of consumer exposure to microbial hazards. As demonstrated in the study, hamburgers yielded most enumerable results. This result is to some extent expected as meat preparations of ground meat also contain pathogenic bacteria in the core of the meat. Besides, the food category with the highest proportion of products not complying with the European Union *Salmonella* criteria is minced meat and meat preparations (EFSA and ECDC, 2014). Moreover, a multistate outbreak of shiga toxin-producing *E. coli* O157:H7 was recently linked to ground beef (hamburgers) (CDC, 2014). However, both meat preparations and well-done steaks showed 12 % survivors what makes them both of particular interest to verify sufficient cooking procedures. Moreover, meat preparations of ground meat are gaining increased popularity which can be seen in the broad variety of hamburgers, sausages, minced meat etc. on the Belgian market. Although, high temperatures (> 100°C) are reached at the surface of intact meat and the inner part is supposed to be sterile, results demonstrated that a residual threat to consumers remains even when steaks are assessed "well-done" (i.e. 6.5 min each side). However, well-done preparation of steaks produced significantly different pathogen reductions in comparison with steaks which were assessed as medium fried (i.e. 4 min each side) or assessed as rare (i.e. 2 min each side), a culinary practice often preferred by Belgian consumers. This study showed thus clearly the effect of consumer cooking practices on survival of pathogens at different levels of doneness. Appropriate heat treatment of fresh raw meat and meat preparations in the kitchen of the consumer is of importance to provide sufficient inactivation of possible present pathogenic bacteria. However, Fischer et al. (2007) observed that although most consumers are knowledgeable about heating in preventing the occurrence of foodborne illness, this knowledge is not necessarily translated into behavior. Most participants perceive a risk–

benefit tradeoff in preparing meat, especially with regard to steak. In the study of Fischer et al. (2007) almost half of the participants downplayed the danger of bacteria for their health from bacteria by mentioning that some exposure to bacteria may be essential to build up resistance. Therefore, even if consumers are aware of a residual risk for public health due to occasional presence and survival of pathogens on prepared meat, behavior will not change easily.

5.5 Conclusion

From the current study it can be concluded that consumers can be exposed to surviving foodborne pathogens after commonly used pan frying of raw meat and meat preparations even if the meat is visually assessed as cooked thoroughly. On several occasions, residual survivors of the initially inoculated (4 log CFU/g) foodborne pathogens could be recovered either by enumeration (detection limit of 1 log CFU/g), especially in hamburgers, or by presence/absence testing per 25 g. Besides, core temperatures of 70°C were not always achieved and a heat treatment equivalent to 2 min at 70°C was not always obtained. Since consumer behavior is not easily changed, it is important to keep the initial contamination level of pathogenic bacteria low (< 1 – 2 log CFU/g) and to respect the cold chain (≤ 4 –7 °C) to keep the residual threat to public health as low as possible.

Acknowledgments

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract PATHOGENCOOK: FOD VVVL 2012) and supported by the China Scholarship Council. We also thank the Veterinary and Agrochemical Research Centre (CODA) for allowing us to use their *Salmonella* strains.

CHAPTER 6

GROWTH POTENTIAL OF *L. MONOCYTOGENES* IN SOFT, SEMI-SOFT AND SEMI-HARD ARTISANAL CHEESES

1

Abstract

Small scale on-farm cheese producers are aware on hygiene requirements during production to prevent *Listeria monocytogenes* contamination, but opportunities for post-contamination may also occur in delicatessens, during the sometimes short further cheese ripening (at maximum 14°C), during storage and display in the refrigeration cabinet (at maximum 7°C), or during slicing. The growth potential of *L. monocytogenes* in three soft cheeses (one white-molded raw cow's milk cheese, one pasteurized cow's milk cheese with spicy herbs, one washed rind pasteurized cow and sheep's milk cheese) and two semi-hard cheeses (one smear-ripened raw cow's milk cheese and one natural-ripened raw cow's milk cheese) was evaluated. *L. monocytogenes* challenge testing was performed according to EU-RL guidelines assessing the growth potential by challenging 3 replicates of each of 3 batches per type of cheese. A *L. monocytogenes* post-contamination was simulated by inoculation either on the cheese slicing surface or the cheese rind. The growth potential was established after 14 days storage at either 7 or 14°C. Substantial growth of *L. monocytogenes* (> 0.5 log CFU/g) was obtained in 79.2 % of all individual challenge tests (n=178) that were performed although huge variation in growth potential was seen among the different cheese types and storage conditions. The growth potential on soft cheeses stored at 7°C ranged from 1.83 to 4.01 log units and from 3.55 to 5.46 log units upon storage at 14°C, whilst on semi-hard cheese, this was in general lower, and ranged from 0.08 to 1.42 log units at 7°C and from 0 to 3.01 log units at 14°C. Overall, increased outgrowth of *L. monocytogenes* was noted when inoculation was performed on the cheese slicing surface compared to the cheese rind. Thus if occasional post-contamination takes place during storage or handling of the cheese, *L. monocytogenes* has the potential to grow to elevated numbers throughout a reasonably expected storage period of up to 14 days notwithstanding the presence of high numbers of indigenous lactic acid bacteria in these cheeses. Also for a defined cheese type both a considerable inter-batch and intra-batch variability was sometimes noted from the replicate testing, indicating no consistent behavior of *L. monocytogenes* in these fermented dairy products. As such it is recommended that measures are taken to prevent post-contamination. Noting the growth potential, absence of *L. monocytogenes* in 25 g of cheese using a multiple sample subunit approach (n= 5) at the time of production is important to ensure compliance to EU legislation 2073/2005.

6.1 Introduction

Soft and semi-hard cheeses are considered important at risk products for foodborne listeriosis and were identified as the vehicle for human *L. monocytogenes* infections in the EU (Fox et al., 2011; Hunt et al., 2012; Schoder et al., 2008; Magalhaes et al., 2015). In particular smear and mold-ripened cheeses and cheeses made from raw milk have been highlighted as potential risk products (Schvartzman et al., 2014; Verraes et al., 2015a). Cheese was involved in 5 out of 23 EU strong evidence *L. monocytogenes* foodborne outbreaks (i.e. those outbreaks where the evidence implicating a particular food vehicle is strong) during the period 2007-2013 (EFSA, 2009-2015). Although foodborne outbreaks from *L. monocytogenes* are not commonly, compared with those caused by other pathogens like *Salmonella* and *Campylobacter*, they receive considerable attention when they do occur because it usually involves seriously affected cases and even deaths (Todd and Notermans, 2011; Allerberger and Wagner, 2010). One of these verified outbreaks was a multinational outbreak in Austria, Germany and the Czech Republic caused by consumption of 'Quargel', an acid curd cheese produced by an Austrian manufacturer, involving 34 cases with 8 deaths (Schoder et al., 2013). Another listeriosis outbreak occurred in Norway, involving 21 cases with 5 deaths and was caused by a soft cheese produced on a small dairy farm (Johnsen et al., 2010).

Still, the prevalence of *L. monocytogenes* reported in cheese at retail and distribution is often low. An EFSA baseline study carried out in 2010 and 2011 on 3 393 soft and semi-soft cheeses at retail level (including 14% of cheeses made from raw milk) showed the EU prevalence of *L. monocytogenes*-contaminated cheese samples to be 0.5 % while the proportion of samples exceeding the level of 100 CFU/g at the end of shelf life was 0.06% (EFSA, 2013b). A low prevalence of *L. monocytogenes* in semi-soft cheese at retail was also noted in a national survey in Sweden in 2010 in which *L. monocytogenes* was detected in 0.4 % of 525 cheese samples (Lambertz et al., 2012). Surveys in Italy showed prevalence of *L. monocytogenes* in cheese between 0.2 % and 6.5 % (Busani et al., 2005; Manfreda et al., 2005; Prencipe et al., 2010) whereas surveys involving some other European countries found 5.5 % to 6.4 % of cheese samples positive for *L. monocytogenes* (Rudolf and Scherer, 2001; Wagner et al., 2007).

Renewed interest in artisanal food products results in a wide range of farmhouse cheeses offered at the farm gate or in delicatessen and cheese shops. The lack of profound knowledge to assess the risks associated with their products in the short supply chain or small shops may present a challenge in complying with and implementing all food safety regulations (Verraes et al 2015b). The presence of *L. monocytogenes* in the farm environment may represent a primary source for the introduction of the pathogen into the food supply chain. Moreover, contaminated raw milk may represent a vehicle for introducing *L. monocytogenes* into food processing plants, where it can be established in the form of biofilms and, therefore, persist for prolonged periods of time. Biofilms are thus often responsible for the contamination of cheese in (post-)processing environments (Santorum et al., 2012; Di Ciccio et al., 2012). Therefore, infrastructure and design and appropriate hygiene and disinfection procedures are essential to prevent contamination of *L. monocytogenes* (Doménech et. al 2013; Schoder et al. 2011). Due to increased efforts on inspection, training and also recalls and outbreaks causing media attention, there is raised awareness among small scale cheese producers in EU with regard to *L. monocytogenes*. The pathogen is well recognized by all involved in cheese production and distribution to be the most concerning microbial hazard due to its potential impact on human health and business. Optimization of farm hygiene management remains an absolute prerequisite for the manufacture of safe foods (Schoder et al., 2011, Todd and Notermans 2011) but opportunities for post-contamination with *L. monocytogenes* may also occur during further distribution of cheese. During storage, display or slicing, post-contamination by *L. monocytogenes* may occur when the bacterium colonizes the environment, equipment, utensils and crates.

Many artisanal soft and semi-hard cheeses are sold in delicatessen shops (Little et al., 2008). Some of these shops may also have a cellar or ripening room for (short) further maturation of some cheeses upon receipt to fulfil some consumer preferences of well-matured soft and semi-hard cheeses. In Belgium, temperatures in these ripening rooms of delicatessen shops are allowed to be up to 14°C. Temperatures in the cold display cabinets of the shops should be maximum 7°C, however in a survey in France (Morelli et al., 2012) it was noted that during the day the temperature of foodstuffs on sale in refrigerated display cabinets in cheese/dairy shops may occasionally rise to 14°C as well.

In the present study the growth potential of *L. monocytogenes* in three soft and semi-soft cheeses and two semi-hard artisanal cheese was evaluated by challenge testing. *L. monocytogenes* challenge testing was performed according to EU-RL guidelines (EU CRL, 2014) assessing the growth potential by challenging 3 replicates of each of 3 batches per type of cheese. A *L. monocytogenes* post-contamination was simulated by inoculation either on the cheese slicing surface or the cheese rind surface and growth potential was established after 14 days storage at either 7 or 14°C.

6.2 Materials and methods

6.2.1 Survey on *L. monocytogenes* in artisanal cheeses from delicatessen shops

Twelve delicatessen shops selling artisanal cheeses located in the city of Ghent, Belgium, were visited to collect cheese samples (n= 60). The cheese samples were sliced by the vendor as ca. 250 g consumer portions from a bigger piece of cheese and packed in a cheese paper wrap to transport to the laboratory within 1 - 2 hour of purchase (transport at ambient temperature). During this convenience sampling a variety of artisanal soft, semi-soft, semi-hard and hard cheeses available for sale in these shops were collected including both cheese made from raw milk and pasteurized milk, some mold-ripened cheeses and cheeses produced either in Belgium, France or Switzerland. Cheese samples were stored in the laboratory at 4°C and were analyzed within 48 h. Sample units of 25 g were homogenized in 225 ml sterile peptone water for enumeration of *L. monocytogenes*, *S. aureus*, generic *E. coli* and lactic acid bacteria. Enumeration of *L. monocytogenes* was performed according to ISO 11290-2 using a reduced detection limit of 10 CFU/g by spreading 1 ml of the primary suspension on three ALOA plates (24 to 48 h incubation at 37°C). The enumeration of *S. aureus* was performed according ISO 6888-1:1999/Amd 1:2003 (24 to 48 h incubation of BP at 37°C). Enumeration of generic *E. coli* was performed according ISO 16649-2:2001 (24h incubation of RAPID' *E. coli* 2 medium at 37°C). Lactic acid bacteria count was determined according to ISO 15214:1998 (72 h incubation of MRS at 30°C). Detection of *L. monocytogenes* was performed using VidasLMO2 (bioMérieux), an AFNOR validated enzyme-linked fluorescent assay (ELFA) (BIO 12/11-03/04). The NaCl

concentration of the cheeses was determined by a titrimetric determination of Cl^-. An amount of the sample (1-2 g) including a minor piece of cheese rind, was dissolved in 100 ml distilled water (100°C). Under constant stirring the suspension was cooled down to 50°C. Afterwards 2 ml K_2CrO_4 (5 %) was added. This solution was then titrated with $AgNO_3$ (0.1N) to determine the concentration of Cl^- in the solution. The remaining samples were mixed and pH was measured with a stab electrode (SevenEasy™ pH-meter, Mettler Toledo, Zaventem, Belgium). From the same mixed samples, the water activity (a_w) was determined with an a_w-kryometer Typ AWK-20 (NAGY messysteme GmbH, Gaufelden, Germany).

6.2.2 Challenge testing

The challenge tests aim to assess the growth potential (δ) of artificially inoculated *L. monocytogenes* on cheese under given storage conditions. Challenge testing was performed on five different types of cheeses (obtained from a local delicatessen shop in Ghent) according to a protocol described in the technical guidance document on shelf-life studies for *L. monocytogenes* in ready-to-eat foods published by the EU Community Reference Laboratory (EU CRL, 2014). In short, three *L. monocytogenes* strains were grown for 24 h at 37°C in BHI broth. A subculture was then grown at 7°C for 4 days in order to adapt the early stationary phase cells to the storage condition of the cheese. Either the cheese rind or the cheese slicing surface was inoculated with 250 µl of the mixed inoculum (ca. 10^4 CFU/ml) to obtain an inoculum level of ca. 50 - 100 CFU/g. For each sampling day and storage temperature, three inoculated test units of 25 g and one blanc were prepared. Samples were kept wrapped in polyethylene stretch foil for 14 days at 7 or 14°C. Analyses were performed at day 0 (before and after inoculation) and at the end of the storage period (day 14). On each day of analysis the competing microbiota (*S. aureus*, generic *E. coli* and lactic acid bacteria), pH, a_w and the NaCl concentration were determined for the blank samples according the methods described in section 6.2.1. Presence/absence testing of *L. monocytogenes* in 25 g was performed before inoculation as well as for the blank samples using an AFNOR validated VIDAS method (Bio-12/9-07/02). Enumeration of *L. monocytogenes* in the inoculated samples was performed according to ISO 11290-2 using a reduced detection limit (10 CFU/g) as described in EC Regulation No. 2073/2005 (Anonymous, 2005).

Thus, for each type of cheese, two types of inoculations were performed: i) at the cheese slicing surface bringing the *L. monocytogenes* in contact with the core of the cheese, ii) at the cheese rind bringing the *L. monocytogenes* in contact with surface microbiota of the cheese. For each type of cheese and inoculation procedure, storage was performed at both 7° and 14°C for 14 days, and this for three batches of the same cheese with three replicates per batch.

The growth potential (δ) is calculated as the difference between the median concentration of *L. monocytogenes* of the three replicates at the end of the challenge test and the median concentration of *L. monocytogenes* of the three replicates at the beginning of the challenge test (day 0, after inoculation) as described in the EU Community Reference Laboratory (EU CRL, 2014). However, to obtain a better view in the variability of the growth potential, the growth potential for each replicate within a batch was calculated as well by calculating the difference between the concentration of *L. monocytogenes* at the end of the test and the concentration at the beginning of the test.

6.2.3 Data analysis

All analyses were performed with SPSS Statistics version 23 at a significance level of 95 % (p = 0.050). Raw data were not normally distributed, so the non-parametric test Kruskal-Wallis was used for statistical analysis. The Bonferroni correction was applied to control the family-wise error rate at 5 % for all multiple pairwise comparisons.

6.3 Results

6.3.1 Survey on *L. monocytogenes* in artisanal (semi-)soft and (semi-)hard cheeses from cheese shops

The sampling in twelve cheese shops in Gent, Belgium, resulted in 32 soft/semi-soft and 28 semi-hard/hard cheeses with an equal distribution of raw milk and pasteurized cheeses in each category of cheese. The “use by” or “best before date” of the cheeses was unknown as those were not mentioned on the sliced and paper wrapped portions. Still, the cheeses

were visually judged before analyses. None of the cheeses showed a deviating odor, undesired mold growth or traces of dehydration (i.e. cracks in cheese rind) indicating that they were still suitable for consumption. A summary of the intrinsic characteristics (pH, a_w, NaCl), the concentration of lactic acid bacteria, *E. coli*, *S. aureus* and the presence of *L. monocytogenes* in the sampled cheeses is presented in Table 6.1.

S. aureus was enumerated (≥ 2.0 log CFU/g) in 6.7 % of the cheeses, mainly soft raw milk cheeses. In one case, a semi-soft pasteurized cheese, *S. aureus* was present in concentrations of 6.1 log CFU/g which exceeds the process hygiene criterion (i.e. 5.0 log CFU/g) as described in EU regulation 2073/2005. *E. coli* was enumerated (≥ 1.0 log CFU/g) in 26.7% of the cheeses, mainly raw milk cheeses with levels ranging from 1.0 log CFU/g to 5.0 log CFU/g and 6 samples exceeding 3 log CFU/g. *L. monocytogenes* could be detected in 25 g of a white-molded soft-ripened raw milk cheese resulting in a prevalence of 1.7 % for this small survey (n= 60, thus 95 % confidence interval ranging from 0.3 to 8.7 %). Enumeration of *L. monocytogenes* in the positive sample revealed a concentration of ca. 5 log CFU/g which definitely exceeds the limit of 2 log CFU/g, the threshold limit for ready-to-eat foods non-complying to the European legislation with regard to *L. monocytogenes* in ready-to-eat foods (Anonymous, 2005). After contacting the owner of the delicatessen shop where this cheese was bought, it was revealed that the cheese was seemingly sold at the day indicated on the packaging as the “best before date”.

Table 6.1. Summary of the results from the survey on *L. monocytogenes* in (semi-)soft and (semi-) hard cheeses.

	Soft and semi-soft		**Semi-hard**	
Heat treatment	None (raw milk)	Pasteurization	None (raw milk)	Pasteurization
pH	4.16 – 7.47	4.29 – 7.22	5.13 – 5.98	5.33 – 6.04
a_w	0.9366 – 0.9926	0.9399 – 0.9805	0.9372 – 0.9651	0.9353 – 0.9630
NaCl (%)	0.58 – 3.67	1.21 – 3.36	1.17 – 2.83	1.74 – 2.80
LAB (log CFU/g)	5.53 – 9.35	5.68 – 9.19	6.29 – 9.02	5.92 – 9.05
E. coli (log CFU/g)	< 1.00 – 5.00	< 1.00 – 3.28	< 1.00 – 3.70	< 1.00
S. aureus log (CFU/g)	< 2.00 – 4.03	< 2.00 – 6.14	<2.00	<2.00
L. monocytogenes	+ (1/16)	-	-	-

6.3.2 Challenge testing

A challenge test is designed to estimate the growth potential by assessing the bacterial contamination differential between the start and the end of the challenge test. These tests can be used to confirm or infirm whether there is significant *Listeria* growth in a given food product. Challenge testing in this study was performed on 5 different types of cheese. The intrinsic characteristics of the different cheeses are summarized in Table 6.2. The cheeses were compared for their intrinsic characteristics on day 0. From those results it can be concluded that there are significant differences ($p < 0.05$) in pH, a_w and percentage NaCl between the different cheeses, even if they belong to the same category (e.g. semi-soft cheese). The growth potential of *L. monocytogenes* on soft, semi-soft and semi-hard cheeses regardless of storage temperature and the diversity of the microbiota and intrinsic characteristics of the these cheeses is illustrated in Figure 6.1.

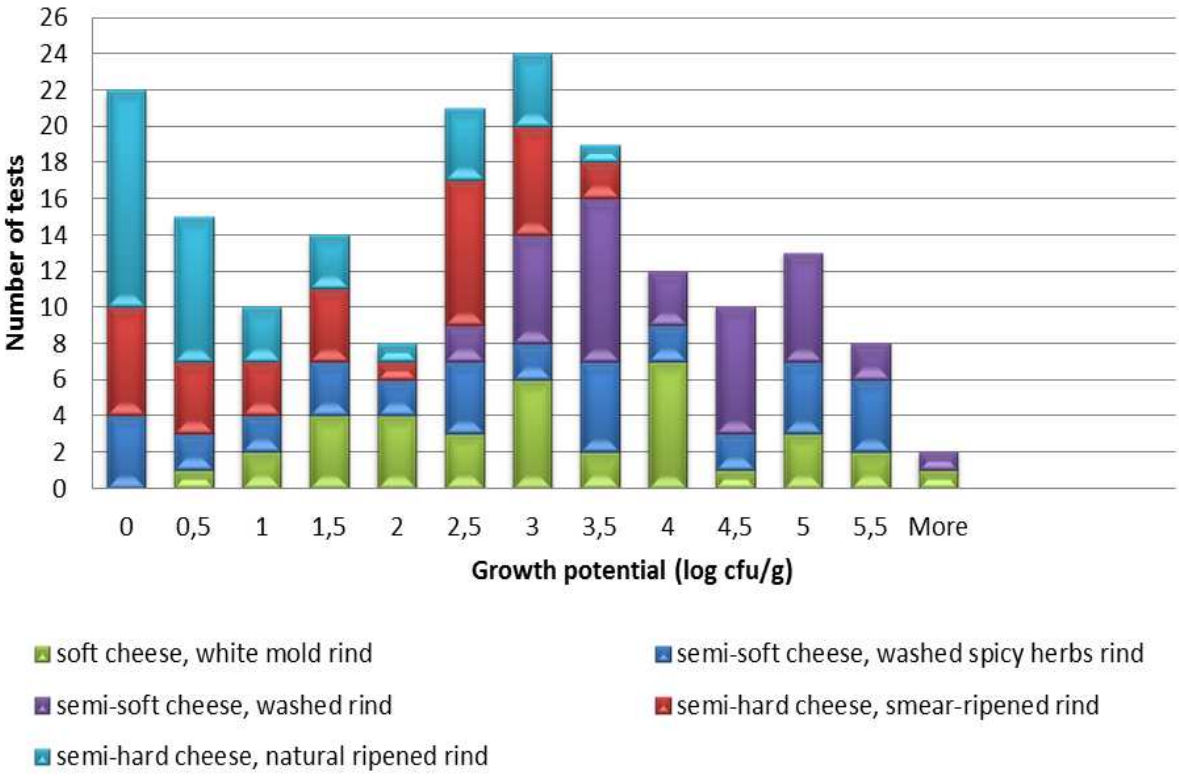


Figure 6.1. Growth potential of *L. monocytogenes* on soft, semi-soft and semi-hard cheeses regardless to storage temperature and contamination profile

Table 6.2. Intrinsic characteristics of the different cheeses involved in challenge testing.

Cheese	Firmness	Crust	Heat treatment	Day	pH	a_w	NaCl (%)	LAB log CFU/g	*E. coli* log CFU/g	*S. aureus* log CFU/g
1	Soft	White *mold*	None (raw milk)	0	6.2 – 7.1	0.95 – 0.96	1.8 – 2.4	7.73 – 8.88	< 1.00 – 4.90	< 2.00
				14	7.2 – 8.5	0.95 – 0.97	2.1 – 2.7	7.88 – 8.95	< 1.00 – 5.51	< 2.00
2	Semi-soft	Spicy herbs	Pasteurisation	0	5.4 – 6.2	0.94 – 0.95	2.6 – 2.9	7.76 – 8.35	< 1.00	< 2.00
				14	5.7 – 6.7	0.94 – 0.96	2.8 – 3.3	8.49 – 8.78	< 1.00	< 2.00
3	Semi-soft	Washed-rind	Pasteurisation	0	5.5 – 7.2	0.96 – 0.97	1.6 – 1.9	7.09 – 8.20	< 1.00	< 2.00
				14	6.5 – 7.3	0.94 – 0.98	1.9 – 2.3	7.26 – 7.95	< 1.00 – 2.3	< 2.00
4	Semi-hard	*Bacterium Linens*	None (raw milk)	0	5.2 – 5.4	0.95 – 0.96	2.0 – 2.6	7.39 – 8.10	2.04 – 4.23	< 2.00
				14	5.5 – 6.7	0.95 – 0.96	2.1 – 2.6	7.35 – 8.16	2.08 – 4.94	< 2.00
5	Semi-hard	Natural-ripened	None (raw milk)	0	5.3 – 6.1	0.94 – 0.96	1.3 – 2.1	8.24 – 8.85	< 1.00 – 3.13	< 2.00
				14	6.2 – 7.3	0.94 – 0.95	1.7 – 2.7	8.11 – 8.75	< 1.00 – 3.12	< 2.00

It can be noted that there is a relatively large variation in growth potential of *L. monocytogenes* in the different types of cheese. *Listeria monocytogenes* growth (> 0.5 log CFU/g) is obtained in 79.2 % of the individual challenge tests (n= 178) and in 78.3 % of the batches (n= 60). More specifically, *Listeria* growth > 0.5 log CFU/g and ≤ 2.0 log CFU/g occurred in 18 % of the cases, *Listeria* growth > 2.0 log CFU/g and ≤ 4.0 log CFU/g was observed in 42.7 % of the tests and *Listeria* growth of more than 4.0 log CFU/g was noted in 18.5 % of the individual challenge tests. There was no substantial growth (≤ 0.5 log CFU/g) in 20.8 % of the challenge tests. The highest growth potential "δ" of *L. monocytogenes* (Table 6.3), determined according to EU-RL guidelines on challenge testing per type of cheese (thus taking into account the results of growth potential for the 3 batches), differed for the soft cheese types and if stored at 7°C ranged from 1.83 to 4.01 log CFU/g whereas stored at 14°C it ranged from 3.55 to 5.46 log CFU/g, whilst this of semi-hard cheeses at 7°C ranged from 0.08 to 1.42 log CFU/g and at 14°C from 0 to 3.01 log CFU/g. Thus, storage temperature (p < 0.05) and the cheese type (soft vs. semi-hard) (p < 0.05) have an influence on the growth potential of *L. monocytogenes.*

Table 6.3. Growth potential of *L. monocytogenes* as obtained during challenge testing.

Cheese	Description	Inoculation surface	Storage (°C)	Growth potential "δ" per batch		
				1	2	3
1	French soft-ripened raw cow's milk cheese, white *mold* rind	Cheese rind	7	1.92	1.20	1.30
			14	3.55	2.66	2.74
		Cutting edge	7	3.82	4.01	> 1.82
			14	5.46	4.93	2.82
2	Belgian semi-soft washed rind pasteurised cow's milk cheese with spicy herbs	Cheese rind	7	2.44	0	0
			14	4.48	3.51	4.82
		Cutting edge	7	0.77	1.83	1.23
			14	3.64	5.18	4.56
3	Italian semi-soft washed rind, pasteurised cows and sheep milk cheese	Cheese rind	7	3.05	2.99	3.02
			14	3.88	4.25	4.53
		Cutting edge	7	3.37	3.16	2.33
			14	5.40	4.82	4.26
4	Belgian semi-hard smear-ripened, raw cow's milk Cheese	Cheese rind	7	0.38	0.40	1.42
			14	3.01	0.70	2.78
		Cutting edge	7	0	0.10	0.90
			14	2.37	2.69	2.22
5	French semi-hard natural-ripened, raw cow's milk Cheese	Cheese rind	7	0	0	0.08
			14	0	0	0
		Cutting edge	7	1.05	0.60	0.50
			14	2.79	2.13	2.37

It could not be observed whether the milk treatment may impact *Listeria monocytogenes* outgrowth as the results may be biased by the cheese type. It was also expected that more growth occurs on the cheese slicing surface than on the cheese rind ($p < 0.05$). Indeed, growth is seen in 88.6 % of the tests performed on the cheese slicing surface (n= 88), while growth on the cheese rind is seen in 70.0 % of the individual challenge tests (n= 90) (Figure 6.2).

Still, both a wide variation in growth potential both intra-batch and inter-batch could be noted, independent of the method of inoculation and temperature of storage. As an example, from Table 6.3 it can be concluded that challenge tests performed on the rind of a semi-hard, natural-ripened, raw cow's milk cheese stored at 14°C will not allow growth of *L. monocytogenes*. However, if detailed results (Annex 6.1) are taken into account substantial growth (1 - 2 log units) is observed in 2 of the 9 test units. Besides, an influence of storage temperature could not be observed in this case. Also no growth of *L. monocytogenes* could be observed on the spicy herbs rind in 2 out of 3 batches of this semi-soft cheese which was stored at 7°C (Table 6.3). However, if looking again into the detailed results of the individual challenge tests (Annex 6.1), growth of *L. monocytogenes* ranging from 0.5 to 3 log units is observed in 5 of the 9 test units. These results demonstrate that cheese support growth of *L. monocytogenes*, but that it is quite variable and it cannot be predicted from prior challenge tests what the behavior and growth potential will be for a next challenge test in another batch or even in another test unit from the same batch of cheese.

6.4 Discussion

A recall in Belgium on May 13th 2015 on an artisanal soft raw milk cheese (Hervé-type) due to the presence of *L. monocytogenes* in 25 g of cheese (FAVV, 2015), raised again the discussion on the food safety of artisanal cheeses in Belgium and whether indeed it was eligible to withdraw these cheeses from the market, in particular if *L. monocytogenes* had not yet exceeded numbers of 100 CFU/g. As mentioned before, the presence of low numbers of *L. monocytogenes* in cheese is not an infrequent event. In the present survey where 60 traditional cheeses had been sampled, one sample of a soft-ripened white-molded

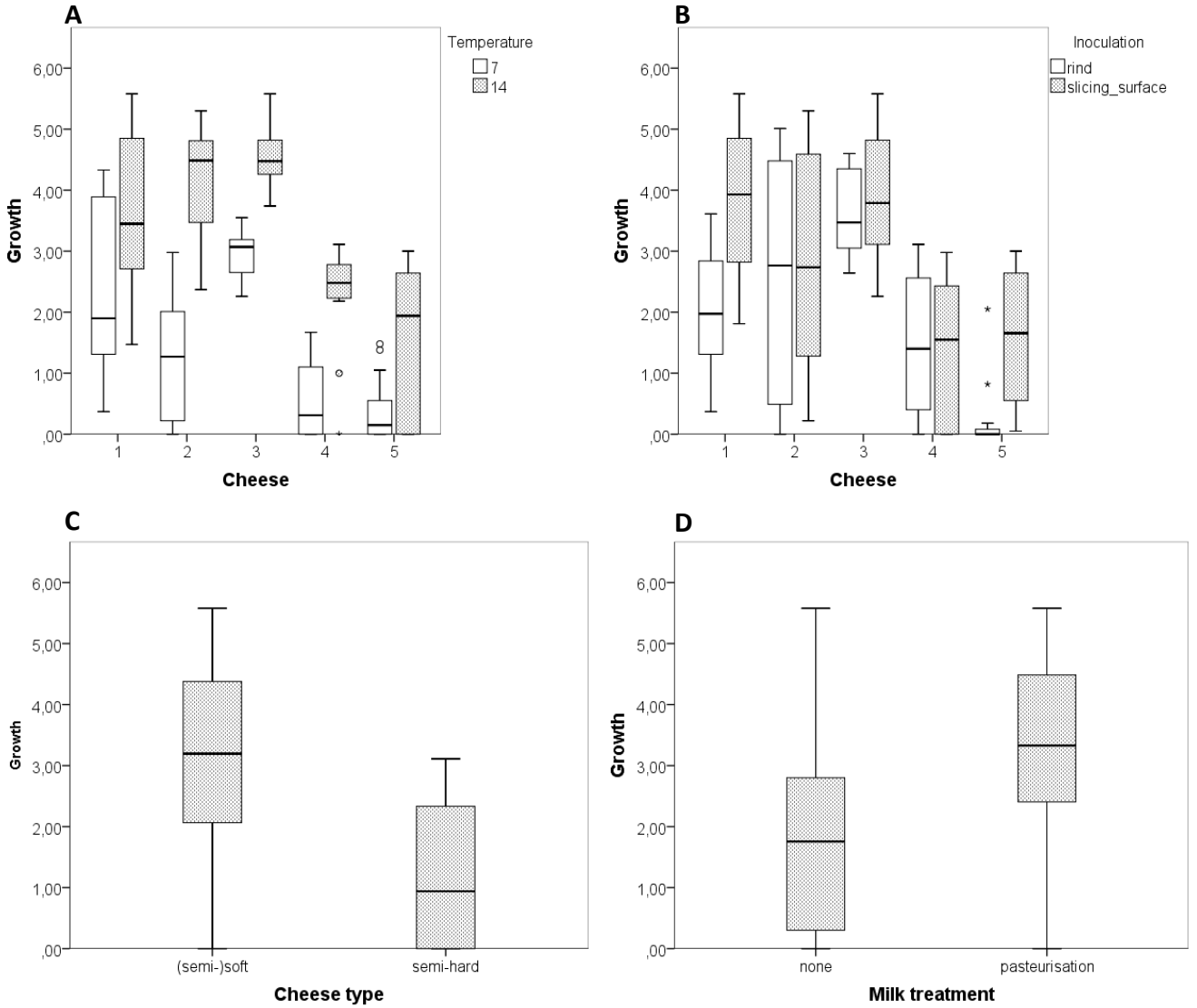


Figure 6.2. Influence of storage temperature (A), inoculation spot (B), cheese type (C), and milk treatment (D) on the growth potential of *L. monocytogenes*.

raw milk cheese was positive for *L. monocytogenes* in high numbers of > 100 000 CFU/g, thus posing an increased risk to consumer health. Unfortunately, cheese from this cheese manufacturer had previously been involved in recalls due to the presence of *L. monocytogenes* in cheese (FAVV, 2012). The positive soft raw milk cheese sample seemed to have been sold at the best before date, but this was not mentioned by the vendor at the time of sale nor could this be noted by the customer when buying the cheese. It has been noticed in a prior study in Belgium that for pre-packed soft cheeses sold at (bigger) retail shops in Belgium there is no consistent use of an 'use by' or 'best before' shelf life label. Only 26 % of pre-packed soft cheeses (n= 270) indicated an 'use by' label, indicating that the expiry date is to be regarded as an ultimate date of consumption (Ceuppens et al. 2016). Thus, many producers leave it up to the consumer's judgement whether or not the cheese is still deemed good for consumption which mainly occurs by a combination of a visual check and smelling (82.5%). Besides, for cheese products in particular, 1 out of 3 consumers was willing to eat an expired product (Van Boxstael et al., 2014).

The observed prevalence of *L. monocytogenes* in samples of soft or semi-soft cheeses in the investigations at retail level reported in 2010 in the EU Summary Report, ranged from 0 % to 0.7 % (EFSA, 2012). Overall, prevalence data of *L. monocytogenes* in cheese can be influenced by the differences in the types of cheeses sampled, whether cheeses were included made from raw or pasteurized milk, but also by the type of distribution channel (local market, small shop or big retail outlet) and by selection of regions where samples were taken (Verraes et al., 2015a). A survey (n= 137) similar to ours was conducted in Greece including soft and semi soft cheeses but also fresh cheeses and sampling in retail shops (Angelidis et al., 2013). None of the samples tested in the survey from Greece were positive for *L. monocytogenes* (per 25 g) (i.e. 0 out of 173 relates to a 95 % confidence interval of 0.0 to 2.2 %). From the collection of previous data from traditional cheeses in Belgium, *L. monocytogenes* was detected per 25 g in 66 of 549 samples originating from the short supply chain of which the *L. monocytogenes* numbers were between 10 and 100 CFU/g for six samples and were higher than 100 CFU/g for four samples (Verraes et al., 2015b). In the present study, the positive cheese sample showed very high numbers of *L. monocytogenes*, but such high numbers in cheese seems to be exceptional. In the data obtained from the Belgian Federal Agency for the Safety of the Food Chain (FASFC)

monitoring and surveillance plan, the prevalence of *L. monocytogenes* (at levels exceeding 100 CFU/g) in raw milk cheeses sampled at farms varied in the period 2008-2011 from 0 to 3.5 % (315 samples in total), whereas none of the 707 raw milk cheese samples taken at retail level in that same period showed *L. monocytogenes* at levels exceeding the threshold limit of 100 CFU/g (FASFC, WIV and CODA, 2010-2011). However, the amount of recalls on cheese, issued by competent food safety authorities as a precautionary measure to prevent human exposure to unacceptable levels of this pathogen in foods, makes us concerned on the presence of *L. monocytogenes* in cheese. For example, FASFC launched 92 recalls involving pathogenic bacteria in a variety of food commodities during the period between 2007 and 2013. Cheese was the food vehicle implicated in 20 out of 41 *L. monocytogenes* related product recalls. At EU level, the Rapid Alert System for Food and Feed (RASFF) reported 3 435 notifications related to pathogenic bacteria in food. Of these, 485 (14.1 %) dealt with non-compliance of *L. monocytogenes* in ready-to-eat foods according to EU 2073/2005 Regulation on Microbiological Criteria for Food stuffs (Anonymous, 2005) and 116 (3.4 %) reports involved *L. monocytogenes* in cheese.

According to Regulation (EC) No 2073/2005, food business operators marketing ready-to-eat foods in which growth of *L. monocytogenes* can occur, need to show that the numbers of this pathogen do not exceed the threshold value of a maximum 100 CFU/g throughout the duration of the shelf life of the food product under consideration. If this evidence of growth restriction of *L. monocytogenes* is not demonstrated, the product will have to comply with the criterion of absence of *L. monocytogenes* per 25 g (taking into consideration a 5-unit sampling plan). For ready-to-eat foods in which no growth can occur, the threshold value of a maximum 100 CFU/g is valid for the duration of the shelf life (thus including the moment of consumption). For traditional cheeses, the resources are generally lacking to set-up challenge testing to asses the growth potential during distribution and storage, thus many of the recalls are based on the finding of *L. monocytogenes* in 25 gram samples. Therefore, it is debated whether these traditional soft and semi-hard cheeses can support the growth of *L. monocytogenes*. If not using commercial starter cultures, there are indigenous lactic acid bacteria that can be isolated from cheese in high numbers. These lactic acid bacteria may protect against *L. monocytogenes* growth during (further) ripening and storage (Montel et al., 2014). Such growth suppression, rather than inactivation, of a target organism has been

referred to as the 'Jameson Effect' (Stephens et al., 1997; Ross et al., 2000; Coleman et al., 2003; Giménez and Dalgaard, 2004; Delignette-Muller et al., 2006).

Challenge testing is a tool to establish the growth potential of *L. monocytogenes*, in particular when the pathogen is present due to post-contamination. Opportunities for post-contamination with *L. monocytogenes* may occur during further distribution of cheese, in particular in delicatessen shops, with sometimes own ripening rooms, and serving counters, where a wide variety of traditional cheeses are displayed and sold in consumer portions and may be handled extensively. This practice provides ample opportunities for contamination by food handlers, equipment and the environment (Tan et al., 2008). Therefore, the behavior of *L. monocytogenes* on cheese as a result of post-contamination during distribution, (short) further ripening, cold display and slicing during serving consumer portions at delicatessen shops was the focus of the present study. Post-contamination was simulated in the lab by inoculation of consumer portions of cheese either on the cheese slicing surface or the cheese rind and the growth potential was established after 14 days storage at either 7 or 14°C. It could not be observed from the challenge tests that growth of *L. monocytogenes* in pasteurized cheesesis better than growth in raw milk cheeses. However, Tiwari et al. (2014) demonstrated that *L. monocytogenes* grew at a slower rate on raw milk cheese compared to pasteurized milk cheese. This difference in growth ability of *L. monocytogenes* may be explained by the presence of the lactoperoxidase enzyme in raw milk cheese which has bacteriostatic properties in milk and milk based products (FAO/WHO, 2005). However, in this study, it was noticed that pasteurized milk cheeses have lower contamination levels of *E. coli* than raw milk cheeses due to the heat treatment during processing. Therefore, more bacterial competition is expected to be present in raw milk cheeses which may be as well an explanation of the lower growth potential of *L. monocytogenes* in these cheeses (Izquierdo et al., 2009; Mellefont et al., 2008; Schvartzman et al., 2011). Although the prevalence on pasteurized cheese may be lower, if there are opportunities for growth of the pathogen, higher numbers of *L. monocytogenes* may be obtained in pasteurized cheeses, making raw milk cheeses and pasteurized cheeses equally important. This is also demonstrated in a study of Gould et al. (2014) where 90 outbreaks in the United States attributed to cheese were analyzed. The study showed that 42 % of the outbreaks were due to cheese made with unpasteurized milk and 49% due to cheese made

with pasteurized milk. Of those outbreaks, 12 were caused by *L. monocytogenes* with unpasteurized milk cheese involved in 4 outbreaks and pasteurized milk cheese in 8 outbreaks.

Overall it is acknowledged that soft raw milk cheese holds the greatest risk for survival and growth of *L. monocytogenes* although the growth potential will depend upon the actual storage temperature (EFSA, 2015; Farrokh et al., 2013; Kagkli et al., 2009; Mataragas et al., 2008; Rosshaug et al., 2012). Hard cheeses are assumed not to support the growth of *L. monocytogenes*, but may support its survival (EFSA, 2015). In an EU survey all tested units (n=2699) complied with the criteria of levels not exceeding 100 CFU/g at processing and retail, except for one single sample of hard cheese made from pasteurized cow's milk sampled at retail (EFSA,2015). Some studies demonstrated that *L. monocytogenes* will die during ripening of hard cheeses (Bachmann and Spahr, 1995; Dalmasso and Jordan, 2014). However, a hard cheese has been demonstrated as the causative food of a listeriosis outbreak in Belgium in 2011 (Yde et al., 2012). It was also found that semi-hard cheeses will not support growth, but only enable survival of *L. monocytogenes* (Bachmann & Spahr, 1995; Valero et al., 2014)). However, in the challenge test results in this study, it was noticed that *L. monocytogenes* on semi-hard cheeses may increase up to 1.7 log CFU/g if stored for 14 days at 7°C and up to 3.5 log CFU/g if stored at 14°C. The growth potential in soft and semi-soft cheeses, on the other hand, is noted to be substantially higher than in semi-hard cheeses. Thus, it can be concluded that soft and semi-soft cheeses present indeed a higher risk with regard to listeriosis.

Whether a certain type of cheese should be categorized as a soft, semi-soft or semi-hard cheese is not always clear to the consumer, as was experienced during the survey in this study. The firmness of a cheese is determined by its percentage moisture, on a fat-free basis, as defined in Codex general standard for cheese (CAC, 2013a). However, if this percentage moisture is not declared, the consumer needs to find more information on the classification on the website of the cheese producer or governmental marketing websites (e.g. Belgian VLAM). Although semi-soft cheeses are characterized by their firm but elastic feel, the difference between a semi-soft and a semi-hard cheese is difficult to recognize. Cheese products might be even more complex as some cheeses (e.g. 'brie or camembert type') may be available as raw or pasteurized milk cheese depending on the producer and

the point of sale. When cheese is bought at a local cheese shop, the consumer can only rely on the information given by the sales person if the label itself is not well visible or no longer present on the cheese at display.

The results in this study demonstrated also that better growth of *L. monoctyogenes* is obtained on a sliced surface of the cheese than on the cheese rind. It has been described that traditional cheeses harbor a rich and diverse microbiota. However, this bio-diversity decreases in cheese cores, where a small number of lactic acid bacteria species are numerically dominant, but persists on the cheese surfaces, which harbor numerous species of bacteria (Montel et al., 2014). A rich and complex microbial community on the cheese rind might help in the controlling of *L. monocytogenes* (Izquierdo et al., 2009; Mellefont et al., 2008). This may explain why growth of *L. monocytogenes* on the cheese rind is unlikely. However, as demonstrated in this study, (reduced) growth may still be possible on the rind of a smear-ripened cheese, a mold-rinded or washed rind cheese. These results are consistent with some studies that demonstrated that no *L. monocytogenes* inhibition was achieved using bacteriocin-producing enterococci on the surface of Taleggio and Camembert cheeses (Giraffa, 1995; Sulzer and Busse, 1991). Independent of the inoculation location, the type of cheese or the type of milk treatment, it was shown that a better growth of *L. monocytogenes* is obtained at 14°C than at 7°C. It is not surprising that a higher storage temperature will result into higher concentrations of *L. monocytogenes* during storage. In a study of Tiwari et al. (2014) it was also shown that in both raw and pasteurized semi-soft washed-rind milk cheeses, the *L. monocytogenes* population increased as the temperature increased.

In this study, the growth potential of *L. monocytogenes* for each batch was determined according the technical guidance document on shelf-life studies for *L. monocytogenes* in ready-to-eat foods published by the EU Community Reference Laboratory (EU CRL, 2014). As the difference between the median of the $\log_{10}$ CFU/g at the end of storage of the three replicates and the median of the $\log_{10}$ CFU/g of the initial concentrations (day 0, after inoculation) of the three replicates was calculated, the maximal growth is not taken into account and due to the occasional high intra-batch variability, the actual growth of *L. monocytogenes* might be underestimated. However, challenge tests for assessing the growth potential as performed in the present study are mainly performed to classify

products into either RTE foods in which growth of *L. monocytogenes* can occur or in RTE foods in which growth of *L. monocytogenes* will not occur during their shelf-life and thus accurate estimation of the growth rate is less important. As such, it was not intended to precisely predict the numbers of *L. monocytogenes* in the cheese under reasonably foreseeable conditions of storage from the time of intermediate storage (including short further ripening) at the delicatessen shops and at consumer's home until consumption. Such growth rate predictions are important for calculating the concentration at the end of the shelf-life from the initial concentration, or determining the concentration at the beginning of the shelf-life in order to comply with the limit of 100 CFU/g at the end of the shelf-life, but were out of the scope of the present study. Challenge testing is only one of the tools available and should be used in addition to predictive modelling, end product control, GMP and HACCP to control the risk for listeriosis due to consumption of RTE food products such as traditional cheeses (Uyttendaele et al., 2004). As for a defined cheese type sometimes both a considerable inter-batch and intra-batch variability was noted from the replicate testing indicating no consistent behavior of *L. monocytogenes* in these fermented dairy products, it is recommended that measures are undertaken to prevent post-contamination. Noting the growth potential, compliance to EU legislation 2073/2005 requires absence of *L. monocytogenes* in 25 g of cheese using a multiple sample subunit approach (n= 5) at the time of production. The low prevalence (i.e. 0.47%) found in the EU baseline survey shows that for soft or semi-soft cheeses it is possible to produce the foods with a rare proportion having counts exceeding the level of 100 CFU/g. It demonstrates that properly designed and implemented food safety management systems by dairy industry across the EU can produce safe compliant food in these categories (EFSA, 2015). Therefore, food business operators in these sectors should be aware of the benefits of diligent application of appropriate protocols to manage this particular risk. Especially good hygiene practices are very important in the prevention of contamination. However, a zero risk will never be obtained as demonstrated by the occasionally higher contaminated cheese samples, which was also found in this survey (EFSA, 2013b; Fretz et al., 2010; Schoder et al., 2013)

6.5 Conclusion

It has been previously demonstrated that the occurrence of *L. monocytogenes* in traditional cheeses is not an infrequent event. Although initially present at low numbers, subsequent outgrow to higher (infectious) numbers poses a risk of listeriosis. In this study, the results of a series of challenge tests conducted on various types of cheese demonstrated that there is a wide range in growth potential of *L. monocytogenes*. Due to this variability, the food business operator may not be able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout the shelf life. Therefore, it is better to focus on prevention through good hygiene practices to comply with a limit of absence in 25 g. Besides, raw milk cheeses and pasteurized cheeses are equally important towards the risk of listeriosis as post-contamination from the (production or storage) environment, utensils or equipment is the main transmission route for introduction of low numbers of *L. monocytogenes* in cheese. As limiting the risk of listeriosis, is a teamwork from farmer to retailer, salesmen in cheese shops should be aware of the risk of post-contamination as well.

Annex 1. Detailed results of challenge testing.

Cheese	Description	Inoculation surface	Storage	Batch	Concentration (log CFU/g)		Growth potential "δ" per batch (log CFU/g)	Highest "δ" among the 3 batches (log CFU/g)
					Day 0	Day end		
1	French soft-ripened, raw cow's milk cheese, white mold rind	Cheese rind	7°C	1	2.20 **2.23** 2.23	2.57 4.26 **4.15**	1.92	1.92
				2	2.23 2.04 **2.15**	2.88 **3.35** 3.58	1.20	
				3	2.08 1.90 **2.00**	**3.30** 3.60 3.00	1.30	
			14°C	1	2.20 **2.23** 2.23	5.81 **5.78** 3.70	3.55	3.55
				2	2.23 2.04 **2.15**	5.64 4.72 **4.81**	2.66	
				3	2.08 1.90 **2.00**	4.95 **4.74** 4.30	2.74	
		Cutting edge	7°C	1	**1.90** 1.78 2.11	5.67 **5.72** 6.03	3.82	4.01
				2	2.08 2.20 **2.08**	6.08 **6.09** 6.41	4.01	
				3	2.11 **2.18** 2.28	< 4.00 **< 4.00** 4.30	>1.82	
			14°C	1	**1.90** 1.78 2.11	7.36 **7.36** 7.05	5.46	5.46
				2	2.08 2.20 **2.08**	7.24 **7.01** 6.93	4.93	
				3	2.11 **2.18** 2.28	5.60 **5.00** < 5.00	2.82	
2	Belgian semi-soft washed rind, pasteurised cow's milk cheese with spicy herbs	Cheese rind	7°C	1	**2.20** 2.15 2.28	**4.64** 5.13 4.38	2.44	2.44
				2	2.11 **1.90** 1.90	< 1.00 **1.30** 4.45	0	
				3	**2.23** 2.30 2.04	2.72 **1.00** < 1.00	0	
			14°C	1	**2.20** 2.15 2.28	**6.68** 6.64 6.82	4.48	4.82
				2	2.11 **1.90** 1.90	**5.41** 5.26 5.88	3.51	
				3	**2.23** 2.30 2.04	4.60 7.11 **7.05**	4.82	
		Cutting edge	7°C	1	2.26 **2.08** 2.00	2.48 **2.85** 3.28	0.77	1.83
				2	2.18 1.90 **2.08**	4.17 **3.91** 3.65	1.83	
				3	2.20 **2.23** 2.32	**3.46** 3.08 3.68	1.23	

			14°C	1	2.26 **2.08** 2.00	**5.72** 5.57 5.75	3.64	5.18
				2	2.18 1.90 **2.08**	7.32 7.20 **7.26**	5.18	
				3	2.20 **2.23** 2.32	**6.79** 5.70 6.96	4.56	
3	Italian semi-soft washed-rind, pasteurised cow's and sheep milk cheese	Cheese rind	7°C	1	2.00 1.85 **1.90**	5.20 4.49 **4.95**	3.05	3.05
				2	1.70 1.60 **1.70**	4.89 **4.69** 4.49	2.99	
				3	2.30 **2.34** 2.43	5.49 5.32 **5.36**	3.02	
			14°C	1	2.00 1.85 **1.90**	**5.78** 6.38 5.64	3.88	4.53
				2	1.70 1.60 **1.70**	5.89 **5.95** 5.98	4.25	
				3	2.30 **2.34** 2.43	6.90 6.75 **6.87**	4.53	
		Cutting edge	7°C	1	2.11 1.90 **2.08**	5.30 **5.45** 5.49	3.37	3.37
				2	2.15 2.32 **2.18**	**5.34** 5.43 4.83	3.16	
				3	3.00 **3.23** 3.23	5.65 **5.56** 5.49	2.33	
			14°C	1	2.11 1.90 **2.08**	7.48 **7.48** 7.32	5.40	5.40
				2	2.15 2.32 **2.18**	7.00 6.83 **7.00**	4.82	
				3	3.00 **3.23** 3.23	7.69 7.26 **7.49**	4.26	
4	Belgian semi-hard smear-ripened, raw cow's milk cheese	Cheese rind	7°C	1	2.46 **2.40** 2.32	**2.78** 2.30 3.79	0.38	1.42
				2	2.00 1.60 **1.90**	2.30 2.70 **2.30**	0.40	
				3	2.00 **1.85** 1.60	3.33 3.14 **3.27**	1.42	
			14°C	1	2.46 **2.40** 2.32	**5.41** 4.58 5.43	3.01	3.01
				2	2.00 1.60 **1.90**	<2.00 **2.60** 4.46	0.70	
				3	2.00 **1.85** 1.60	4.48 **4.63** 4.65	2.78	
		Cutting edge	7°C	1	2.00 2.20 **2.11**	1.48 1.60 **1.48**	0	0.90
				2	**1.90** 1.78 1.95	**2.00** <2.00 2.70	0.10	

				3	2.20 **2.18** 1.95	**3.08** - -	0.90	
			14°C	1	2.00 2.20 **2.11**	**4.48** 4.61 4.41	2.37	2.69
				2	**1.90** 1.78 1.95	**4.59** 4.76 4.38	2.69	
				3	2.20 **2.18** 1.95	4.79 **4.40** 4.18	2.22	
5	French, semi-hard natural-ripened, raw cow's milk cheese	Cheese rind	7°C	1	**2.18** 2.30 2.11	2.30 **2.00** <2.00	0	0.08
				2	1.85 **1.95** 2.18	<2.00 **<2.00** <2.00	0	
				3	2.18 **2.18** 2.08	1.95 **2.26** 2.26	0.08	
			14°C	1	**2.18** 2.30 2.11	4.23 **<2.00** <2.00	0	0
				2	1.85 **1.95** 2.18	<2.00 **<2.00** 3.00	0	
				3	2.18 **2.18** 2.08	**2.08** 2.20 1.95	0	
		Cutting edge	7°C	1	2.15 **2.15** 2.11	**3.20** 3.53 3.11	1.05	1.05
				2	1.00 2.00 **1.70**	2.48 **2.30** 2.00	0.60	
				3	2.04 1.90 **1.95**	2.48 **2.45** 2.00	0.50	
			14°C	1	2.15 **2.15** 2.11	4.38 5.08 **4.94**	2.79	2.79
				2	1.00 2.00 **1.70**	3.64 **3.83** 4.56	2.13	
				3	2.04 1.90 **1.95**	4.11 4.90 **4.32**	2.37	

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 Management of microbiological food safety

Ensuring that food is safe along the entire food supply chain is a major concern for competent authorities, food industry associations and food business operators (FBOs). As FBOs are best placed to govern food safety and to prevent foodborne outbreaks, they are urged to develop and implement a food safety management system (FSMS) when active in food processing and food distribution (Anonymous, 2002; Anonymous, 2004b; CAC, 2003). This FSMS has to be validated and verified for its proper functioning as the quality and safety of the food is largely affected by the performance of this FSMS (Jacxsens et al., 2015, Luning et al., 2015). The verification is mostly performed by (internal) auditing the FSMS system and reviewing Critical Control Points (CCP) monitoring records. However microbiological testing can also have an important role in FSMS validation and verification (Buchanan & Schaffner, 2015; Kvenberg and Schwalm, 2000; Martins and Germano, 2008; Osimani et al., 2013; Zwietering et al., 2016). As there is a need to empower micro and small-sized enterprises, especially those involved into business-to-consumer (B2C) activities, in diagnosing and improving their FSMS themselves, it was decided in this PhD thesis to focus on microbiological analysis for the validation and verification of FSMS in these settings.

7.1.1 Microbiological analysis to verify food safety management in FSO

Microbiological testing for verification of a FSMS in the frame of self-checking as explained in **CHAPTER 1** involves the set-up and elaboration of a sampling plan. The sampling can be performed according a risk-based or a statistical approach. Risk-based sampling uses a baseline number for sample size/frequency based upon risk and performance, and that number can change based on prior inspection results, i.e. it may be reduced due to good results or tightened due to poor results. At the level of the government, a risk-based approach means that each company is controlled with a frequency of inspections depending on the type of activity which it carries and the risk associated with this activity. It may also take into account the current consumption pattern and volumes of produced food products. At the level of the FBO, risk-based sampling means that food products with a smaller risk towards food safety will be sampled less frequent than high risk food products. Statistically-

based sampling gathers data from each individual lot from a probability standpoint to ensure that the sample is an unbiased representation of the entire batch/population. This method may provide a more concrete representation and will therefore be used in governmental monitoring and surveillance programs. However, it is not always feasible within the limitation of resources for microbiological analysis for FSO, neither within the framework of the present research work to elaborate and implement a statistically underpinned sampling plan as described by e.g. Augustin and Minvielle (2008), Baird-Parker (1995), Green (1991) and Legan et al. (2001). Often, one is bound in (food safety) risk management or (research) project management to make choices and restrict sampling and testing. If one is aware about the bias or limitations introduced by this enforced restricted sampling frequency this does not necessarily detract that still useful knowledge on the food safety (or food quality) of a foodstuff or production process in a particular context or setting is and can be obtained.

Due to the limitation of resources for microbial analysis, but also due to the high variety of incoming raw materials and served food products, a risk-based sampling method is thus preferred above a statistical approach. Therefore, the sampling plans elaborated in this PhD study were based on risk analysis, especially with respect to the priority of the food product/pathogen combination, sampling locations and frequency of sampling (**CHAPTER 2, 3 and 4**). Consequently, one of the core aspects of this thesis was to develop an approach for “risk classification” of the diversity of food products present in FSO. This approach is based upon i) epidemiological association of the food type with reported foodborne outbreaks, ii) the reported prevalence of foodborne pathogens and level of hygiene indicators in the food type, and iii) the potential of microorganisms (pathogens but also spoilage microflora) to grow or survive during storage and/or further processing. As such, the variety of products on the inventory lists of the FSO, but also the variety of end products produced within the FSO could be subdivided into risk categories. The products classified in the high risk category could then be taken up in the sampling plan for microbiological analysis. However, this categorizing of all food items on the inventory list of the institutional FSO, by itself, is a challenging issue. Depending on the ingredients of the food recipe, the product may end up in different categories. In this PhD study for example, the chocolate mousse was classified into the group of milk and dairy products. The chocolate mousse

might indeed not be a pure dairy product. However, the classification of the products (as mentioned in **CHAPTER 3**) is based on commodity type and the processing and preservation method applied. As gelatin, one of the ingredients, is of animal origin, you might classify chocolate mousse as a composite food as composite foods are defined as those food products containing ingredients from more than one of the seventeen commodities. However, the ratio between the different commodities in composite foods is more equal and that distinguish them from products like chocolate mousse. As the main ingredient(s) (and thus the ingredient(s) causing most likely a problem) are of dairy origin, it was decided to classify chocolate mousse in this commodity (i.e. milk and dairy products). In case it would have been artisanal chocolate mousse containing raw egg white, it would be proposed to classify it in Ready-To-Eat refrigerated food products with substantial raw ingredients, a category with a higher risk score. The specific micro-organisms that should be considered are dependent on the microbiological ecology of the food under consideration and on the knowledge of prior and further conditions of processing, storage and distribution (Buchanan and Schaffner, 2015).

It is recommended with respect to the sampling locations to perform microbiological analysis on raw materials to verify raw material control in the FSMS (e.g. compliance to specifications and selection of suppliers). Raw material control is indeed a point of attention in FSO as experienced in this PhD study. Most of the time, verification of suppliers is performed by visual control for quality and by temperature check when the products are delivered. However, sampling is providing objective results and reveals some information on the quality and safety of the products (which cannot be visually checked). As illustrated in **CHAPTER 2 and 3**, *L. monocytogenes* was detected in e.g. raw meat spread, which would have been left undetected in case of visual control. At all times the obtained results were compared with microbiological criteria and guidelines as set to be applicable at the end of shelf life as the products were analyzed in the food service operation when they were supposed to be eaten (i.e. somewhere between production and end of shelf life). Although this assumes worst case, if raw materials would exceed these threshold values, and if temperature of intermediate storage and transport had been respected, it could be concluded that initial quality of raw materials delivered by the supplier would be insufficient and therefore they could not be in accordance with the specifications set to the suppliers.

The systematic approach in the elaboration of a sampling plan allows the FSO to provide information on the variability of the microbiological contamination of raw materials and thus the consistency (or not) of the quality of the raw materials supplied. As FSO (and FBO in general) would become more pro-active in controlling their suppliers with microbiological analysis, it will gradually lead to an improvement of food safety and quality of food products along the farm-to-fork chain. The elaboration of a microbiological sampling plan in the frame of self-checking would also fit within the efforts taken by the Belgian government. The Belgian food safety agency is namely stimulating B2C organisations, such as FSO, to implement a certified self-checking system by rewarding them with a "Smiley". This Smiley is a certificate in the form of a sticker that shows that the company has a trustworthy system of hygiene, based on HACCP. The possession of a Smiley implies confidence to consumers, but also that the company can benefit from a considerable discount on annual FASFC levies. It also implies that the FASFC will carry out fewer inspections in these organisations because their efforts in the field of hygiene have already been inspected resulting in a smaller risk for food safety. Another point of interest is that due to the setting-up of the sampling plan in **CHAPTER 4**, the awareness of the responsible for menu selection increased, resulting in a more critical selection of raw materials and food products to be served to vulnerable people.

It is also preferred to perform microbiological analysis on intermediate products at a stage in the production process where a manual operation and/or physical intervention process occurred (e.g. after a thermal treatment step or after a manual handling step such as dressing up of plates), but also on finished products at the time they are offered to the consumer. Sampling of finished products reflect in this case the integration of the microbiological status of raw ingredients with the effectiveness of the preventive and control measures during manufacturing.

It was also decided in this PhD study to perform environmental sampling during food processing. When environmental testing in FSO is integrated it is mostly to verify that the food processing plant sanitation program is actually effective at controlling the pathogen(s) of concern (or their indicator organisms) (Kvenberg & Schwalm, 2000; Tompkin, 2002). However, samples performed on surfaces of equipment after cleaning and disinfection, but before food processing have a high level of compliance (Tompkin, 2004). Therefore, it is

recommended to sample the environment during processing of foods (with at least 2h of prior production) or at the end of the production run (but before cleaning and disinfection), because cells remaining in harborage sites (biofilms) will be more accessible to sampling once dislodged during processing because equipment vibrates or because foods and liquids come in contact with harborage sites. Thus not only the sampling location but also the time of sampling in the production process affect the probability of detecting a persistent 'in-house' bacterial strain and thus increases the likelihood of detecting the source of (continuous) cross-contamination. As food handlers can be asymptomatic carriers of foodborne pathogens, it is also of interest to verify hand hygiene (or overall personal hygiene) and thus to perform sampling and testing on hands or gloves of food handlers. Especially in food service operations, where manual manipulations are very important and thus transmission of pathogenic micro-organism may easily occur between food handlers and food or surfaces, this type of sampling location is highly recommended (Boxman et al., 2011 and 2015; Stals et al., 2015). In **CHAPTER 2**, hand hygiene was identified as a point of attention. To evaluate the general hand hygiene of the food handlers, extra swab samples of gloves and hands were collected by the responsible during a 6-month period and analyzed for TVC and the hygiene indicators *E. coli*, *S. aureus* and *Enterobacteriaceae*. As such, the sampling and testing to verify hygiene practices, increased the awareness of food handlers and nowadays improvements on hand hygiene within the food service operation are noted. No distinction in hand hygiene was made between employees working with or without gloves. In both cases, good hygiene practices are necessary. However, it is noticed that samples taken from employees with hand gloves have higher TVC values than samples taken from bare hands.

Overall, the sampling plans elaborated in this PhD study may have a limitation of statistical power due to the (low) sampling frequency and cannot guarantee food safety and food quality. However, in determining the frequency of testing for verification purposes, it is important to emphasize that the goal is not to determine the safety of a specific lot (i.e. batch control), but to determine if the food safety system is still functioning as intended. Besides, to ensure safety of the food product with sampling and microbiological analysis only, every part of the batch has to be tested, leaving no product to sell (Zwietering et al. 2014). More important, the sampling plans are able to detect gross errors in the

management of food safety and for sure positive samples detected indicate a situation out of control which need corrective actions. As illustrated in **CHAPTER 3**, the limited number of product samples was able to detect *L. monocytogenes* in e.g. raw meat spread. The food service operation will react on each unsatisfactory sample that they detect and take corrective actions. If it is noticed that a particular supplier does not respond to these complaints as a result of the sampling plan or cannot comply with the imposed specifications, it can be decided by the food service operation to remove this supplier from the supplier list. This happened in the case of the supplier of the raw meat spread contaminated with *L. monocytogenes*. This creates a pressure on the supplier to improve their FSMS as well. The proposed sampling approach can be implemented downstream the farm-to-form chain as well. As illustrated by Daelman et al. (2013a and 2013b) in assessing the overall microbiological quality and safety of cooked chilled foods. This production process uses, just as in case of institutional FSOs, a variety of incoming raw materials and needs to apply various 'food preparation and assembling' steps to come to a ready-to-eat product. The approach is also used in food processing companies with a relative modest food product variation, as they focus on the production of a single food stuff such as poultry meat preparations, dairy products or pork or lamb meat preparations (Jacxsens et al. 2009; Sampers et al. 2010; Osés et al., 2012a and 2012b). The sampling plan has also some additional benefits as it gathers knowledge and provides an actual outcome. The results can for example be used in communication to suppliers and staff members of the food service operation. As such it will increase the awareness and adhered importance to food safety and food quality, which might increase the "food safety culture". It has been noted that this type of sampling plan is judged by food businesses as still taking up too much resources, although this type of systematic approach in sampling throughout the food production system can be used to identify remaining points of attention or critical points in the implemented FSMS. However, in due time, sampling is continued at a regular basis (with a defined frequency) for these particular identified sampling locations (incoming raw materials, environment or intermediate food products) and the microbiological parameters (and associated selection method of analysis) considered to be most practical and expected to contribute most to improve the degree of food safety or food quality of the product or production process.

7.1.2 Microbiological analysis to validate food safety management systems

A FSMS shifts the focus of microbiological sampling and testing of final food products towards monitoring in-process control measures. This monitoring of CCPs is mostly accomplished by using process parameters, such as measuring the temperature at which a food product is held and the time it is held at a particular temperature (Kvenberg and Schwalm, 2000). This gives indirect information of the microbiological status of foods or intermediate products. The main reason to use indirect methods of measurement is that microbiological sampling and testing to monitor CCPs is not always feasible as results of tests for pathogens often cannot be obtained in time. However, microbiological analysis are needed to validate these control measures. As in particular for micro and small-sized enterprises there might be a lack of resources and knowledge to take a collaborative approach for validation of identified critical processing and storage conditions, it was decided in the present PhD study to focus on the use of challenge testing to provide insight in some implemented control measures used in two sectors involving many small businesses in a business-to-consumer operation. These validation experiments in the lab mimic the actual situation of food handling and storage as good as possible. A comparison between the actual and the expected results based on scientific literature or predictive models allows then the validation of the control measures.

7.1.2.1 Validation of heat inactivation procedures

In **CHAPTER 5** challenge testing was performed to validate heat inactivation procedures as the heat treatment of meat and meat preparations is a major critical control point in the sector of food service operations (hotels, restaurants, catering) to significant reduce numbers of pathogenic bacteria possibly being present in the meat. These heat inactivation procedures in FSOs depend on safe harbors for thermal treatment, for example, 2 minutes at 70°C for safe cooking of burgers. Those safe harbors are in fact widely accepted control measures which are based on the outcome of deterministic models using the mean values of parameters linked to target organisms' growth and survival characteristics and incorporate simplified thermal inactivation kinetics (Bean et al., 2012). Despite, they are often used without further validation because of historical track record of providing safe

foods. However in this PhD study, two situations were observed after the validation experiments.

Firstly, a situation for which the thermal treatment of the meat and meat preparations was equivalent to 2 min at 70 °C (i.e. thoroughly cooking) but seemingly not always a 6 log reduction was obtained as one would expected. It needs to be taken into account that safe harbors such as 2 minutes 70°C are set quite conservative in order to achieve a 6 log reduction. Information used to establish cooking recommendations has largely been derived from D-values in laboratory experiments (ICMSF, 2005). Since the late 1990s, a number of studies have evaluated the heat resistance of *S. enterica* and *L. monocytogenes* in buffers or broth (Juneja et al., 2001; Miller et al., 2009; Sorqvist, 2003), and in meat and meat products (Halder et al., 2010; Juneja et al., 2001; Murphy et al., 2006; Vasan et al., 2014), but data collected using actual consumer-based handling and cooking processes are comparatively scarce. Thermal inactivation studies in the laboratory are usually performed at isothermal conditions, yet the cooking processes being used in hotels, restaurants and catering or by consumers at home are generally non-isothermal: burgers are usually thermally treated for several minutes on each side in a frying pan in hot butter before being served for consumption. Furthermore, micro-organisms in ground meat are immobilized and constrained to grow as colonies rather than planktonically, which may also have an effect on the observed thermal inactivation profiles. Besides, a 6 log reduction is only needed in a worst case scenario of initial high levels of pathogen contamination, which needs thorough cooking of the meat to render it a safe food. That the request of a 6 or 7 log reduction is derived from deterministic worst case approaches is illustrated in the following example. *Salmonella* spp. was identified by a US Food Safety and Inspection Service risk assessment as the pathogen of concern for their Lethality Performance Standards for meat and poultry products (USFSIS, 1998). The standards define an objective of a 7 log reduction of *Salmonella* in Ready-to-Eat (RTE) poultry products and a 6.5 log reduction of *Salmonella* in RTE beef products. The rationale for these performance standards was based upon i) the establishment of a worst-case population of *Salmonella* spp. by animal species, considering baseline survey levels and probability distributions, and ii) the probability of survival of *Salmonella* spp. in 100 g of finished product after the specific lethality processes were calculated. For poultry products a worst-case level of 37 500 *Salmonella*/g was calculated

based on data from baseline surveys in the poultry industry. In a serving size of 143 g of raw product (assuming a serving size of 100 g of the cooked product) there would be approximately 5 362 500 (6.7 log) *Salmonella* spp. A 7-log reduction of *Salmonella* is therefore considered sufficient to obtain the acceptable level of protection with some safety margin. Likewise 6.2 log was determined to be the worst-case level for beef products, and 6.5 log lethality was determined to provide an acceptable level of protection. The assumptions behind these standards are now being debated in light of many regulatory changes concerning the management of the safety of the whole food chain which have shifted the focus from end-product control and focus on a preventive approach including a greater effort on improvements in hygiene and application of HACCP principles throughout the whole meat and poultry processing chain from farm to fork.

Secondly, another situation is the one that refers to serving meat that has not been thoroughly cooked. Thus a situation for which the thermal treatment of the meat and meat preparations was not equivalent to 2 min at 70 °C. In this situation, it is indeed expected that there is occasional survival of foodborne pathogens. However, it is a part of the culinary preferences of consumers in Belgium, especially for intact beef meat. Some consumers might also prefer or eat insufficiently cooked burgers (comminuted meat) which is not desirable. Serving these types of meat, in particular rare burgers, to susceptible persons (YOPI) is debatable and is indeed part of a debate currently going on also in the UK (https://www.food.gov.uk/news-updates/news/2015/14362/new-proposals-on-serving-rare-burgers).

This PhD study demonstrated thus that the capacity of a physical intervention should first be validated in the own production process and should not only be tested by monitoring the core temperatures of meat and meat preparations, but should also be verified over time with microbiological analysis to confirm the performance of the control measure. In this situation, CCP monitoring measuring time-temperature combinations indicated that the process was functioning properly, but validation using microbiological challenge testing indicated that a microbiological indicator or pathogen could be present at an unacceptable level (in particular if prior good hygienic practices and storage at proper refrigerator temperatures would not have been respected). However, safe harbors assume thermal processing to be the sole intervention for assuring meat safety and often do not take into

account the effect of control measures in the food chain before and after thermal processing (Fryer and Robbins, 2005; Gaze, 2006). Therefore, in both cases, food safety relies on good hygiene at farm and slaughterhouse to minimize pathogen contamination on the meat and respect of cold chain during further distribution and storage to keep the initial level of pathogens as low as possible. As such also less than 'the worst case needed 6 log reduction" could be sufficient to ensure food safety. This farm to fork approach would be strengthened by assessing the quality of incoming raw material and thus to verify supplier selection with a systematic sampling approach as described above.

7.1.2.2 Validation of storage conditions in controlling growth of *L. monocytogenes*

Another important control measure in safeguarding the food supply chain and prevention of foodborne infections and intoxications is to respect appropriate cold temperatures and restriction of shelf life duration to prevent the growth of pathogenic bacteria to an unacceptable level. This is in particular applicable in ready-to-eat food products with prolonged storage under refrigeration, such as soft and semi-soft or semi-hard cheese as those food products are important at risk products for foodborne listeriosis (Fox et al. 2011; Hunt et al., 2012; Schoder et al., 2008; Magalhaes et al., 2015). In Belgium many artisanal soft and semi-hard cheeses are sold in delicatessen shops and some shops actually promote themselves as being specialists in best knowledge on how to handle and select a variety of tasteful traditional cheeses to reply to consumer's requests on high quality food. These shops are also allowed to have a cellar or ripening room (under well-defined conditions on how to arrange and maintain these rooms under good practices) for (short) further maturation of some cheeses upon receipt to fulfil some consumer preferences of well-matured soft and semi-hard cheeses. In Belgium, temperatures in these ripening rooms within delicatessen shops is allowed to be up to 14°C. It is debated if under these temperatures *L. monocytogenes* would be able to grow (and more likely to grow than storage upon 7°C) in the presence of active indigenous lactic acid bacteria being present as part of the (natural) ferment in these cheeses. Also the performed inoculation procedure is often debated in performing challenge testing, in particular for this rather heterogeneous artisanal cheese product for which the microbiota of the surface or rind can differ, but also can differ according to the produced batch. Besides the microbiota might evolve during

ripening of the cheese. Therefore, challenge testing was performed in **CHAPTER 6** to evaluate the growth potential of *L. monocytogenes* to assess if this micro-organism is able to grow further to elevated numbers in the produced product throughout the shelf life, and thus if current storage conditions (i.e. 7 and 14°C) are effective in controlling the growth of this pathogen. As during storage, display or slicing post-contamination may occur from *L. monocytogenes* colonizing the environment, it was decided to simulate this by inoculation either on the cheese slicing surface or the cheese rind surface.

The results of a series of challenge tests conducted on various types of cheese within **CHAPTER 6** of this PhD study demonstrated that there is a wide range in growth potential of *L. monocytogenes* and that current storage conditions are not able to control this hazard. The microbiological criterion defined for *L. monocytogenes* as stated in Regulation EC No. 2073/2005 defines an end product threshold value of "< 100 CFU *L. monocytogenes* cells per ml or per g" but is only applicable when the producer can proof that *L. monocytogenes* is not able to grow further to elevated numbers in the product throughout the shelf life or the ultimate date of consumption (i.e. use by date). Due to this variability, the food business operator may not be able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout the shelf life. As a consequence, cheese will be classified, according to EU-RL Technical Guidance document of conducting shelf life studies for *L. monocytogenes* in RTE foods, as a high risk product and should not be served to vulnerable people (EU-RL, 2014).

If growth occurs there is also an estimate of the order of magnitude of how many log units increase of *L. monocytogenes* could be expected throughout the tested storage conditions. However, in the recommended calculation to assess the growth potential in a batch as prescribed in the EU-RL Guidance document one takes "the difference between the median of results at the end of the challenge test and the median of results at the beginning of the challenge test of three replicate test units within a batch". As such the maximal outgrowth that may have been noted in one of three replicates within a batch is not taken into account. Although over the three batches that are included in challenge testing for a food type, the maximum of the growth potential of the three individual batches is taken into consideration to report the 'overall growth potential' for a food type, it was noted that occasionally the actual growth of *L. monocytogenes* might be underestimated due to

considerable inter-batch and intra-batch variability observed. In particular for these soft and semi-soft cheeses the inter-batch and intra-batch variability noted seemed to be higher than in case of *L. monocytogenes* challenge testing for other types of food such as cooked meat products, cold-smoked salmon or mayonnaise-based deli-salads for which the service lab at the Lab of Food Microbiology and Food Preservation has more experience with (Uyttendaele et al. 2004; Vermeulen et al. 2011). This observation indicates no consistent behavior of *L. monocytogenes* in these fermented dairy products. This might be due to complexity and biodiversity of the indigenous microbiota in these cheeses (Montel et al., 2014; Delcenserie et al., 2014). Still, if it cannot be shown that storage conditions are not effective in controlling the growth of *L. monocytogenes*, it is necessary to focus on prevention through good hygiene practices to comply with a limit of absence in 25 g of cheese using a multiple sample subunit approach (n= 5) at the time of production. However, ensuring the absence of *L. monocytogenes* in artisanal cheeses might be difficult to achieve. Besides, challenge testing using an artificial inoculum, may overestimate the actual growth of *L. monocytogenes* in naturally contaminated foods. Therefore, there is still a need to collect as much as possible information from follow-up of naturally *L. monocytogenes* contaminated cheese products throughout their shelf life. This is referred to as 'durability testing'. Schoder et al. (2013) could recover some cheeses from the Quargel outbreak in Austria, Germany and Czech Republic between 2009 and 2010 and used these cheeses to estimate the contamination at end of production based on the results obtained of the recalled cheeses. The growth simulations suggested that a very low initial contamination level (e.g. <1 CFU/g or 5 CFU/100 g) could justify the levels of *L. monocytogenes* enumerated in recalled samples of Quargel cheese. In our case study, described in **CHAPTER 6**, a naturally contaminated soft cheese was found with *L. monocytogenes* levels of > 10 000 CFU/g at the ultimate date of consumption (although the shelf life label indicated a "best before date" and not a 'use by date'). These examples illustrate that there is a reason for concern on food safety of artisanal soft and semi-soft cheeses. Due to increased efforts on inspection, training and also recalls and outbreaks causing media attention, there is raised awareness among small scale cheese producers in EU with regard to *L. monocytogenes*. However, these types of risk products should preferably NOT be served in institutional food service operations, such as hospitals, elderly homes, crèches or primary schools as they are mainly serving food to vulnerable persons.

7.2 Conclusion

Sampling and microbiological analysis should be performed by FSO to identify hazards and to validate the control measures within a FSMS. As the heat treatment of meat and meat preparations is a major critical control point in FSO enabling to significantly reduce numbers of pathogenic bacteria possibly being present in the meat, microbiological analysis were performed in this PhD study to actually validate the current procedures in place in terms of pathogen reduction. To prevent insufficient reduction it is important to keep the initial level of pathogens as low as possible. This stresses the need of supplier verification to obtain information on the microbiological quality and safety of incoming raw materials by setting up a 'horizontal' sampling plan. Another important control measure in safeguarding the food supply chain and prevention of foodborne infections and intoxications is to respect appropriate cold temperatures and restriction of shelf life duration to prevent the growth of pathogenic bacteria. Results of a series of challenge tests conducted on various types of cheese within this PhD study demonstrated that there is a wide range in growth potential of *L. monocytogenes* insinuating that current storage conditions are not able to control this hazard if post-contamination occurs. As a consequence, cheese will be classified as a high risk product and should not be served to vulnerable people. The results from the two validation studies support the categorization of these type of food products, (insufficiently) cooked meat and soft and semi-soft or semi-hard artisanal cheeses, as risk products thus indeed favouring these type of food as priority foods to be taken up in monitoring and surveillance plans, whether by competent authorities or by food business operators in the verification of their supplier selection or food safety management system. Focused microbiological testing programs based on the risk categorisation of incoming raw materials and food served to the consumer are desirable in many micro and small-sized enterprises, as they are able to simultaneously verify the effectiveness of control measures and procedures in place and at the same time minimize the cost of sampling and testing. In addition to verify the effectiveness of a FSMS in the internal auditing process, the setting up of a sampling plan may serve to build a systematic track record which can be used in case of complaints or external audits. From the results obtained on site during the elaboration of the sampling plans, it can be concluded that although the sampling plans have intrinsic limitations in assessing the quality and safety of the foods sampled due the low numbers of

samples, it was shown useful to reveal major non-compliances and opportunities to improve the FSMS. With the low numbers of samples and sampling plans only detecting gross errors, positive results should be seen as a very severe outcome, but absence in a limited number of samples is no guarantee of food safety. It is well known that microbial hazards are present in the food supply chain and even a well-functioning regulatory and management system accompanied with sampling and testing cannot ensure zero risk.

7.3 Recommendations and future perspectives

- The approach of either vertical or horizontal sampling for respectively validation and verification of the implemented FSMS should be applied in more FSO. If the data are then shared within an industry association, it could provide an added value to the sector and one would build a powerful database. As such it could be noted whether some particular sampling locations or microbial hazards are recurring in several of these food businesses and are thus microbiological issues that need to be discussed at the level of an industry association (e.g. needing extra attention in a hygiene code, or needing additional research in the root-cause analysis of this problem, or might be linked to a change in a production technology or sourcing region, or change of method of analysis, or a more stringent microbiological threshold limit being set etc.). Microbiological analysis per se cannot only serve the individual company on performance of food safety managements' systems, but by sharing data, microbiological analysis can provide information and generate insight and findings for a sector as a whole independent from the individual company.

- It is also recommended to use microbiological analysis to validate if control measures taken to ensure food safety are adequate in the (own) production process. In particular, when deviating from established time-temperature combinations, it is necessary to demonstrate, in a measurable way, that the control measures e.g. time and temperature of cookers or time and temperature of storage conditions during distribution can control the hazards. These results can also be taken up in sector guides to provide more specific information.

- Validation studies can also help to support the categorization of food products to be taken up in monitoring and surveillance plans. In the current PhD study, (insufficiently) cooked meat and soft and semi-soft or semi-hard artisanal cheeses were confirmed to be risk products thus indeed favoring these type of food as priority foods to be taken up in monitoring and surveillance plans whether by competent authorities or by food business operators in the verification of their supplier selection or FSMS.

- As risk products should not be served to vulnerable people (i.e. YOPI group) it is recommended to communicate better on these risk products. In analogy with the allergens policy, it can be useful to put a symbol or warning on the food packaging of high risk products to warn people of the YOPI group. As such, the consumer will be more informed, but also in FSO more attention would be paid to the selection of raw materials used or served in the FSO.

REFERENCE LIST

Advisory Committee on the Microbiological Safety of Food (ACMSF). (2007). Report on safe cooking of burgers. Food Standards Agency, 1–40.

Airey, S. and Greaves, A. (2005). HACCP for small business in the UK. In: Maunsell, B., Declan, J.B. (Eds.), Restaurant and Catering Food Safety: Putting HACCP on the Menu. European Union Risk Analysis Information Network (EU-RAIN), Dublin, Ireland, ISBN 1 84170 439 3, pp. 138-157.

Akaike, H. (1973). Information theory and an extension of themaximum likelihood principle. Second International Symposium on Information Theory. Akademinai Kiado,. 267–281.

Albersmeier, F., Schulze, H., Jahn, G. and Spiller, A. (2009). The reliability of third-party certification in the food chain: From checklists to risk-oriented auditing. Food Control, 20, 927–935.

Allerberger, F. and Wagner, M. (2010). Listeriosis: a resurgent foodborne infection. Clinical Microbiology and Infection, 16, 16-23.

Alvarez-Ordonez, A., Leong, D., Hickey, B., Beaufort, A., Jordan, K. (2014). The challenge of challenge testing to monitor *Listeria monocytogenes* growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. Food Research International, 75, 233–243.

Andritsos, N.D., Mataragas, M., Paramithiotis, S. and Drosinos, E.H. (2013). Quantifying Listeria monocytogenes prevalence and concentration in minced pork meat and estimating performance of three culture media from presence/absence microbiological testing using a deterministic and stochastic approach. Food Microbiology, 36, 395–405.

Angelidis, A., Papageorgiou, D., Tyrovouzis, N. and Stoforos, N. (2013). Kinetics of Listeria monocytogenes cell reduction in processed cheese during storage. Food Control, 29, 18-21.

Anonymous, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Union, 31, 1-24.

Anonymous. (2003a). Directive 2003/99/EC of the European Parliament of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Official Journal of the European Union, 325, 31-41.

Anonymous. (2003b). Koninklijk Besluit van 14 november 2003 betreffende autocontrole, traceerbaarheid en meldingsplicht in de voedselketen. Available online http://www.favv.be/autocontrole-nl/wetgeving/_documents/2003-11-14_AR_autocontrole.pdf.

Anonymous. (2004a). Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal of the European Union, 165, 1-141.

Anonymous. (2004b). Regulation EC No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union, 139, 1-54.

Anonymous. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, 338, 1–26.

Anonymous. (2009). Koninklijk Besluit van 26 April 2009 betreffende microbiologische criteria voor voedingsmiddelen. Belgisch Staatsblad, June 8.

Association Française de Normalisation. (1996). AFNOR validation. Microbiologie des aliments. Dénombrement en anaérobiose des bactéries sulfito-réductrices par comptage des colonies (nu XP V 08-061). Association Française de Normalisation, Paris.

Association Française de Normalisation. (1999). AFNOR validation. COLI-ID. Milieu (gélose) (nu BIO 12/6-01/99). Association Française de Normalisation, Paris.

Association Française de Normalisation. (2002). AFNOR validation. Microbiologie des aliments. Dénombrement des levures et moisissures par comptage des colonies à 25°C (nu NF V08-059). Association Française de Normalisation, Paris.

Association Française de Normalisation. (2004). AFNOR validation. VIDAS Listeria monocytogenes II (VIDAS LMO2). Ref. 30704. Enrichment stage at 37°C (nu BIO 12/11-03/04). Association Française de Normalisation, Paris.

Association Française de Normalisation. (2005). AFNOR validation. VIDAS Easy *Salmonella*. Ref. 06984 O (nu BIO-12/16-09/05). Association Française de Normalisation, Paris.

Augustin, J.C. and Minvielle, B. (2008). Design of control charts to monitor the microbiological contamination of pork meat cuts. Food Control 19, 82–97.

Bachmann, H. P. and Spahr, U. (1995). The fate of potentially pathogenic bacteria in Swiss hard and semi-hard cheeses made from raw milk. Journal of Dairy Science,78, 476-483.

Baert, L., Uyttendaele, M., Stals, A., Van Coillie, E., Dierick, K. and Debevere, J. (2009). Reported foodborne outbreaks due to noroviruses in Belgium: the link between food and patient investigations in an international context. Epidemiology and Infection, 137, 316–325.

Baird-Parker, A.C. (1995). Development of industrial procedures to ensure the microbiological safety of food. Food Control, 6, 29-36.

Bayliss, C.L. and Petitt, S.B. (1997). The significance of coliforms, Escherichia coli and the Enterobacteriaceae in raw and processed food. In Coliforms and *E. coli* Problem or solution? D. Kay and C. Fricker (Eds.) Cambridge, Royal Society of Chemistry, pp. 49-53.

Bayliss, C., Uyttendaele, M., Joosten, H. and Davies, A. (2011). The Enterobacteriaceae and their significance to the food industry. ILSI Europe Report Series, December. Available online: http://www.ilsi.org/Europe/Documents/EP%20Enterobacteriaceae.pdf

Bean, D., Bourdichon, F., Bresnahan, D., Davies, A., Geeraerd, A., Jackson, T., Membré, J.M., Pourkomailian, B., Richardson, P., Stringer, M., Uyttendaele, M. and Zwietering, M. (2012). Risk assessment approaches to setting thermal processes in food manufacture. ILSI Europe report series, April. Available online: http://www.ilsi.org/Pages/ViewItemDetails.aspx?WebId={84d7fa4a-0fd5-40cd-a49a-2da6fcdfd654}&ListId={0348eb34-df85-49dd-9ade-77ed136643f1}&ItemID=272

Bearth, A., Cousin, M.E. and Siegrist, M., (2014) Poultry consumers' behaviour, risk perception and knowledge related to *Campylobacter*iosis and domestic food safety. Food Control 44, 166–176.

Bergsma, N.J., Fischer, A.R.H., Van Asselt, E.D., Zweitering, M.H. and De Jong, A.E.I. (2007). Consumer food preparation and its implication for survival of *Campylobacter* jejuni on chicken. British Food Journal, 109, 548–561.

Boxman, I., Dijkman, R., Verhoef, L., Maat, A., van Dijk, G., Vennema, H. and Koopmans, M. (2009). Norovirus on swabs taken from hands illustrate route of transmission, a case study. Journal of food protection, 72, 1753-1755.

Boxman, I., Verhoef, L., Dijkman, R., Hagele, G., Loeke, N. and Koopmans, M. (2011).Year-round prevalence of norovirus in the environment of catering companies without a recently reported outbreak of gastroenteritis. Applied and Environmental Microbiology, 77, 2968-2974.

Boxman, I, Verhoef, L., Hagele, G., Klunder, K., Loeke, N., Vennema, H., Jansen, C. and Koopmans, M. (2015). Environmental testing for norovirus in various institutional settings using catering companies as sentinels for norovirus prevalence among the general population. Food Control 47, 98–102.

British Columbia Centre for Disease Control (BCCDC). (2010). Environmental hygiene monitoring—a guide for environmental health officers. Version October 5, 2010. Available at: http://www.bccdc.ca/NR/rdonlyres/EF1461BE-0301-4A59-8843-420072412721/0/EnvMonitoringHygieneGuideforEHOs.pdf

Buchanan, R.L. and Schaffner, D. (2015). FSMA: Testing as a Tool for Verifying Preventive Controls. Food Protection Trends, May-June, 228-237.

Bunning, V.K., Crawford, R.G., Tierney, J.T. and Peeler, J.T. (1990). Thermotolerance of Listeria monocytogenes and *Salmonella* typhimurium after sublethal heat shock. Applied and Environmental Microbiology, 56, 3216–3219.

Busani, L.; Cigliano, A.; Taioli, E.; Caligiuri, V.; Chiavacci, L.; Di Bella, C.; Battisti, A.; Duranti, A.; Gianfranceschi, M.; Nardella, M.C.; Ricci, A.; Rolesu, S.; Tamba, M.; Marabelli, R. and

Caprioli, A. (2005). Prevalence of *Salmonella* enterica and Listeria monocytogenes contamination in foods of animal origin in Italy. Journal of Food Protection, 68, 1729-1733.

Cairns, B. J. and Payne, R. J. H. (2009). Sudden increases in Listeriosis rates in England and Wales, 2001 and 2003. Emerging Infectious Diseases, 15, 465-468.

Caponigro,V., Ventura, M., Chiancone, I., Amato, L., Parente, E. and Piro, F. (2010). Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer. Food Microbiology, 27, 1071-1077.

Carpentier, B. and Cerf, O. (2011). Review—Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology, 145, 1–8.

Cassin, M.H., Lammerding, A.M., Todd, E.C., Ross, W. and McColl, R.S., (1998). Quantitative risk assessment for Escherichia coli O157:H7 in ground beef hamburgers. International Journal of Food Microbiology, 41, 21–44.

Centers for Disease Control and Prevention (CDC). 2014. Multistate outbreak of shiga toxin-producing Escherichia coli O157:H7 Infections Linked to Ground Beef (Final Update). Available at:. http://www.cdc.gov/ecoli/2014/O157H7-05-14/index.html.

Commission of the European Communities (CEC). (2006). Guidance document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs. Available online: http://ec.europa.eu/food/safety/official_controls/legislation/docs/sampling_testing_en.pdf

Ceuppens, S., Van Boxstael, S., Westyn, A., Devlieghere, F. and Uyttendaele, M. (2016). The heterogeneity in the type of shelf life label and storage instructions on refrigerated foods in supermarkets in Belgium and illustration of its impact on assessing the Listeria monocytogenes threshold level of 100 CFU/g. Food Control, 59, 377-385.

Christison, C. A., D. Lindsay, and A. Von Holy. (2008). Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. Food Control 19, 727–733.

Claeys, E., Lauwers, N. and Demeyer, D. (1998). Kwaliteit en technologie van vlees. 90-804576-2-0, p. 195.

Clavero, M.R., Monk, J.D., Beuchat, L.R., Doyle, M.P. and Brackett, R.E. (1994). Inactivation of Escherichia coli O157:H7, *Salmonella*e, and *Campylobacter* jejuni in raw ground beef by gamma irradiation. Applied and Environmental Microbiology, 60, 2069–2075.

Codex Alimentarius Commission (CAC). (1997). Principles and guidelines for the establishment and application of microbiological criteria related to foods. CAC/GL 21 – 1997. Available online: http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

Codex Alimentarius Commission (CAC). (2003). Recommended International Code of practice. General Principles of Food Hygiene CAC/RCP1-1969 (Rev.. Ed. .4), 1-31.

Codex Alimentarius Commission (CAC). (2004). General guidelines on sampling. CAC/GL 50-2004. Available online: http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

Codex Alimentarius Commission (CAC). (2008). Guidelines for the validation of food safety control measures. CAC/GL 69 – 2008. Available online: http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

Codex Alimentarius Commission (CAC). (2013a). Codex general standard for cheese. CODEX STAN 283-1978. Formerly CODEX STAN A-6-1973. Adopted in 1973. Revision 1999, Amendments 2006, 2008, 2010, 2013. Available online: http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

Codex Alimentarius Commission (CAC). (2013b) Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods. CAC/GL 21-1997. Available online: http://www.codexalimentarius.org/standards/list-standards/en/?no_cache=1

Coleman, M., Sandberg, S. and Anderson, S. (2003). Impact of microbial ecology of meat and poultry products on predictions from exposure assessment scenarios for refrigerated storage. Risk Analysis, 23, 215-228.

Condron, R., Farrokh, C., Jordan, K., McClure, P., Ross, T. and Cerf, O. (2015). Guidelines for experimental design protocol and validation procedure for the measurement of heat

resistance of microorganisms in milk. International Journal of Food Microbiology, 192, 20-25.

Cook, A., Odumeru, J., Lee, S. and Pollari, F., (2012). *Campylobacter*, *Salmonella*, Listeria monocytogenes, Verotoxigenic Escherichia coli, and Escherichia coli prevalence, enumeration, and subtypes on retail chicken breasts with and without skin. Journal of Food Protection, 75, 34–40.

Cormier, R.J., Mallet, M., Chiasson, S., Magnùsson, H. and Valdimarsson, G. (2007). Effectiveness and performance of HACCP-based programs. Food Control 18, 665-671.

Daelman, J., Jacxsens, L., Devlieghere, F. and Uyttendaele, M., (2013a). Microbial safety and quality of various types of cooked chilled foods. Food Control 30, 510-517.

Daelman, J., Jacxsens, L., Lahou, E., Devlieghere, F. and Uyttendaele, M. (2013b). Assessment of the microbial safety and quality of cooked chilled foods and their production process. International Journal of Food Microbiology 160, 93-200.

Dahms, S. (2004). Microbiological sampling plans – Statistical aspects. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 95, 32–44.

Dalmasso, M. and Jordan, K. (2014). Absence of growth of Listeria monocytogenes in naturally contaminated Cheddar cheese. Journal of Dairy Research, 81, 46-53.

de Jong, A.E.I., Van Asselt, E.D., Zwietering, M.H., Nauta, M.J. and de Jonge, R. (2012). Extreme heat resistance of foodborne pathogens *Campylobacter* jejuni, *E. coli* and *Salmonella* typhimurium on chicken breast fillet during cooking. International Journal of Microbiology, 2012, 1–10.

de Valk, H. (2000). Outbreak of listeriosis linked to the consumption of pork tongue in jelly in France. Eurosurveillance 4 (9) Available online: http://www. eurosurveillance.org/ViewArticle.aspx?ArticleId=1650.

Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey, D., Korsak, N. and Daube, G. (2014). Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. Journal of Dairy Science, 97, 6046-6056.

Delignette-Muller, M.L., Cornu, M., Pouillot, R. and Denis, J.B. (2006) Use of Bayesian modelling in risk assessment: application to growth of Listeria monocytogenes and food flora in cold-smoked salmon. International Journal Food Microbiology, 106, 195–208

Denny, J., & McLauchlin, J. (2008). Human Listeria monocytogenes infections in Europe: an opportunity for improved European surveillance. Eurosurveillance, 13. Available online http://www.eurosurveillance.org/viewarticle.aspx?ArticleId=8082.

Devlieghere, F., Debevere, J., Jacxsens, L., Uyttendaele, M. and Vermeulen, A. (2011). Levensmiddelenmicrobiologie en –conservering. Die Keure, Brugge, Belgium. ISBN 978 90 4861 191 1

Di Ciccio, P., Conter, M., Zanardi, E., Ghidini, S., Vergara, A., Paludi, D., Festino, A. and Lanieri, A. (2012). Listeria monocytogenes: Biofilms in food processing. Italian Journal of Food Science, 24, 203-213.

Dierick, K., & Botteldoorn, N. (2007). Annual Report on foodborne outbreaks and antimicrobial resistance in zoonotic agents in Belgium 2007. Scientific Institute of Public Health. Depotnummer: D/2007/2505/54. Available online http://bacterio.wiv-isp.be/reporting/reportspdf/VTI%202006.pdf.

Dierick, K., Botteldoorn, N., Denayer, S., & Naranjo, M. (2009). Annual Report on foodborne outbreaks and antimicrobial resistance in zoonotic agents in Belgium 2007. Scientific Institute of Public Health. Depotnummer: D/2008/2505/35. Available online http://bacterio.wiv-isp.be/reporting/reportspdf/tia-nl007.pdf.

Dimitrijevíc, M., Anderson, R. C., Karabasil, N., Pavlicevic, N., Jovanovic, S., Nedeljkovic-trailovic, J., Teodorovic V., Markovic M. and Dojcinovic S. (2011). Environmental prevalence and persistence of Listeria monocytogenes in cold-smoked trout processing plants. Acta Veterinaria, 61, 429-442.

Doménech, E., Amorós, J.A., Pérez-Gonzalvo, M. and Escriche, I. (2011). Implementation and effectiveness of the HACCP and pre-requisites in food establishments. Food control, 22, 1419-1423.

Doménech, E., Amorós, J. and Escriche, I. (2013). Effectiveness of Prerequisites and the HACCP Plan in the control of microbial contamination in ice cream and cheese companies. Foodborne Pathogens and Disease, 10, 222-228.

Doménech-Sánchez, A., Laso, E., Pérez, M. J. and Berrocal, C. I. (2011). Microbiological levels of randomly selected food contact surfaces in hotels located in Spain during 2007–2009. Foodborne Pathogens and Disease, 8, 1025–1029.

Doyle, M.E. and Mazzotta, A.S. (2000). Review of studies on the thermal resistance of *Salmonella*e. Journal of Food Protection, 63, 779–795.

Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W. and Scott, V.N. (2001). Heat resistance of Listeria monocytogenes. J. Food Prot. 64, 410–429.

Duffy, L.L., Small, A. and Fegan, N. (2010). Concentration and prevalence of Escherichia coli O157 and *Salmonella* serotypes in sheep during slaughter at two Australian abattoirs. Australian Veterinary Journal, 88, 399–404.

Dufour, C. (2011). Application of EC regulation no. 2073/2005 regarding Listeria monocytogenes in ready-to-eat foods in retail and catering sectors in Europe. Food Control, 22, 1491-1494.

Dwinger, R.H., and De Smet, K. (2016.) Legal Requirements for food hygiene. In: Caballero, B., Finglas, P., and Toldrá, F. (eds.) The Encyclopedia of Food and Health vol. 3, p. 529-538. Oxford: Academic Press.

Espié, E. and Weill, F.X. (2003). Outbreak of multidrug resistant *Salmonella* Newport due to the consumption of horsemeat in France. Eurosurveillance, 7 (27). Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2252.

EU CRL for Listeria monocytogenes. (2014). Technical guidance document for conducting shelf life studies on Listeria monocytogenes in ready-to-eat foods. pp. 1–47. Available online:http://ec.europa.eu/food/food/biosafety/*Salmonella*/docs/technical_guidance_listeria_en.pdf

European Food Safety Authority (EFSA). (2006). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2005. EFSA Journal, 94, 1-288.

European Food Safety Authority (EFSA). (2007). The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA Journal, 130, 1-352.

European Food Safety Authority (EFSA). (2008). Overview of methods for source attribution for human illness from foodborne microbiological hazards. Scientific opinion of the panel on biological hazards. EFSA Journal, 764, 1-43.

European Food Safety Authority (EFSA). 2009. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agentsin the European Union in 2007. EFSA Journal, 223, 1-320.

European Food Safety Authority (EFSA). (2010). The Community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA Journal, 8(7):1658

European Food Safety Authority (EFSA). (2011). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA Journal, 9(3):2090

European Food Safety Authority (EFSA). (2012). Scientific opinion on the development of a risk ranking framework on biological hazards. EFSA Journal, 10, 2724.

European Food Safety Authority (EFSA). (2013a). Analysis of the baseline survey on the prevalence of Listeria monocytogenes in certain ready-to-eat foods in the EU, 2010-2011 Part A: Listeria monocytogenes prevalence estimates. EFSA Journal, 11:3241

European Food Safety Authority (EFSA). (2013b). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011.

EFSA (2014a). Analysis of the baseline survey on the prevalence of Listeria monocytogenes in certain ready-to-eat foods in the EU, 2010-2011 Part B: analysis of factors related to prevalence and exploring compliance. EFSA Journal, 12, 3810.

European Food Safety Authority (EFSA). (2014b). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA Journal, 12, 1–312.

European Food Safety Authority (EFSA). (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal, 13(1):3991

Egan, H., Ronald, S.K., Sawyer, R. and Pearson, D. (1981). Pearson's Chemical Analysis of Foods. 8th ed. Churchill Livingstone, Edinburgh, New York.

Evers, E.G. (2001). Statistische schatting van de positieve fractie en concentratie van micro-organismen in voedingsmiddelen in relatie tot steekproefomvang. Warenchemicus 31, 55-62.

Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., Thevenot, D., Condron, R., De Reu, K., Govaris, A., Heggum, K., Heyndrickx, M., Hummerjohann, J., Lindsay, D., Miszczycha, S., Moussiegt, S., Verstraete, K., and Cerf, O. (2013). Review of Shiga-toxin-producing Escherichia coli (STEC) and their significance in dairy production. International Journal of Food Microbiology, 162, 190-212.

Food and Agriculture Organisation and World Health Organisation (FAO/WHO). (2004). Risk assessment of Listeria monocytogenes in ready-to-eat foods. In Microbiological risk assessment series 5. Retrieved 27.06.13 from http://www.who.int/foodsafety/publications/micro/mra_listeria/en/.

Food and Agriculture Organisation and World Health Organisation (FAO/WHO). (2005). Benefits and potential risks of the lactoperoxidase system of raw milk preservation. Rome, Italy: Report of an FAO/WHO technical meeting FAO Headquarters.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2004). Activiteitenverslag 2003. Available online: http://www.afsca.be/jaarverslagen/_documents/AV_2003_s.pdf.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2006a). Gids voor de invoering van een autocontrolesysteem in de Horecasector (G-023), 23/08/2006.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2006b). Activiteitenverslag 2005. Available online: http://www.favv.be/jaarverslagen/_documents/AV_05_Nl_S.pdf.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2007). Activiteitenverslag 2006. Available online: http://www.favv.be/jaarverslagen/_documents/AV_2006_Nl_S_new.pdf.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2008a). Autocontrolegids voor de sector van de grootkeukens en verzorginstellingen (G-025), 22/01/2008.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2008b). Activiteitenverslag 2007. Available online: http://www.afsca.be/home/pub/_documents/2008-08-28_AV2007_v2_nl.pdf.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2008c). Productterugroepingen. Available online: http://www.afsca.be/productterugroepingen/.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), (2009a). Activiteitenverslag 2008. Available online: http://www.favv.be/jaarverslagen/_documents/2009-07-07_AV08_nl.pdf.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV). (2009b). VVI7: Inspecties infrastructuur, inrichting en hygiëne in de sectoren distributie, horeca en gemeenschapskeukens. Available online: http://www.afsca.be/wetenschappelijkcomite/barometer/voedselveiligheid/_documents/VVI7.pdf

Federaal Agentschap voor de Veiligheid van de voedselketen (FAVV). (2010a). Actiegrenzen voor microbiologische contaminanten in levensmiddelen. Available online: http://www.favv-afsca.be/thematischepublicaties.

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), (2010b). Activiteitenverslag 2009. Available online: http://www.favv-afsca.fgov.be/jaarverslagen/

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), (2011). Activiteitenverslag 2010. Available online: http://www.favv-afsca.fgov.be/jaarverslagen/

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), (2012). Activiteitenverslag 2011. Available online: http://www.favv-afsca.fgov.be/jaarverslagen/

Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), (2013). Activiteitenverslag 2012. Available online: http://www.favv-afsca.fgov.be/jaarverslagen/

Federaal Agentschap voor de Veiligheid van de voedselketen (FAVV). (2014). Activiteitenverslag 2013. Available online: http://www.favv-afsca.fgov.be/jaarverslagen/

Federaal Agentschap voor de Veiligheid van de voedselketen (FAVV). (2015). Activiteitenverslag 2014. Available online: http://www.favv-afsca.fgov.be/activiteitenverslag/2014/default.asp

Federal Agency for the safety of the food chain (FASFC). (2012). Facts and figs. 2011. pp. 1–52 Available online: http://www.afsca.be/publications-en/_documents/2012-08-30_ff2011_en.pdf).

Federal Agency for the safety of the food chain (FASFC). (2013). Facts and figs. 2012. pp. 1–64 Available online: http://www.afsca.be/publications-en/_documents/2013-08-22_F-F2012EN_S.pdf).

Federal Agency for the Safety of the Food Chain (FASFC), Scientific Institute for Public Health (WIV) and Veterinary and Agrochemical Research Centre (CODA).(2010-2011). Trends and sources 2010-2011. Available online: www.coda-cerva.be/images/pdf/trends%20and%20sources%202010-2011.pdf

Fischer, A.R.H., De Jong, A.E.I., Van Asselt, E.D., De Jonge, R., Frewer, L.J., Nauta, M.J., 2007. Food safety in the domestic environment: An interdisciplinary investigation of microbial hazards during food preparation. Risk Anal. 27, 1065–1082.

Food Safety Authority of Ireland (FSAI). Guidance Note No. 18: Validation of Product Shelf-life (Revision 2). Food Safety Authority of Ireland, Dublin, Ireland. ISBN 1-904465-33-1

Fox, E., Leonard, N. and Jordan, K. (2011). Molecular Diversity of Listeria monocytogenes Isolated from Irish Dairy Farms. Foodborne Pathogens and Disease, 8, 635-641.

Franco, A., Lovari, S., Cordaro, G., DiMatteo, P., Sorbara, L., Iurescia, M., Donati, V., Buccella, C. and Battisti, A. (2009). Prevalence and concentration of verotoxigenic Escherichia coli O157:H7 in adult sheep at slaughter from Italy. Zoonoses Public Health, 56, 215–220.

Frank, C., Werber, D., Cramer, J.P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., Spode, A., Wadl, M., Zoufaly, A., Jordan, S., Kemper, M.J., Follin, P., Muller, L., King, L.A., Rosner, B., Buchholz, U., Stark, K. and Krause, G. (2011). Epidemic profile of shiga-toxin-producing Escherichia coli O104: H4 outbreak in Germany. The New England Journal of Medicine, 365, 1771–1780.

Fretz, R., Sagel, U., Ruppitsch, W., Pietzka, A., Stöger, A., Huhulescu, S., Heuberger, S., Pichler, J., Munch, P., Pfaff, G., Stark, K., Prager, R., Flieger, A., Feenstra, O. and Allerberger, F. (2010). Listeriosis outbreak caused by acid curd cheese 'Quargel', Austria and Germany 2009. Eurosurveillance, 15. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19477

Fryer, P. J. and Robbins, P. T. (2005). Heat transfer in food processing: ensuring product quality and safety. Applied Thermal Engineering, 25, 2499–2510.

Gandhi, M., and Chikindas, L. (2007). Listeria: a foodborne pathogen that knows how to survive. International Journal of Food Microbiology, 113, 1-15.

Garayoa, R., Vitas, A.I., Díez-Leturia, M. and García-Jaloó I. (2011). Food safety and the contract catering companies: food handlers, facilities and HACCP evaluation. Food Control 22, 2006–2012.

Gaze, J. (2006). Pasteurisation: A Food Industry Practical Guide (Second Edition), Guideline No. 51. Campden & Chorleywoord Food Research Association, Chipping Camden.

Geeraerd, A.H., Valdramidis, V.P. and Van Impe, J.F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. International Journal of Food Microbiology, 102, 95–105.

Gillespie, I.A., McLauchlin, J., Grant, K.A., Little, C.L., Mithani, V., Penman, C., Lane, C. and Regan, M. (2006). Changing pattern of human listeriosis, England and Wales, 2001-2004. Emerging Infectious Diseases, 12, 1361-1366.

Gillespie, I.A., Mook, P., Little, C.L., Grant, K.A. and Adak, G.K. (2010). Listeria monocytogenes infection in the over-60s in England between 2005-2008: a retrospective case-control study utilizing market research panel data. Foodborne Pathogens and Disease, 7, 1373-1379.

Giménez, B. and Dalgaard, P. (2004). Modelling and predicting the simultaneous growth of Listeria monocytogenes and spoilage micro-organisms in cold-smoked salmon. Journal of Applied Microbiology, 96, 96-109.

Giraffa, G. (1995). Enterococcal bacteriocins: their potential as anti-Listeria factors in dairy technology. Food Microbiology, 12, 291-299.

Gonzales-Barron, U., Zwietering, M.H., Butler, F. (2013). A novel derivation of a within-batch sampling plan based on a Poisson-gamma model characterizing low microbial counts in foods. International Journal of Food Microbiology 161, 84-96.

Gonzàlez-Miret, M.L., Coello, M.T., Alonso, S. and Heredia, F.J. (2001). Validation of parameters in HACCP verification using univariate and multivariate statistics. Application to the final phases of poultry meat production. Food Control 12, 261-268.

Gould, L.H., Mungai, E. and Behravesh, C.B. (2014). Outbreaks attributed to cheese: differences between outbreaks caused by unpasteurized and pasteurized dairy products, United States, 1998-2011. Foodborne Pathogens and Disease, 11, 545-551.

Goulet, V., Hedberg, C., Le Monnier, A. and de Valk, H. (2008). Increasing incidence of listeriosis in France and other European countries. Emerging Infectious Diseases, 14, 734-740.

Goulet, V., Hebert, M., Hedberg, C., Laurant, E., Vaillant, V., de Valk, H., Desenclos, J.C. (2012). Incidence of Listeriosis and Related Mortality Among Groups at Risk of Acquiring Listeriosis. Clinical Infectious Diseases, 54, 652-660.

Green, S.B. (1991). How many subjects does it take to do a regression analysis? Multivariate Behavioural Research, 26, 499-510.

Green, L.R., Selman, C.A., Radke, V., Ripley, D., Mack, J. C., Reimann, D.W., Stigger, T., Motsinger M. and Bushnell L. (2006). Food worker hand washing practices: an observation study. Journal of Food Protection, 69, 2417-2423.

Greig, J.D. and Ravel, A. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. International Journal of Food Microbiology, 130, 77-87.

Griffith, C. (2000). Food safety in catering establishments. In: Farber, J.M., Todd, E.C.D. (Eds.), Safe Handling of Foods. Marcel Dekker, New York, pp. 235-256.

Guillier, L., Danan, C., Bergis, H., Deignette-Muller, M.L., Granier, S., Rudelle, S., Beaufort, A. and Brisabois, A. (2013). Use of quantitative microbial risk assessment when investigating foodborne illness outbreaks: the example of a monophasic *Salmonella* typhimurium 4,5,12:i- outbreak implicating beef burgers. International Journal of Food Microbiology, 166, 471-478.

Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D. and De Zutter, L. (2008). Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. Applied and Environmental Microbiology, 74, 5483–5489.

Halder, A., Black, D.G., Davidson, P.M. and Datta, A. (2010). Development of associations and kinetic models for microbiological data to be used in comprehensive food safety prediction software. Journal Food Science, 75, R107–R120.

Hansen, N.H. and Riemann, H. (1963). Factors affecting the heat resistance of nonsporing organisms. Journal of Applied Bacteriology, 26, 314–333.

Hedin, G., Rynbäck, J. and Loré, B. (2010). New technique to take samples from environmental surfaces usingflockednylonswabs. Journal of Hospital Infection, 75, 314-317.

Hendriksen, R.S., Vieira, A.R., Karlsmose, S., Lo Fo Wong, D.M.A., Jensen, A.B., Wegener, H.C. and Aarestrup, F.M. (2011). Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results

of quality assured laboratories from 2001 to 2007. Foodborne Pathogens and Diseases, 8, 887–900.

Hernández Pezzi, G., Martínez, A., Oliva, J.M., Pañella, E. and Soler, P. (2001). Outbreak of *E. coli* O157 infection in Spain. Eurosurveillance 5 (1) pii=1828. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1828

Hertzman, J. and Barrash, D. (2007). An assessment of food safety knowledge of catering employees. Britisch Food Journal, 109, 562–576.

Hoelzer, K., Sauders, B. D., Sanchez, M. D., Olsen, P. T., Pickett, M. M., Mangione, K. J., Rice, D.H., Corby, J., Stich, S., Fortes, E.D., Roof, S.E., Grohn, Y.T., Wiedmann, M. and Oliver, H.F. (2011). Prevalence, distribution, and diversity of Listeria monocytogenes in retail environments, focusing on small establishments and establishments with a history of failed inspections. Journal of Food Protection, 74, 1083-1095.

Holvoet, K., Jacxsens, L., Sampers, I. and Uyttendaele, M. (2012). Insight into the prevalence and distribution of microbial contamination to evaluate water management in the fresh produce processing industry. Journal of Food Protection, 75, 671-681.

Hunt, K., Drummond, N., Murphy, M., Butler, F., Buckley, J. and Jordan, K. (2012). A case of bovine raw milk contamination with Listeria monocytogenes. Irish Veterinary Journal, 65 (13).

International Commission on Microbiological Specifications for Foods (ICMSF). (1986). Microorganisms in Food 2: Sampling for Microbiological Analysis: Principles and Specific Applications. University of Toronto Press, Toronto, ISBN 0802056938.

International Commission on Microbiological Specifications for Foods (ICMSF). (2002). Microorganisms in Food 7: Microbiological Testing in Food Safety Management, first ed. Kluwer Academic/Plenum Publishers, New York. ISBN 030642627.

International Commission on Microbiological Specifications for Foods (ICMSF). (2005). Microorganisms in Food 6. Mircobial Ecology of Food Commodities, second ed. Kluwer Academic/Plenum Publishers, New York. ISBN 030648675.

International Commission on Microbiological Specifications for Foods (ICMSF). (2011). Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance. Springer. 1st edition, ISBN 978-1-4419-9373-1.

International Organization for Standardization (ISO). (1998). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30uC. ISO 15214:1998. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (1998). Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of Listeria monocytogenes. Part 2. Enumeration method. ISO 11290-2:1998/Amd 1: 2004. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (1999). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1. Technique using Baird-Parker agar medium. ISO 6888-1:1999/Amd 1:2003. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (2001). Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Escherichia coli O157. ISO 16654:2001. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (2003). Microbiology of food and animal feeding stuffs. Protocol for the validation of alternative methods. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (2003). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30°C. ISO 4833:2003. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (2004). Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of

Enterobacteriaceae. Part 2. Colonycount method. ISO 21527-2:2004. International Organization for Standardization, Geneva.

International Organization for Standardization (ISO). (2004). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus. Colony-count technique at 30uC. ISO 7932:2004. International Organization for Standardization, Geneva.

Izquierdo, E., Marchioni, E., Aoude-Werner, D., Hasselmann, C. and Ennahar, S. (2009). Smearing of soft cheese with Enterococcus faecium WHE 81, a multi-bacteriocin producer, against Listeria monocytogenes. Food Microbiology, 26, 16–20.

Jacxsens, L., Devlieghere, F. and Debevere, J. (2002). Temperature dependence of the microbial safety, the microbial and sensorial quality of equilibrium modified atmosphere packaged fresh-cut produce. Postharvest Biology and Technology, 26, 59-73.

Jacxsens, L., Devlieghere, F., Ragaert, P., Vanneste, E. and Debevere, J. (2003). Relation between microbiological quality, metabolite production and sensorial quality of equilibrium modified atmosphere packaged fresh-cut produce. International Journal of Food Microbiology, 83, 263-280.

Jacxsens, L., Devlieghere, F. and Uyttendaele, M. (2009a). Quality management systems in the food industry. Book in the framework of Erasmus. ISBN 978-90-5989-275134, 113-125

Jacxsens, L., Kussaga, J., Luning, P.A., Van der Spiegel, M. and Uyttendaele, M. (2009b). A microbial assessment scheme to support microbial performance measurements of food safety management systems. International Journal of Food Microbiology, 134, 113-125.

Jacxsens, L., Uyttendaele, M., Devlieghere, F., Rovira, J., Oses-Gomez, S. and Luning, P.A. (2010). Food safety performance indicators to benchmark food safety output of food safety management systems. International Journal of Food Microbiology, 141, S180-S187.

Jacxsens, L., Kirezieva, K., Luning, P., Ingelrham, J., Diricks, H. and Uyttendaele, M. (2015). Measuring microbial food safety output and comparing self-checking systems of food business operators in Belgium. Food Control, 49, 59-69.

James, S. J. and Evans, J.A. (2006). Predicting the reduction in microbes on the surface of foods during surface pasteurisation—the BUGDEATH project. Journal of Food Engineering, 76, 1–6.

Jay, J.M. (2000). Modern Food Microbiology. 6th ed. Aspen Publishers, Inc., Gaithersburg, Maryland.

Johnsen, B.O., Lingaas, E., Torfoss, D., Strøm, E.H. and Nordøy, I. (2010). A large outbreak of Listeria monocytogenes infection with short incubation period in a tertiary care hospital. Journal of infection, 61, 465–470.

Jones, T.F. and Angulo, F.J. (2006). Eating in restaurants: a risk factor for foodborne disease? Clinical Infectious Diseases, 43, 1324-1328.

Jones, S.L.,. Parry, S.M, O'Brien, S.J. and Palmer, S.R. (2008a). Are staff management practices and inspection risk ratings associated with foodborne disease outbreaks in the catering industry in England and Wales? Journal of Food Protection, 71, 550–557.

Jones, S.L.,. Parry, S.M, O'Brien, S.J. and Palmer, S.R. (2008b). Operational practices associated with foodborne disease outbreaks in the catering industry in England and Wales. Journal of Food Protection,71, 1659–1665.

Jongenburger, I., den Besten, H.M.W. and Zwietering, M.H. (2015). Statistical Aspects of Food Safety Sampling. Annual Review of Food Science and Technology, 6, 479–503.

Joris, M., Pierard, D. and De Zutter, L. (2011). Occurrence and virulence patterns of *E. coli* O26, O103, O111 and O145 in slaughter cattle. Veterinary Microbiology 151, 418–421.

Juneja, V.K., Eblen, B.S. and Ransom, G.M. (2001). Thermal inactivation of *Salmonella* spp. In chicken broth, beef, pork, turkey, and chicken: Determination of D- and Z-values. J FOOD SCI 66, 146–152.

Käferstein, F. (2003). Actions to reverse the upward curve of foodborne illness. Food Control 14, 101–109.

Kagkli, D.M., Vassilios, I., Stergiou, V., Lazaridou, A. and Nychas, G.J. (2009). Differential Listeria monocytogenes strain survival and growth in Katiki, a traditional greek soft cheese, at different storage temperatures. Applied and Environmental Microbiology, 75, 3621-3626.

Kells, J. and Gilmour, A. (2004). Incidence of Listeria monocytogenes in two milk processing environments, and assessment of Listeria monocytogenes blood agar for isolation. International Journal of Food Microbiology, 91, 167-174.

Kirkpatrick, B.D. and Tribble, D.R. (2011). Update on human *Campylobacter* jejuni infections. Current Opinion in Gastroenterology, 27, 1.

Kokkinakis, E., Boskou, G., Fragkiadakis, G. and Kokkinaki, A. (2008). Changes in vegetable microbiological quality introduced by processing methods. International Journal of Food Science and Technology, 43, 603–609.

Kokkinakis, E. and Fragkiadakis, G. (2007). HACCP effect on microbiological quality of minimally processed vegetables: a survey in six mass-catering establishments. I International Journal of Food Science and Technology 42, 18–23.

Kvenberg, J.E. and Schwalm, D.J. (2000). Use of microbial data for HACCP-food and drug administrative perspective. Journal of Food Protection, 63, 810-814.

Lachat, C., Van der Maas, R. and Kolsteren, P. (2010). Buitenshuis eten: een gezonde gewoonte? Voeding NU 4:15–17.

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F. and Uyttendaele, M. (2012). Microbiological performance of a food safety management system in a food service operation. Journal of Food Protection, 75, 706-716.

Lambertz, S., Nilsson, C., Bradenmark, A., Sylven, S., Johansson, A., Jansson, L.M. and Lindblad, M. (2012). Prevalence and level of Listeria monocytogenes in ready-to-eat foods in Sweden 2010. International Journal of Food Microbiology, 160, 24-31.

Legan, J.D., Vandeven, M., Dahms, S. and Cole M. (2001). Determining the concentration of micro-organisms controlled by attributes sampling plans. Food Control, 12, 137-147.

Lianou, A., Stopforth, J.D., Yoon, Y., Wiedmann, M. and Sofos, J.N. (2006). Growth and stress resistance variation in culture broth among Listeria monocytogenes strains of various serotypes and origins. Journal of Food Protection, 69, 2640–2647.

Lianou, A. and. Sofos, J.N. (2007). A review of the incidence and transmission of Listeria monocytogenes in ready-to-eat products in retail and food service environments. Journal of Food Protection, 70, 2172–2198.

Lianou, A. and Koutsoumanis, K.P. (2013). Evaluation of the strain variability of *Salmonella* enterica acid and heat resistance. Food Microbiology, 34, 259–267.

Little, C., Rhoades, J., Sagoo, S., Harris, J., Greenwood, M., Mithani, V., Grant, K. and McLauchlin, J. (2008). Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. Food Microbiology, 25, 304-312.

Little, C. and S. Sagoo. (2009). Evaluation of the hygiene of ready-to-eat food preparation areas and practices in mobile food vendors in the UK. International Journal of Environmental Health Research, 19, 431–443.

Lues, J.F.R, and Van Tonder, I. (2007). The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. Food Control, 326-332.

Lund, B.M., and O'Brien, S.J. (2009). Microbiological safety of food in hospitals and other healthcare settings. Journal of Hospital Infection, 73, 109-120.

Lund, B.M., (2014). Microbiological food safety and a low-microbial diet to protect vulnerable people. Foodborne Pathogens and Disease, 11, 413–424.

Luning, P.A., Bango, L., Kussaga, J., Rovira, J. and Marcelis, W.J. (2008). Comprehensive analysis and differentiated assessment of food safety control systems: a diagnostic instrument. Trends in Food Science & Technology, 19, 522-534.

Luning, P.A., Marcelis, W.J., Rovira, J., Van der Spiegel, M., Uyttendaele, M. and Jacxsens, L. (2009). Systematic assessment of core assurance activities in a company specific food safety management system. Trends in Food Science and Technology, 20, 300-312.

Luning, P.A., Marcelis, W.J., Rovira, J., Van Boekel, M.A., Uyttendaele, M. and Jacxsens, L. (2011). A tool to diagnose context riskiness in view of food safety activities and microbiological safety output. Trends in Food Science and Technology, 22, S67-S79.

Luning, P.A., Jacxsens, L., Rovira, J., Osés, S.M., Uyttendaele, M. and Marcelis, W.J. (2011). A concurrent diagnosis of microbiological food safety output and FSMS performance: cases from meat processing industries. Food Control, 22, 555–565.

Luning, P., Chinchilla, A., Jacxsens, L., Kirezieva, K. and Rovira, J. (2013). Performance of safety management systems in Spanish food service establishments in view of their context characteristics. Food Control 30, 331-340.

Luning, P., Kirezieva, K., Hagelaar, G., Rovira, J., Uyttendaele, M. and Jacxsens, L. (2015). Performance assessment of food safety management systems in animal-based food companies in view of their context characteristics: A European study. Food Control, 49, 11-22.

Magalhães, R., Almeida, G., Ferreira, V., Santos, I., Silva, J., Mendes, M., Pita, J., Mariano, G., Mâncio, I., Sousa, M., Farber, J., Pagotto, F. and Teixeira, P. (2015). Cheese-related listeriosis outbreak, Portugal, March 2009 to February 2012. Eurosurveillance, 20. Available online: http://www.eurosurveillance.org/images/dynamic/EE/V20N17/art21104.pdf

Manfreda, G., De Cesare, A., Stella, S., Cozzi, M. and Cantoni, C. (2005). Occurrence and ribotypes of Listeria monocytogenes in Gorgonzola cheeses. International Journal of Food Microbiology, 102, 287-293.

Martinez-Tomé, M., Vera, A. and Murcia, M. (2000). Improving the control of food production in catering establishments with particular reference to the safety of salads. Food Control 11:437–445.

Martins, E.A. and Germano, P.M.L. (2008). Microbiological indicators for the assessment of performance in the hazard analysis and critical control points (HACCP) system in meat lasagne production. Food Control, 19, 764-771.

Marzano, M.A. and Balzaretti, C.M. (2011). Cook-serve method in mass catering establishments: is it still appropriate to ensure a high level of microbiological quality and safety? Food Control, 22, 1844–1850.

Mataragas, M., Stergiou, V. and Nychas, G.J. (2008). Modeling survival of Listeria monocytogenes in the traditional Greek soft cheese Katiki. Journal of Food Protection, 71, 1835-1845.

Maudoux, J.P., Saegerman, C., Rettigner, C., Houins, G., Van Huffel, X. and Berkvens, D. (2006). Food safety surveillance through a risk based control programme: approach employed by the Belgian Federal Agency for the Safety of the Food Chain. Veterinary Q. 28, 140-154.

Mellefont, L., McMeekin, T. and Ross, T. (2008). Effect of relative inoculum concentration on Listeria monocytogenes growth in co-culture. International Journal of Food Microbiology 121, 157–168.

Metelmann, C., Schulz, K., Geldschläger-Canda, R., Plötz, S., and Handrick, W. (2010). Listeriose bei erwachsenen e fallberichte und literature-übersicht. Wiener klinische Wochenschrift, 122, 354-359.

Milillo, S.R., Friedly, E.C., Saldivar, J.C., Muthaiyan, A., O'bryan, C., Crandall, P.G., Johnson, M.G. and Ricke, S.C. (2012). A review of the ecology, genomics, and stress response of Listeria innocua and Listeria monocytogenes. Critical Reviews in Food Science and Nutrition. 52, 712–725.

Miller, F.A., Gil, M.M., Brandão, T.R.S., Teixeira, P., and Silva, C.L.M. (2009). Sigmoidal thermal inactivation kinetics of Listeria innocua in broth: Influence of strain and growth phase. Food Control, 20, 1151-1157.

Montel, M., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D., Desmasures, N., Berthier, F. (2014). Traditional cheeses: rich and diverse microbiota with associated benefits. International Journal of Food Microbiology, 177,136-54.

Moore, G. and Griffith, C. (2007). Problems associated with traditional hygiene swabbing: the need for in-house standardization. Journal of Applied Microbiology, 103, 1090-1103.

Morelli, E., Noel, V., Rosset, P., Poumeyrol, G.. (2012). Performance and conditions of use of refrigerated display cabinets among producer/vendors of foodstuffs. Food Control, 26, 363-368.

Mossel, D.A.A. (1982). Marker (index and indicator) organisms in food and drinking water. Semantics, ecology, taxonomy and enumeration. Antonie van Leeuwenhoek, 48, 609-611.

Mossel, D.A. and Struijk, C.B., (1995). Public health implication of refrigerated pasteurized ('sous-vide') foods. International Journal of Food Microbiology, 13, 187–206.

Muñoz, P., Rojas, L., Bunsow, E., Saez, E., Sánchez-Cambronero, L., Alcalá, L., Rodríguez-Creixems, M. and Bouza, E. (2011). Listeriosis: an emerging public health problem especially among the elderly. Journal of Infection, 64, 19-33.

Murphy, R.Y., Martin, E.M., Duncan, L.K., Beard, B.L. and Marcy, J.A. (2004). Thermal process validation for Escherichia coli O157:H7, *Salmonella*, and Listeria monocytogenes in ground turkey and beef products. Journal of Food Protection, 67, 1394–1402.

Murphy, R.Y., Beard, B.L., Martin, E.M., Duncan, L.K., and Marcy, J.A. (2006). Comparative study of thermal inactivation of Escherichia coli O157:H7, *Salmonella*, and Listeria monocytogenes in ground pork. Journal of Food Science, 69, FMS97-FMS101.

Neal, J.A., Binkley, M., and Henroid, D. (2012). Assessing factors contributing to food safety culture in retail food establishments. Food Protection Trends, 32, 468-476.

Nguyen, H.T., Corry, J.E. and Miles, C.A. (2006). Heat resistance and mechanism of heat inactivation in thermophilic *Campylobacter*s. Applied and Environmental Microbiology, 72, 908–913.

Nyachuba, D. G. (2010). Foodborne illness: is it on the rise? Nutrition Reviews, 68, 257–269.

O'Brien, S.J., Gillespie, I.A., Sivanesan, M.A., Elson, R., Hughes, C. and Adak, G.K., (2006). Publication bias in foodborne outbreaks of infectious intestinal disease and its implications for evidence-based food policy. England and Wales 1992-2003. Epidemiology and Infection, 134, 667-674.

Olaimat, A.N. and Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: a review. Food Microbiology, 32, 1-19.

Oliveira, M., J. Usall, I. Vina?s, M. Anguera, F. Gtius, and M. Abadias. (2010). Microbiological quality of fresh lettuce from organic and conventional production. Food Microbiology, 27, 679–684.

Osés, S.M., Luning, P.A., Jacxsens, L., Santillana, S., Jaime, I. and Rovira, J. (2012a). Microbial performance of food safety management systems implemented in the lamb production chain. International Journal of Food Microbiology, 75, 95-103.

Osés, S.M., Luning, P.A., Jacxsens, L., Santillana, S., Jaime, I., and Rovira. J. (2012b). FSMS performance in the lamb chain. Food Control, 25, 493–500.

Osimani, A., Aquilanti, L., Tavoletti, S. and Clementi, F. (2013). Evaluation of the HACCP system in a university canteen: microbiological monitoring and internal auditing as verification tools. International journal of environmental research and public health 10, X-Y. doi:10.3390/IJERPH100X000x

Painter, J.A., Ayers, T., Woodruff, R., Blanton, E., Perez, N., Hoekstra, R.M., Griffin, P.M. and Braden, C. (2009). Recipes for foodborne outbreaks: a scheme for categorizing and grouping implicated foods. Foodborne Pathogens and Diseases, 6, 1259-1264.

Panisello, P. J., & Quantick, P. C. (2001). Technical barriers to hazard analysis critical control point (HACCP). Food Control, 12, 165-173.

Pappelbaum, K., Grif, K., Heller, I., Wurzner, R., Hein, I., Ellerbroek, L. and Wagner, M. (2008). Monitoring hygiene on- and at-line is critical for controlling Listeria monocytogenes during produce processing. Journal of Food Protection, 71, 735-741.

Powell, D.A., Jacob, C.J. and Chapman, B.J. (2011). Enhancing food safety culture to reduce rates of foodborne illness. Food Control 22, 817-822.

Prencipe, V., Migliorati, G., Matteucci, O., Calistri, P. and Di Giannatale, E. (2010). Assessment of hygienic quality of some types of cheese sampled from retail outlets. Veterinaria italiana, 46, 233-242.

Quinn, B.P. and Marriott, N.G. (2002). HACCP plan development and assessment: a review. Journal of Muscle Foods, 13, 313-330.

Rayner, J., R. Veeh, and J. Flood. (2004). Prevalence of microbial biofilms on selected fresh produce and household surfaces. International Journal of Food Microbiology, 95, 29–39.

Roccato, A., Uyttendaele, M., Cibin, V., Barrucci, F., Cappa, V., Zavagnin, P., Longo, A. and Ricci, A. (2015). Survival of *Salmonella* typhimurium in poultry-based meat preparations during grilling, frying and baking. International Journal of Food Microbiology, 197, 1–8.

Rodriguez, M., Valero, A., Carrasco, E., Pérez-Rodríguez, F., Posada, G. D. and Zurera, G. (2011). Hygienic conditions and microbiological status of chilled ready-to-eat products served in southern Spanish hospitals. Food Control, 22, 874-882.

Ropkins, K., and A. J. Beck. (2000). Evaluation of worldwide approaches to the use of HACCP to control food safety. Trends in Food Science & Technology, 11, 10–21.

Ross, R., Stanton, C., Hill, C., Fitzgerald, G. and Coffey, A. (2000). Novel cultures for cheese improvement. Trends in Food Science and Technology, 11, 96-104.

Ross, T., Fratamico, P.M., Jaykus, L. and Zwietering, M.H. (2011). Statistics of sampling for microbiological testing of foodborne pathogens. In Rapid Detection, Characterization, and Enumeration of Foodborne Pathogens, ed. J.Hoorfar, pp. 103–20. Washington, DC: ASM Press

Rosshaug, P.S., Detmer, A., Ingmer, H. and Larsen, M.H. (2012). Modeling the growth of Listeria monocytogenes in soft blue-white cheese. Applied and Environmental Microbiology, 78, 8508-8514.

Rudolf, M. and Scherer, S. (2001). High incidence of Listeria monocytogenes in European red smear cheese. International Journal of Food Microbiology, 63, 91-98.

Sampers, I., Jacxsens, L., Luning, P.A., Marcelis, W.J., Dumoulin, A. and Uyttendaele, M. (2010). Performance of food safety management systems in poultry meat preparation processing plants in relation to *Campylobacter* spp. contamination. Journal of Food Protection, 73, 1447–1457.

Sampers, I., Toyofuku, H., Luning, P.A., Uyttendaele, M. and Jacxsens, L., (2011). Semiquantitative study to evaluate the performance of a HACCP-based food safety management system in Japanese milk processing plants. Food Control, 23, 227-233.

Sampers, I., Berkvens, D., Jacxsens, L., Ciocci, M.C., Dumoulin, A. and Uyttendaele, M. (2012). Survey of Belgian consumption patterns and consumer behaviour of poultry meat to provide insight in risk factors for *Campylobacter*iosis. Food Control, 26, 293–299.

Santorum, P., Garcia, R., Lopez, V. and Martinez-Suarez, J. (2012). Review. Dairy farm management and production practices associated with the presence of Listeria monocytogenes in raw milk and beef. Spanish Journal of Agricultural Research, 10, 360-371.

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M., (2011). Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases journal, 17, 16–22.

Scallan, E., Hoekstra, R., Mahon, B., Jones, T. and Griffin, P., (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. Epidemiol. Infect., 143, 2795–2804.

Schaffner, D. W., S. Sithole, and R. Montville. (2004). Use of microbial modeling and Monte-Carlo simulation to determine microbial performance criteria on plastic cutting boards in use in foodservice kitchens. Food Protection Trends, 24, 14–19.

Schoder, D., Zangana, A., Paulsen, P., Winter, P., Balimgartner, W. and Wagner, M. (2008). Ovine Listeria monocytogenes mastitis and human exposure via fresh cheese from raw milk - the impact of farm management, milking and cheese manufacturing practices. Milchwissenschaft-Milk Science International, 63, 258-262.

Schoder, D., Melzner, D., Schmalwieser, A., Zangana, A., Winter, P. and Wagner, M. (2011). Important Vectors for Listeria monocytogenes Transmission at Farm Dairies Manufacturing Fresh Sheep and Goat Cheese from Raw Milk. Journal of Food Protection, 74, 919-924.

Schoder, D., Skandamis P. and Wagner M. (2013). Assessing in-house monitoring efficiency by tracing contamination rates in cheese lots recalled during an outbreak of listeriosis in Austria. International Journal of Food Microbiology, 167, 353–358.

Schvartzman, M. S., Maffre, A., Tenenhaus-Aziza, F., Sanaa, M., Butler, F. and Jordan, K. (2011). Modelling the fate of Listeria monocytogenes during manufacture and ripening of smeared cheese made with pasteurised or raw milk. International Journal of Food Microbiology, 145, 31-38.

Schvartzman, M., Gonzalez-Barron, U., Butler, F., Jordan, K. (2014). Modeling the growth of Listeria monocytogenes on the surface of smear- or mold-ripened cheese. Frontiers in Cellular and Infection Microbiology, 4.

Seward, S. (2000). Application of HACCP in food service. Irish Journal of Agricultural and Food Research, 39, 221–227.

Shojaei, H., Shooshtaripoor, J. and. Masoud, A. (2006). Efficacy of simple hand-washing in reduction of microbial hand contamination of Iranian food handlers. Food Research International, 39, 525–529.

Simoes, M., Simoes, L.C. and Vieira, M.J. (2010). A review of current and emergent biofilm control strategies. Food Science and Technology, 43, 573–583.

Smelt, J.P. and Brul, S. (2014). Thermal inactivation ofmicroorganisms. Critical Reviews in Food Science and Nutrition, 54, 1371–1385.

Smith, B.A., Fazil, A. and Lammerding, A.M. (2013). A risk assessment model for Escherichia coli O157:H7 in ground beef and beef cuts in Canada: evaluating the effects of interventions. Food Control, 29, 364–381.

Söderström, A., Österberg, P., Lindqvist, A., Jönsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Löfdahl, S., Kaijser, B. and De Jong, B., (2008). A large Escherichia coli O157 outbreak in Sweden associated with locally produced lettuce. Foodborne Pathogens and Diseases, 5, 339–349.

Soon, J.M., Seaman, P. and Baines, R.N. (2013). Escherichia coli O104:H4 outbreak from sprouted seeds. International Journal of Hygiene and Environmental Health, 216, 346-354.

Sorqvist, S. (2003). Heat resistance in liquids of Enterococcus spp., Listeria spp., Escherichia coli, Yersinia enterocolitica, *Salmonella* spp. and *Campylobacter* spp. Acta Veterinaria Scandinavica, 44, 1-19.

Stals, A., Jacxsens, L., Baert, L., Van Coillie, E. and Uyttendaele, M. (2015). A quantitative exposure model simulating human norovirus transmission during preparation of deli sandwiches. International Journal of Food Microbiology, 196, 126-136.

Stephens, P., Joynson, J., Davies, K., Holbrook, R., Lappin-Scott, H. and Humphrey, T. (1997). The use of an automated growth analyser to measure recovery times of single heat injured *Salmonella* cells. Journal of Applied Microbiology, 83, 445-455.

Stollewerk, K., Jofre, A., Comaposada, J., Arnau, J. and Garriga, M. (2012). The effect of NaCl-free processing and high pressure on the fate of Listeria monocytogenes and *Salmonella* on sliced smoked dry-cured ham. Meat Science, 90, 472-477.

Straver, J.M., Janssen, A.F.W., Linnemann, A.R., van Boekel, M.A.J.S., Beumer, R.R. and Zwietering, M.H. (2007). Number of *Salmonella* on chicken breast filet at level and its implications for public health risk. Journal of Food Protection, 70, 2045–2055.

Strohbehn, C., Sneed, J., Paez, P. and Meyer, J. (2008). Hand washing frequencies and procedures used in retail food services. Journal of Food Protection, 71, 1641–1650.

Sulzer, G. and Busse, M. (1991). Growth inhibition of Listeria spp. on Camembert cheese by bacteria producing inhibitory substances. International Journal of Food Microbiology, 14, 287-296.

Swanson, K. M. J. and Anderson, J.E. (2000). Industry perspectives on the use of microbial data for hazard analysis and critical control point validation and verification. Journal of Food protection, 63, 815–818.

Takhar, P.S., Head, K.L., Hendrix, K.M. and Smith, D.M., (2009). Predictive modeling of *Salmonella* species inactivation in ground pork and turkey during cooking. International Journal of Food Engineering, 5, 1–11.

Tamplin, M.L. (2002). Growth of Escherichia coli O157:H7 in raw ground beef stored at 10 °C and the influence of competitive bacterial flora, strain variation, and fat level. Journal Food Protection, 665, 1535–1540.

Tan, A., Beaton, S., Dimovski, K., Hogg, G., di Paola, V. and Haines, H. (2008). Pathogen survival in portioned retail soft cheeses. The Australian Journal of Dairy Technology, 63,

Tanik, M. (2010). Improving ‘‘order handling’’ process by using QFD and FMEA methodologies: a case study. International Journal of Quality & Reliability Management, 27, 404–423.

Taylor, E., Herman, K., Ailes, E., Fitzgerald, C., Yoder, J., Mahon, B. and Tauxe, R. (2012). Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. Epidemiology and Infection, 1, 1–10.

Tiwari, U., Walsh, D., Rivas, L., Jordan, K. and Duffy, G. (2014). Modelling the interaction of storage temperature, pH, and water activity on the growth behaviour of Listeria monocytogenes in raw and pasteurised semi-soft rind washed milk cheese during storage following ripening. Food Control, 42, 248-256.

Todd, E.C.D. and Notermans, S. (2011). Surveillance of listeriosis and its causative pathogen, Listeria monocytogenes. Food Control, 22: 1484–1490.

Tompkin, R. B. (2002). Control of Listeria monocytogenes in the food-processing environment. Journal of Food Protection, 65, 709-725.

Tompkin, R.B. (2004). Environmental sampling – a tool to verify the effectiveness of preventive hygiene measures. Mitt. Lebensm. 95, 45-51.

Tuntivanich, V., Orta-Ramirez, A., Marks, B.P., Ryser, E.T. and Booren, A.M., (2008). Thermal inactivation of *Salmonella* in whole muscle and ground turkey breast. Journal Food Protection, 71, 2548–2551.

Tuominen, P. and Maijala, R. (2009). Evaluating food safety management in professional kitchens. Archiv für Lebensmittelhygiene, 60, 151–160.

Tzschoppe, M., Martin, A. and Beutin, L. (2012). A rapid procedure for the detection and isolation of enterohaemorrhagic Escherichia coli (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. International Journal of Food Microbiology, 152, 19-30.

USDA (United States Department of Agriculture), 2003. Color of Cooked Meat as it Relates to Doneness. Food Safety and Inspection Service, United States Department of Agriculture, technical publication Available online: http://www.fsis.usda.gov/OA/pubs/colortech.htm

U.S. Food Safety and Inspection Service (USFSIS). (1998). Performance Standards for the Production of Certain Meat and Poultry Products. Federal Register, 64 (3), 732-749.

Uyttendaele, M., de Troy, P. and Debevere, J. (1999). Incidence of *Salmonella*, *Campylobacter* jejuni, *Campylobacter* coli, and Listeria monocytogenes in poultry carcasses and different types of poultry products for sale on the Belgian retail market. Journal of Food Protection 7, 711–829.

Uytendaele, M., Rajkovic, A., Benos, G., Francois, K., Devlieghere, F. and Debevere, J. (2004). Evaluation of a challenge testing protocol to assess the stability of ready-to-eat cooked meat products against growth of Listeria monocytogenes. International Journal of Food Microbiology, 90, 219-236.

Uyttendaele, M., Busschaert, P., Valero, A., Geeraerd, A. H., Vermeulen, A., Jacxsens, L., Goh K.K., De Loy, A., Van Impe, J.F. and Devlieghere, F. (2009). Prevalence and challenge tests of Listeria monocytogenes in Belgian produced and retailed mayonnaise-based deli-salads, cooked meat products and smoked fish between 2005 and 2007. International Journal of Food Microbiology, 133, 94-104.

Uyttendaele, M., L. Jacxsens, A. De Loy-Hendrickx, F. Devlieghere, and J. Debevere. (2010). Microbiologische richtwaarden en wettelijke microbiologische criteria. Laboratory of Food Microbiology and Food Preservation, University of Ghent, Ghent, Belgium.

Valero, A., Hernandez, M., De Cesare, A., Manfreda, G., Gonzalez-García, P. and Rodríguez-L'azaro, D. (2014). Survival kinetics of Listeria monocytogenes on raw sheep milk cured cheese under different storage temperatures. International Journal of Food Microbiology, 184, 39-44.

van Asselt, E.D. and Zwietering, M.H., (2006). A systematic approach to determine global thermal inactivation parameters for various food pathogens. International Journal of Food Microbiology, 107, 73–82.

Van Boxstael, S., Devlieghere, F., Berkvens, D., Vermeulen, A. and Uyttendaele, M. (2014). Understanding and attitude regarding the shelf life labels and dates on pre-packed food products by Belgian consumers. Food Control, 37, 85-92.

Van Coillie, E., Werbrouck, H., Heyndrickx, M., Herman, L. and Rijpens, N. (2004). Prevalence and typing of Listeria monocytogenes in ready-to-eat food products on the Belgian market. Journal of Food Protection, 67, 2480-2487.

Van Laack, R.L.J.M., Berry, B.W. and Solomon, M.B. (1996). Variations in Internal color of cooked beef patties. Journal Food Science, 61, 410–414.

Van Schothorst, M., Zwietering, M.H., Ross, T., Buchanan, R.L. and Cole, M.B. (2009). Relating microbiological criteria to food safety objectives and performance objectives. Food Control 20, 967-979.

Vandekinderen, I., Van Camp, J., Devlieghere, F., Veramme, K., Bernaert, K., Denon, Q., Ragaert, P. and De Meulenaer, B. (2009). Effect of decontamination on the microbial load, the sensory quality and the nutrient retention of ready-to-eat white cabbage. European Food Research and Technology, 229, 443–455.

Vandevijvere, S., Lachat, C., Kolsteren, P. and Van Oyen, H. (2009). Eating out of home in Belgium: current situation and policy implications. British Journal of Nutrition, 102, 921–928.

Vasan, A., Geier, R., Ingham, S.C. and Ingham, B.H. (2014). Thermal tolerance of O157 and non-O157 Shiga toxigenic strains of Escherichia coli, *Salmonella*, and potential pathogen surrogates, in frankfurter batter and ground beef of varying fat levels. Journal of Food Protection, 77, 1501-1511.

Vermeulen, A., Devlieghere, F., De Loy-Hendrickx, A. and Uyttendaele, M. (2011). Critical evaluation of the EU-technical guidance on shelf life studies for *L. monocytogenes* on RTE-foods: a case study for smoked salmon. International Journal of Food Microbiology, 145, 176-185.

Verraes, C., Vlaemynck, G., Van Weyenberg, S., De Zutter, L., Daube, G., Sindic, M., Uyttendaele, M. and Herman, L. (2015a). A review of the microbiological hazards of dairy products made from raw milk. International Dairy Journal, 50, 32-44.

Verraes, C., Uyttendaele, M., Clinquart, A., Daube, G., Sindic, M., Berkvens, D. and Herman, L. (2015b). Microbiological safety and quality aspects of the short supply chain: SWOT analysis of the Belgian case study. British Food Journal, 117, 2250-2264.

Vitullo, M., Ripabelli, G., Fanelli, I., Tamburro, M., Delfine, S., Sammarco, M., (2011). Microbiological and toxicological quality of dried herbs. Letters in Applied Microbiology, 52, 573-580.

VLAM (Vlaams Centrum voor Agro- en Visserijmarketing vzw). (2014). Thuisverbruik van vers vlees in België in 2013. Available online: http://www.vlam.be/public/uploads/files/feiten_en_cijfers/vlees/thuisverbruik_vlees_en_vleeswaren_2013.pdf

Wagner, M., Auer, B., Trittremmel, C., Hein, I., Schoder, D. (2007). Survey on the *Listeria contamination* of ready-to-eat food products and household environments in Vienna, Austria. Zoonoses and public health, 54, 16-22.

Walker, E., Pritchard, C. and Forsythe, S. (2003). Food handlers' hygiene knowledge in small food businesses. Food Control, 14, 339–343.

Whittington, R.J. and Waldron, A. (2010). Thermal profiles of domestically baked leg-of-lamb roasts and implications for microbiological safety. Food Control 21, 1087–1091.

Wiegand, K.M., Ingham, S.C. and Ingham, B.H. (2009). Survival of Escherichia coli O157:H7 in ground beef after sublethal heat shock and subsequent isothermal cooking. Journal of Food Protection, 72, 1727–1731.

Wilson, E.B. (1927). Probable Inference, the Law of Succession, and Statistical Inference. Journal of the American Statistical Association, 22, 209-212.

Worsfold, D. and Griffith, C.J. (2003). A survey of food hygiene and safety training in the retail and catering industry. Nutrition and Food Science, 33, 68–79.

Worsfold, D. (2001). A guide to HACCP and function catering. Journal of the Royal Society for the Promotion of Health, 121, 224-229.

Yde, M., Naranjo, M., Mattheus, W., Stragier, P., Pochet, B., Beulens, K., De Schrijver, K., Van den Branden, D., Laisnez, V., Flipse, W., Leclercq, A., Lecuit, M., Dierick, K., Bertand, S. (2012). Usefulness of the European Epidemic Intelligence Information System in the Management of an Outbreak of Listeriosis, Belgium, 2011. Eurosurveillance, 17. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20279

Zwietering, M.H., Ross, T. and Gorris, L.G.M. (2014). Microbiological testing, sampling plans, and microbiological criteria. In Encyclopedia of Food Safety, Vol. 4, ed. YMotarjemi,MA, Waltham, pp. 244–53. Waltham, MA:

Zwietering, M.H., Jacxsens, L., Membre, J.M., Nauta, M. and Peterz, M. (2016). Relevance of microbial finished product testing in food safety management. Food Control, 60, 31-43.

CURRICULUM VITAE

Evy Lahou was born on the 22nd of January 1986 in Diest, Belgium. She obtained her high school degree in Science and Mathematics at Voorzienigheid Diest in 2004 and graduated as a Master of Science in Biomedical Sciences at Ghent University in 2009. In October 2009, she started as an assistant at the Laboratory of Food Microbiology and Food Preservation (LFMFP) at the faculty of Bioscience Engineering at Ghent University under the supervision of Prof. dr. ir. Mieke Uyttendaele (promoter) and Prof. dr. ir. Liesbeth Jacxsens (co-promoter). The research is dealing with sampling and microbiological analysis as an indicator of effective food safety management. As such, she gathered knowledge of different aspects of microbiological analysis (i.e. methods and sampling), microbiological safety (i.e. pathogens and food products) and quality management.

During this research she participated in various national and international conferences and published in international peer reviewed journals. Furthermore, she guided several students with their master thesis and she provided educational support during practical and theoretical course sessions.

Evy Lahou is geboren op 22 januari 1986 te Diest. In 2004 behaalde zij het diploma Wetenschappen-Wiskunde aan de Voorzienigheid Diest. Vijf jaar later promoveerde zij tot Meester in de exacte wetenschappen (MSc) in de Biomedische Wetenschappen aan de Universiteit Gent. In oktober 2009 startte zij als assistent aan het Laboratorium voor Levensmiddelenmicrobiologie en –conservering van de faculteit Bio-ingenieurswetenschappen verbonden aan de Universiteit Gent onder begeleiding van Prof. dr. ir. Mieke Uyttendaele (promoter) en Prof. dr. ir. Liesbeth Jacxsens (co-promoter). Haar onderzoek betreft monstername en microbiologische analyse als indicatoren voor een efficiënt voedselveiligheid managementsysteem. Hierdoor verkreeg zij inzicht in de verschillende aspecten van een microbiële analyse (m.a.w. microbiologische methoden en monstername), microbiologische veiligheid (m.a.w. ziekteverwekkende micro-organismen en levensmiddelen) en kwaliteitsbeheer.

Tijdens dit onderzoek nam zij deel aan verscheidene nationale en internationale conferenties en publiceerde zij wetenschappelijke artikels in internationale tijdschriften. Tevens begeleidde zij verscheidene studenten in het behalen van hun masterthesis en ondersteunde zij de onderwijsactiviteiten van het laboratorium.

Publications in A1 peer-reviewed journals

Lahou, E. and Uyttendaele, M. (2016). Growth potential of *Listeria monocytogenes* in soft, semi-soft and semi-hard artisanal cheeses. *Submitted for publication in International Journal of Food Microbiology*

Wang, X., **Lahou, E**., De Boeck, E., Devlieghere, F., Geeraerd, A., Uyttendaele, M. (2015). Growth and inactivation of *Salmonella enterica* and *Listeria monocytogenes* in broth and validation in ground pork meat during simulated home storage abusive temperature and home pan-frying. *Frontiers in Microbiology* 6:1161. doi:10.3389/fmicb.2015.01161

Lahou, E., Wang, X., De Boeck, E., Verguldt, E., Geeraerd, A., Devlieghere, F. and Uyttendaele, M. (2015). Effectiveness of inactivation of foodborne pathogens during simulated home pan frying of steak, hamburger or meat strips. *International Journal of Food Microbiology* 206, 115-129.

Lahou, E., Jacxsens, L., Verbunt, E. and Uyttendaele, M. (2015). Evaluation of the food safety management system in a hospital food service operation towards *Listeria monocytogenes. Food Control* 49, 75-84.

Lahou, E., Jacxsens, L., Van Landeghem, F., and Uyttendaele, M. (2014). Microbiological sampling plan based on risk classification to verify supplier selection and production of served meals in food service operation. *Food Microbiology* 41, 60-75

Lahou, E. and Uyttendaele, M. (2014). Evaluation of three swabbing devices for detection of *Listeria monocytogenes* on different types of food contact surfaces. *Int. J. Environ. Res. Public Health* 11, 804-814.

Daelman, J., Jacxsens, L., **Lahou, E**., Devlieghere, F., and Uyttendaele, M. (2013). Assessment of the microbial safety and quality of cooked chilled foods and their production process. *International journal of food microbiology* 160, 93-200.

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F., and Uyttendaele, M. (2012). Microbiological performance of a food safety management system in a food service operation. *Journal of food protection* 75, 706-716

Extended abstracts of symposia

Lahou, E., Uyttendaele, M. (2015). Growth potential of *Listeria monocytogenes* in soft and semi-hard artisanal cheeses. Oral presentation. BSFM Twentieth Conference on Food Microbiology, October 8-9, Brussels, Belgium.

Lahou, E., Uyttendaele, M. (2015). Growth potential of *Listeria monocytogenes* in soft and semi-hard artisanal cheeses. Oral presentation. IAFP European symposium on food safety, April 20-22 , Cardiff, Wales.

Lahou, E., Jacxsens, L., Carlier, E., Uyttendaele, M. (2012). Impact of the slow cooking technique on the microbiological safety of foods. Poster presentation. BSFM Seventeenth Conference on Food Microbiology, September 20-21, Brussels, Belgium.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2012). The development and elaboration of a risk-based sampling plan to control *Listeria monocytogenes* in a hospital food service operation. Oral presentation. IAFP Annual meeting, July 22-25, Providence, Rhode Island, USA.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2012). The evaluation of the food safety management system of a hospital food service operation towards *L. monocytogenes*. Oral presentation. Food Safety Management 2012, June 19-20, Chipping Campden, United Kingdom.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2012). Evaluation of three swabs-types for recovery of *Listeria monocytogenes* on different food contact surfaces. Oral presentation. IAFP European symposium on Food Safety, May 21-23, Warschau, Poland.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2012). Risk-based sampling plan to control *Listeria monocytogenes* in a hospital food service operation. *Communications in agricultural and applied biological sciences* 77, 51-54. Oral presentation. 18th PhD Symposium on Applied Biological Sciences, February 10, Leuven, Belgium.

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F., Uyttendaele M. (2011). Development of a horizontal MAS and a vertical MAS for institutional catering to measure microbiological performance of the food safety management system. Poster presentation. BSFM Sixteenth conference on Food Microbiology, September 22-23, Brussels, Belgium.

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F., Uyttendaele, M. (2011). Development of a horizontal MAS and a vertical MAS for institutional catering to measure microbiological performance of the food safety management system. Poster presentation. IAFP Annual meeting, July 31 – August 3, Milwaukee, Wisconsin, USA.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2011). Evaluating food safety management performance in a food service establishment according a microbiological assessment scheme. IAFP European symposium on food safety, May 18-20, Ede, The Netherlands.

Daelman, J., Jacxsens, L., **Lahou, E**., Uyttendaele, M. (2010) A microbial assessment scheme of the cooked chilled food production. *Communications in agricultural and applied biological sciences* 76,

119-123. Oral presentation. 16th PhD Symposium on Applied Biological Sciences, December 20, Ghent, Belgium.

Lahou, E., Jacxsens, L., Daelman, J., Van Landeghem, F., Stals, A., Uyttendaele, M. (2010). Evaluating food safety management performance in a food service establishment according a microbiological assessment scheme. Poster presentation. 16th PhD Symposium on Applied Biological Sciences, December 20, Ghent, Belgium.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2010). Assessing food safety management performance in a catering establishment according a microbiological assessment scheme. Poster presentation. BSFM Fifteenth Conference on Food Microbiology, September 16-17, Ghent, Belgium.

Dissemination

Lahou, E., Jacxsens, L., Uyttendaele, M. (2010). Case studie: staalnameplan in de catering. Oral presentation. Open opleiding " Onderbouwing microbiologische staalnameplannen en hun interpretatie in het kader van validatie en verificatie van kwaliteitszorgsystemen, December 9, Ghent, Belgium.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2011). *Listeria monocytogenes* in grootkeukens. Oral presentation. PR-event Johnson Diversey, May 6, Diegem, Belgium.

Lahou, E., Uyttendaele, M. (2012). Classical methods in food microbiology: the reference methods. Oral presentation. Opleiding Life Technologies, November 13, Ghent, Belgium.

Lahou, E., Jacxsens, L., Uyttendaele, M. (2012). Environmental sampling for detection of *Listeria monocytogenes* in food processing area. BSFM Seventeenth Conference on Food Microbiology, September 20-21, Brussels, Belgium.

Doctoral schools program

Specialist courses
2010 Kwaliteitsbeheer en risicoanalyse
2012 Intensive training on Mycotoxin Analysis
2014 Statistics - Nonparametric Methods

Personal skills training
2011 Populair-wetenschappelijk schrijven
2011 Personal Effectiveness
2012 Advanced Academic English: Writing Skills
2012 Basisassistententraining
2012 Het Feedbackgesprek
2013 FLAMES Summer School in Methodology and Statistics

Supervision of undergraduate students

AJ 2010-2011 *Ellen Verbunt*
Risico-gebaseerd monsternameplan voor *Listeria monocytogenes* in grootkeukens van ziekenhuizen.

AJ 2011-2012 *Evelien Carlier*:
Consumptiedata van groenten en fruit, identificatie van trends en hun impact op de microbiologische veiligheid.
Paulien Vanhalst:
Alternatieve methoden voor de detectie van *Listeria monocytogenes* in vis visserijproducten en omgevingsmonsters.

AJ 2012-2013 *Elien De Boeck*:
Verificatie van hitte-inactivatie van pathogenen in broth en vleesproducten

AJ 2013-2014 *Hanne Van Yperzele*:
Groeipotentieel van *Listeria monocytogenes* in zachte en half-harde ambachtelijke kazen

AJ 2014-2015 *Michel Verhagen*:
Microbiologische kwaliteit en veiligheid van maaltijdsalades