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# Artisanal food production and consumer behaviour: impact on microbial food safety

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In 2012 it was my first time in Ghent, a beautiful city where you can still breathe the medival past and at the same time a vivid and active present. Since then, I bound myself to this city of east Flanders and at the end of 2013 I started to be a PhD student at Ghent University.
This PhD thesis is the result of four years of intensive work supported by the people I met during those years. A PhD is not just being focus on your scientific research but it is thanks to the scientific research that you go through a process of life that allows you to interact with different people and put question marks on your certainties and leads you to develop a critical mind, which becomes part of a way of thinking and acting. Therefore, I want to thank all the people that I met during those years.

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## List of abbreviations

| | |
|---|---|
| AIC | Akaike Information Criterion |
| ALOA | Agar Listeria Ottaviani Agosti |
| ALOP | Appropriate Level of Protection |
| APC | Aerobic Plate Count |
| $a_w$ | Water activity |
| BE | Belgium |
| BHI | Brain Heart Infusion |
| BPW | Buffered Pepton Water |
| CA | Competent Authority |
| CFU | colony forming units |
| DAEC | Diffusely Adherent *Escherichia coli* |
| DALYs | Disability Adjusted Life Years |
| DEFeRA | Department for Environment Food and Rural Affairs |
| DNA | deoxyribonucleic acid |
| EAEC | Enteroaggregative *Escherichia coli* |
| EB | *Enterobacteriaceae* |
| EC | European Commission |
| ECDC | European Centre for Disease Prevention and Control |
| EFSA | European Food Safety Authority |
| EHEC | Enterohaemorrhagic *Escherichia coli* |
| EIEC | Enteroinvasive *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| ETEC | Enterotoxigenic *Escherichia coli* |
| EU | European Union |

| | |
|---|---|
| EURL | European Union Reference Laboratory |
| EURL-Lm | European Union Reference Laboratory for *Listeria monocytogenes* |
| EuroFIR FP6 | European Food Information Resource Framework Program 6 |
| FASFC SciCom | Scientific Committee of the Belgian Agency for the Safety of the Food Chain |
| FBO | Food Business Operator |
| FR | France |
| FSA | Food Standard Agency |
| FSAI | Food Safety Authority of Ireland |
| FSMS | Food Safety Management System |
| GHP | Good Hygienic Practices |
| GMP | Good Manufacturing Practices |
| GR | Greece |
| HACCP | Hazard Analysis Critical Control Point |
| HUS | Haemolitic Uremic Syndrome |
| IQR | Inter Quartile Range |
| IR | Ireland |
| ISO | International Organization for Standardization |
| IT | Information Technology |
| IT | Italy |
| IZSVe | Istituto Zooprofilattico Sperimentale delle Venezie |
| LAB | Lactic Acid Bacteria |
| LFS | Local Food System |
| LOD | Limit of Detection |
| MPN | Most Probable Number |
| NACMF | National Advisory Committee on Microbiological Criteria for Foods |

| | |
|---|---|
| NL | Netherlands |
| NOFIMA | Norwegian Institute of Food, Fisheries and Aquaculture Research |
| P | Portugal |
| PCR | Polymerase Chain Reaction |
| PDO | Protected Designation of Origin |
| PGI | Protected Geographical Indication |
| PRPs | Pre Requisite Programs |
| PSS | Physiological Saline Solution |
| QMRA | Quantitative Microbiological Risk Assessment |
| REPFED | Refrigerated Processed Food of Extended Durability |
| RMSE | Root Mean Squared Error |
| RTE | Ready To Eat |
| SD | Standard Deviation |
| SFSC | Short Food Supply Chain |
| SME | Small and Medium Enterprises |
| SOPs | Standard Operating Procedures |
| SP | Spain |
| STEC | Shiga Toxin-Producing *Escherichia coli* |
| *stx* | Shiga toxin-coding gene |
| SW | Sweden |
| UK | United Kingdom |
| US | United States |
| USDA | United States Department of Agriculture |
| VTEC | Verocytotoxin Producing *Escherichia coli* |
| WHO | World Health Organisation |

# OBJECTIVES AND OUTLINE

Food-borne diseases are a source of concern for public health as well as socio-economic implications. Bacteria and viruses are the most common cause of food poisoning, both in developing and industrialized countries (Havelaar et al., 2015). In the EU, 4,362 food-borne outbreaks, including waterborne outbreaks, were reported in 2016. Decreasing EU trend for confirmed human salmonellosis cases since 2008 continued, but the proportion of foodborne cases related to other foodborne pathogens (e.g. *Campylobacter*, *Listeria monocytogenes*) increased. (EFSA & ECDC, 2016).

Since 2000, first with the White Paper and subsequently with EC Regulation 178/2002, the approach adopted by the European legislation in relation to food safety has been the strategy "from farm to fork", which attributed the main responsibility of food safety to the Food Business Operator (FBO). In order to manage safety within the production, processing and distribution of food, the application of a self-checking system based upon good hygienic practices along with the HACCP (hazard analysis and critical control point) principles and the use of process hygiene and food safety criteria for verification of well-functioning of the preventive approach, are the main tools available for the FBOs. However, these tools are used only until the consumer purchases the food product. After purchase, it is the consumer who decides how to store and handle foods.

Household is by far the most frequent place of exposure in case of strong-evidence outbreaks and *Salmonella* is strongly associated with the domestic environment (EFSA & ECDC, 2016). Often outbreaks occurring in a household setting are associated with the one or more of the most common faults in domestic food hygiene practices, such as inappropriate storage, inadequate cooking, and/or cross-contamination (Redmond & Griffith, 2003). However, also other factors might contribute to the occurrence of food-borne illness outbreaks at home. Most food is prepared at home, thus in a non-professional setting, thereby increasing the likelihood for food handling mistakes to occur. In addition, most consumers consider the domestic environment (home) as a safe place (optimistic bias) (Byrd-Bredbenner, Berning, Martin-Biggers, & Quick, 2013), thus underestimating the role of personal handling of products in contamination in the domestic environment.

Apart from consumer behaviour at home, changing consumer's choices in terms of food type and food composition and trends towards natural and local foods may have an impact on food safety as well. In particular, there is an increased demand for ready-to-eat food products, minimally processed but still with prolonged shelf-life (Daelman, Jacxsens, Devlieghere, & Uyttendaele, 2013). These are often mildly processed foods with no or few preservatives such as pre-packed cooked meat products, bagged fresh-cut salads, soft

cheeses, smoked fish, etc., which mainly rely on the respect of the cold chain to ensure safety and quality of the product until the end of the shelf-life. In addition, these products have a high risk for *L. monocytogenes* and can be a source of human listeriosis, which is showing an increasing trend in EU despite legislation and efforts by the food industry to decrease the occurrence of the pathogen in foods (EFSA & ECDC, 2016).

Moreover, there is an increasing food market segmentation with consumers asking for food products as organic food, artisanal food, often bought at the farm or local markets (Falguera, Aliguer, & Falguera, 2012), which are seen as places to buy 'good food' but also as means to express consumer values associated with food choices (e.g. resource conservation, animal welfare, the revival of a sense of community, enjoyment of cooking) (Guerrero et al., 2010). Little information is available on the safety of these 'terroir' products, mainly marketed by micro or small-scale producers, *versus* the conventional food supply chain. Due to the lack of expertise and financial resources, specialization towards food safety management is mostly not possible (Ball, Wilcock, & Aung, 2010; Verraes, Uyttendaele, et al., 2015). Consequently, these micro and small-scale producers are sometimes struggling with the complexity of the food safety management rules.

Therefore, in order to reach an appropriate level of protection, the competent authority (CA), should combine and balance the above-mentioned aspects in their efforts to ensure food safety. A deepened knowledge of the impact of consumer behaviour could be useful in order to address better food safety controls. Moreover, in many regions of Europe, competent authorities seek to develop a collaborative approach with farmer associations or FBOs to develop tools or guidelines to provide support. In particular, this proactive and collaborative approach has been directed to enhance food safety of the micro and small-scale producers producing artisanal food, increasingly preferred by consumers.

Bearing in mind the above-mentioned considerations, the **objectives** of the present **PhD** study were the following:

1) **Identification of tools and suggestions to support and enhance food safety during small-scale production of artisanal food.** As a case-study, the production process of an artisanal fermented meat product (salami & soppresse) of the Veneto region in the north of Italy was considered. The associated microbiological hazards were identified and the extension services undertaken by the regional competent authority to facilitate the set-up and implementation of an appropriate food safety management system for these small-scale producers were investigated (**CHAPTER 2**). In addition, the food hygiene knowledge and practices of these small-scale producers of artisanal salami and soppresse, as a result of

the aforementioned collaborative approach between producers and the regional competent authority was evaluated in a follow-up study (**CHAPTER 3)**.

2) **Time-temperature abuse at retail and consumer level: how to deal with it?** According to EU legislation, FBOs are responsible of the products they put on the market and have to guarantee their safety. The safety has to be assured for the entire shelf-life of the product and in plausible conditions of storage and use. This means that in order to establish the safety of a food product, the FBO has to take into account plausible conditions of time-temperature abuse that can occur along the food chain, especially at consumer level. In **CHAPTER 4**, the effect of temperature abuse was studied in several types of artisanal raw milk cheeses artificially (challenge tests) or naturally (durability studies) contaminated with L. monocytogenes. Moreover, in order to improve the effectiveness of challenge tests (which can be useful for shelf-life determination) and public health risk assessment, simplified probability distributions of domestic refrigerator temperatures for EU countries and of storage times of chilled food are provided in **CHAPTER 5**.

3) **Study the effect of the consumer food handling practices in the domestic environment**. The domestic environment represents one of the main setting for food-borne outbreaks. Therefore, the knowledge of the effect of consumer's behaviour on microbial growth, survival or death in foods is of paramount importance in order to appropriately define label instructions on packed food, address educational campaigns on food safety and to implement risk assessment models in the framework of risk analysis. Consequently, different types of poultry-based meat preparations were prepared in the lab to mimic home preparation (grilling, pan frying, oven baking) (**CHAPTER 6**) or subjected to domestic storage and thawing practices (**CHAPTER 7**) to understand its influence on survival or growth of *Salmonella* spp., which is the first reported agent in strong-evidence food-borne outbreaks.

A schematic overview of the contents of this PhD is provided in Figure 1.

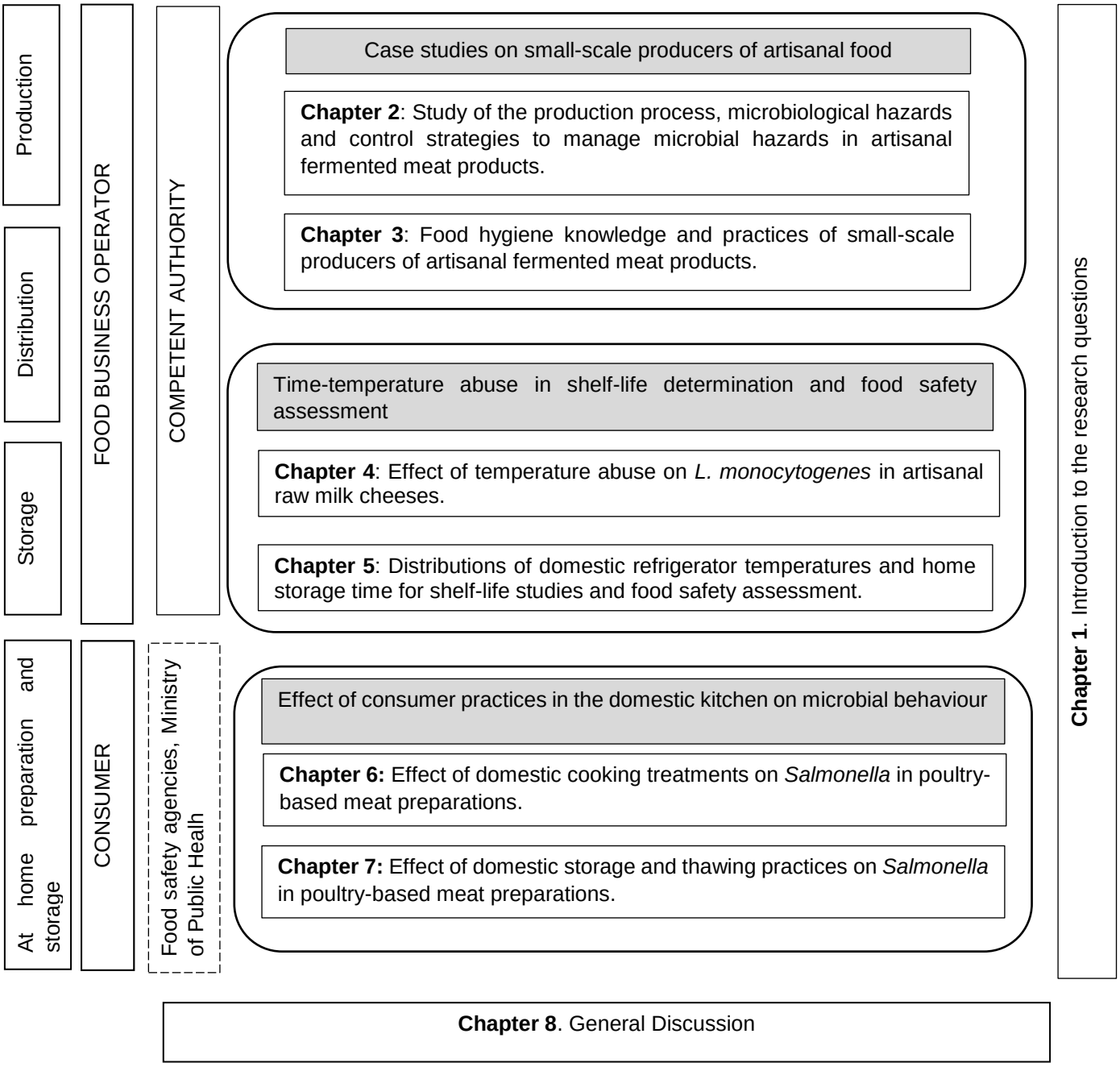


**Figure 1**. Outline of this PhD thesis

**CHAPTER 1** introduces several topics, which create the background of the research questions. Therefore, information on the most important food-borne agents, which are responsible for the majority of food-borne outbreaks and cases in EU are provided. In addition, an overview of the practices most frequently adopted by the consumer in the domestic environment is given. At the same time, the responsibility of FBOs towards food safety is taken into account and the challenges related to micro and small-scale producers and on the inclusion of reasonably foreseen time-temperature abuse in shelf-life

determination and food safety assessment are discussed. Finally, the role and responsibility of the competent authority to ensure food safety is highlighted.

In **CHAPTER 2**, the production process of artisanal fermented meat products (salami and soppresse) in the north of Italy and the control strategies adopted to manage their associated microbiological hazards, in strict collaboration with the regional competent authority are described.
Following the collaborative approach established between the competent authority and the small-scale producers, the results of a questionnaire that was developed in order to investigate the level of food hygiene knowledge and good practices of small-scale producers of artisanal salami and soppresse in the Veneto region of Italy is summarised in **CHAPTER 3**.

The time-temperature abuse of food, which can occur during intermediate storage or storage at retail or consumer phase was analysed through the case study of *L. monocytogenes* in artisanal raw milk cheeses, described in **CHAPTER 4**. The methodology of challenge testing was used to assess the effect of temperature abuse on the growth potential of *L. monocytogenes* in both artisanal Belgian and Italian raw milk cheeses, including also some naturally contaminated cheese obtained (durability studies).
Moreover, the time-temperature abuse was further investigated with a focus of differences between reasonably foreseen storage conditions to occur in countries of the north *versus* the south of Europe. General rules which could be used either in shelf-life testing or risk assessment were derived and presented in **CHAPTER 5**.

**Chapter 6 and Chapter 7** are dedicated to study the effect of consumer's practices in the domestic environment on microbial behaviour. In particular, in **CHAPTER 6** the effect of several types of food preparation techniques, both done according to label instructions of the manufacturer and under conditions of 'abuse' and not following label instructions, on *Salmonella* Typhimurium artificially inoculated in various poultry-based meat preparations was investigated. The effect of domestic temperature abuse and different consumers' thawing methods on *Salmonella* in naturally or artificially contaminated poultry-based meat preparations are described in **CHAPTER 7**.

In **CHAPTER 8**, the conclusions and recommendations are reported. Overall, improvements to food safety and responsible use of food products demands action from all stakeholders in the food chain, producers, consumers and competent authorities. In fact, the changing

consumer's preferences towards food products and thus the raising of new typologies of food products and FBOs (e.g. artisanal/farmstead small-scale producers) require that the principle of flexibility stated by EU legislation is translated into effective food safety policies. Therefore, the development of a simplified FSMS for small-scale FBOs, based on the application of PRPs and the identification of few effective control points, which can be managed by the application of simple but science-based tools, as being studied in this PhD, will provide a valuable contribution to food safety within small-scale artisanal food products. The effect of consumer's behaviour in shaping the risks when handling food has been clearly highlighted in this study. Therefore, effective educational campaigns (which require the collaboration between natural and social sciences) delivering fit for purpose messages targeting different groups of consumers at risk are needed in order to increase the level of consumer's knowledge of food handling practices as well as affect the consumer's attitudes and behaviours in the domestic environment.

Finally, the CA do not only decide on what is or is not considered acceptable in terms of food safety but also provide support to both producers and consumers. In fact, the CA can build a cooperative approach with the FBO, leading to an exchange of knowledge and information useful for assuring food safety. Moreover, with the support of the scientific community, the CA can provide information, through guidelines and manuals to both FBO and consumer, thus contributing to raise the awareness of their role and responsibility in the framework of food safety.

# SUMMARY

Assuring food safety is a shared responsibility that requires the interplay of all actors in the food chain. This PhD thesis has investigated how consumer preferences towards different kinds of food products (e.g., artisanal food) and consumer behaviour in the domestic environment can affect the safety of food products and how the other two actors of the food chain, namely the food business operator and the competent authority, through a cooperative approach – supported by scientific research - can contribute to the enhancement of the safety of food products.

This PhD starts with a literature review of the research areas (CHAPTER 1) and ends with a discussion (CHAPTER 8), that synthesizes the main findings and insights of the performed study. Overall, the PhD research is developed and organised around two areas:

**1. Investigation of small/micro-scale production system for identification of effective tools to support food safety of artisanal products.**

Through the case-study of artisanal salami and soppresse produced in Veneto region of Italy (CHAPTER 2), it was investigated how the production process could be monitored and controlled to prevent the occurrence of unacceptable levels of microbial hazards. The data collected with the strict collaboration of the regional competent authority showed that the fermentation process was characterized by considerable variability between producers in relation to the ripening conditions, which was reflected in a wide range of pH and $a_w$ values along the different steps of the production process. Moreover, microbiological analyses pointed out that *L. monocytogenes* detected at levels above 10 CFU/g in 23% (5/22) of salami and soppresse at the end of the ripening period was the main pathogen of concern. In addition, the collected data allowed to observe that a weight loss value of at least 25% would result in an $a_w \leq 0.92$ for salami and soppresse, and thus multiplication of *L. monocytogenes* to elevated numbers would be avoided in the case of accidental contamination. The study highlighted that the cooperation between FBO and CA, the training and education of the producers (performed by the regional CA as a continuous effort throughout several years) and the identification of simple tools, as the one proposed in this study (measurement of weight loss) represent a winning strategy to prevent bringing to market potentially hazardous artisanal food products.

In order to investigate the effectiveness of the educational programs and the assistive approach developed by the Italian CA towards these artisanal small-scale producers, a questionnaire was developed and administered to the small-scale salami and soppresse producers in the Veneto region of Italy (CHAPTER 3). The questionnaire was aiming to

investigate the level of knowledge and practices of the above-mentioned producers. It consisted of 24 questions (mainly multiple-choice questions) divided over 4 parts (Part I - general information; Part II – basic knowledge on food safety; Part III – resources, infrastructure and control measures; Part IV: opinions and future needs). Thirty-four of in total 70 small-scale producers returned the questionnaire. Overall, these small-scale producers had a good knowledge of the main principles of food safety (e.g. main food-borne pathogens, temperature range for bacterial growth or inactivation; health conditions not acceptable for food handling; importance of hands washing; difference between detergents and disinfectants). In addition, most of the respondents were daily using the tools and guidelines provided by the regional authorities in order to control and assess the safety of their products. Almost all respondents were satisfied with the financial resources allocated in order to support them and had a good collaboration with the competent authority. Moreover, participants would like to invest the further available resources in training and education. Still a point of attention seemed to be the documentation and record keeping needed. This study showed that the efforts spent in order to improve the food hygiene knowledge of these small-scale salami and soppresse producers was taken up in their everyday practices, thus answering to the effective needs. Knowledge is the starting point for the development of the awareness and motivation among producers to implement food safety management systems.

**2. Assess and ensure food safety when food products are set to the market.**

Once food products are ready to be released to the market, food safety still needs to be ensured and FBO but also consumers are responsible to ensure it. Time and temperature abuse which occur at retail and consumer's home are the two main factors which affect microbial behaviour in food. Therefore, FBO have to take into account the effect of reasonably foreseeable time-temperature abuse on microorganisms when establishing shelf-life or when assessing the risk for public health.

The effect of temperature abuse was studied in several types of artisanal raw milk cheeses either using artificially contaminated (challenge tests) or naturally contaminated (durability studies) cheese with *L. monocytogenes*. In CHAPTER 4, methodological details on how to conduct challenge tests and durability studies in different types of raw milk cheese (from soft to semi-hard) were provided. Moreover, the time and temperature (abuse) conditions (9, 7 and 14 days; 7, 12 and 14°C) applied to the artificially inoculated cheeses showed growth of *L. monocytogenes* in two of five analysed types of cheese being studied.

The two cheeses supporting growth were white-moulded soft to semi-soft cheeses stored for 9 days at 7 and 12°C, for which the combination of the physicochemical characteristics of pH and water activity were expected to facilitate growth of *L. monocytogenes*.
On the other side, the absence of growth of *L. monocytogenes* was observed in three semi-hard cheeses. In particular, no growth of the pathogen was reported in the Belgian red-smear semi-hard cheese and this result was also confirmed by a durability study performed on this type of cheese. Therefore, red-smear cheese is not by definition a risky product supporting the growth of *L. monocytogenes* to elevated levels. However, durability studies showed that *L. monocytogenes* was quite homogenously present at low levels (< 10 CFU/g) in the naturally contaminated batches of the red-smear semi-hard cheese and was surviving (and thus not decreasing) during further ripening and storage. Therefore, this type of cheese would still be a risky product for pregnant women and other vulnerable groups in contracting listeriosis and thus absence of the pathogen per 25 g needs to be preferred. It is therefore recommended to aim for the absence of the pathogen per 25 g of raw cheese independent of the type of cheese (and verify this after production as mentioned in legislation using a multi-unit (n = 5) sampling plan).

Moreover, even if challenge tests and durability studies represent important tools to assess food safety, the approach used, still based on single point (conservative) estimates of temperature and storage time instead of distributions, could lead to an overestimation of exposure to elevated levels of *L. monocytogenes*. Therefore, in CHAPTER 5, data on domestic refrigerator temperatures and storage times of chilled food in European countries were analysed in order to draw general rules, which could be used either in shelf-life testing or risk assessment. Knowing that collecting data is time and money consuming, in the absence of data, and at least for the European market and for refrigerated products the following rules were shown to be valid. In fact, the overall variability in temperature of European domestic refrigerators is described by a normal distribution: N (7.0, 2.7)°C for southern countries, and, N (6.1, 2.8)°C for the northern countries. The storage time was described by an exponential distribution corresponding to the use-by date period divided by 4.

Finally, the effect of consumer's behaviour in the domestic environment on microbial growth/ survival/ inactivation was investigated. In CHAPTER 6, several types of heat treatments (according to label instructions and not following label instructions) were reproduced in an experimental kitchen, in order to assess the presence and numbers of *Salmonella* Typhimurium artificially inoculated in five types of poultry-based meat

preparations (burgers, sausages, ready-to-cook-kebabs, quail roulades and extruded roulades) that are likely to be contaminated *by Salmonella*. Three contamination levels (10 CFU/g; 100 CFU/g and 1,000 CFU/g) and three cooking techniques (grilling, frying and baking) were applied. Results showed that heat treatments performed according to label instructions eliminated *Salmonella* Typhimurium (absence per 25 g) for contamination levels of 10 and 100 CFU/g but not for contamination levels of 1,000 CFU/g. After improper heat treatment, 26 of 78 samples were *Salmonella*-positive (23 of these 26 samples were before heat treatment artificially contaminated with bacterial loads between 100 and 1,000 CFU/g). Thus, following label instructions mostly, but not always, produced safe cooked poultry-based meat preparations. On the other side, disrespect of the label recommendations and thus the application of inadequate heat treatment was not able to assure complete elimination of *Salmonella* from the products (i.e. absence per 25 g) even if the initial contamination level was low (10 CFU/g). Of all types of meat considered, *Salmonella* was most often recovered from kebabs, and from all types of heat treatment applied, pan-frying resulted most often in residual *Salmonella* being present.

The impact of temperature abuse, which occurs during domestic storage and defrosting of food, on the presence and numbers of *Salmonella* spp. was studied in different types of poultry-based meat preparations (CHAPTER 7). Naturally or artificially contaminated burgers, sausages and kebabs were stored at several refrigerator temperatures (4°C versus 8 or 12°C), with or without prior temperature abuse (25°C for 2 h, simulating transport of meats from shop to home). In addition, other experimental trials were set up in order to submit the above-mentioned food products to thawing overnight in refrigerator at 8°C or on the kitchen countertop at 23°C. Artificially contaminated products showed a significant ($p < 0.05$) growth of *Salmonella* Typhimurium at 12°C (i.e. from ca. 8 most probable number [MPN]/g to > 710 MPN/g) in kebabs after 7 and 10 days but more moderate growth in sausages (i.e. from ca. 14 MPN/g to a maximum of 96 MPN/g after 9 days storage). Storage of naturally contaminated burgers or sausages (contamination at or below 1 MPN/g) at 4, 8, or 12°C and short time of temperature abuse (2 h at 25°C) did not facilitate an increase in the presence and numbers of *Salmonella*. Thawing overnight in the refrigerator led to either a moderate reduction or no change of *Salmonella* Typhimurium numbers in burgers, sausages and kebabs. The above-mentioned results (i.e. domestic cooking and temperature abuse) highlighted the role that the consumer has in shaping the risks associated with microbial hazards adopting either adequate or improper behaviors. This provides information for food businesses and competent authorities for improving the

information reported on the labels, adding clear indications on how to properly cook, store and handle food products. At the same time, as already stated, consumers have to be educated and approached in a way in order to effectively take up the provided information.

This PhD study has as such contributed to strategies to control of food safety in dealing with small-scale food production of artisanal food:

1) Using the case study of artisanal production of salami and soppresse in the Veneto region in Italy, the collaborative approach between FBO, CA and scientific research has been shown to represent a valuable strategy to address the needs of small-scale food business operators to develop a simplified FSMS, based on the effective application of good hygienic practices and the monitoring of some key control points by the use of tailor-made tools and procedures.

2) From experimental studies in the lab on simulated home-storage and preparation of poultry-based meat preparations it was shown that the consumer behaviour related to food storage and food preparation can heavily affect food safety. Therefore, improvements of the information addressing consumers by FBO (e.g. clear and appropriate label instructions) and the development of educational campaigns by CA targeting appropriate food handling practices by the consumers need to be pursued.

3) Defining the conditions of reasonably foreseen abuse at consumer level in for example refrigerated storage of foods for determining shelf-life and performing microbiological risk assessment is debated. Further technical details provided and experimental work being performed on challenge testing and durability tests for determining the growth potential of *L. monocytogenes* in raw milk cheeses and approaches on dealing with distributions of time and temperature instead of using single point estimates to be used for estimating the risk of encountering elevated numbers of *L. monocytogenes* in refrigerated foods were suggested that can support FBO and CA to elaborate protocols when having to take into account the consumer behaviour in assessing food safety.

# SAMENVATTING

Het garanderen van de voedselveiligheid is een gedeelde verantwoordelijkheid tussen de verschillende actoren in de voedselketen, die hieromtrent dan ook onderling dienen in dialoog te gaan. In deze doctoraatsthesis werd onderzocht hoe het consumentgedrag bij de opslag en bereiding van levensmiddelen mede een effect kan hebben op en dient in rekening gebracht te worden door andere actoren bij de borging van voedselveiligheid. Gezien de toenemende voorkeur van consumenten voor bepaalde artisanale of lokale voedingswaren werd tevens onderzocht hoe een gezamenlijke aanpak van de bevoegde overheid met de exploitanten van vaak kleinschalige levensmiddelenbedrijven of hoeveproducenten kan bijdragen tot het garanderen van de voedselveiligheid bij de productie en het vermarkten van dergelijke artisanale voedingswaren.

Deze doctoraatsthesis begint met een literatuuroverzicht van het onderzoeksgebied (HOOFDSTUK 1) en eindigt met een discussie (HOOFDSTUK 8) waarin de hoofdbevindingen en inzichten van de studie samengevat worden. Deze doctoraatsthesis kan grosso modo onderverdeeld worden in twee delen:

**1. De voorkeur van consumenten voor artisanale voeding en het bijhorend onderzoek naar productiesystemen van kleinschalige producenten ter identificatie van effectieve tools ter ondersteuning van de microbiologische veiligheid van artisanale producten.**

Aan de hand van een case-study, nl. de productie van artisanale salami en “soppresse”, in de Veneto regio in Italië, werd onderzocht hoe het productieproces gemonitord en gecontroleerd kan worden om microbiologische veilige producten te verkrijgen (HOOFDSTUK 2). De data, verkregen tijdens de studie met medewerking van de kleinschalige producenten en opgezet in nauwe samenwerking met het regionale bevoegde autoriteiten, toonden aan dat het fermentatieproces gekenmerkt werd door een hoge variabiliteit tussen de producenten. Dit was gerelateerd aan de rijpingscondities, en kwam mede tot uiting in een breed bereik van de zuurtegraad (pH) en wateractiviteit ($a_w$) opgemeten in de verschillende stappen van het productieproces. Microbiologische analyses toonden *Listeria monocytogenes* aan als het belangrijkste te beheersen gevaar. Immers deze pathogeen was aanwezig in aantallen van meer dan 10 kve/g in 23% (5/22) van de salami en “soppresse” monsters op het einde van hun rijpingsperiode. Uit de verzamelde data kon wel een controlemaatregel gedistilleerd worden. Immers, in het algemeen, resulteerde een gewichtsverlies van minstens 25% tijdens de rijping in een $a_w \leq 0.92$ voor

salami en “soppresse”. Beneden deze wateractiviteitwaarde van 0.92 wordt de groei van *L. monocytogenes* geïnhibeerd en dus aanwezigheid van hoge (risicovolle) aantallen vermeden in het geval van occasionele contaminatie met de pathogeen. Deze studie toonde aan dat de samenwerking tussen exploitant en bevoegde overheid, ondersteund door wetenschappelijk onderzoek, toelaat kritische punten te identificeren in het artisanale productieproces alsook eenvoudige controlemaatregelen (in dit geval meten van gewichtsverlies tijdens rijping) te definiëren voor borging van voedselveiligheid.

Continue en voortgezette inspanning over de jaren heen om in dialoog te gaan met, en training en opleiding te voorzien voor deze kleinschalige producenten door de regionale overheid in Italië wordt beschouwd als een succesvolle strategie naar bewustwording omtrent voedselveiligheid bij deze producenten van artisanale producten. Om de effectiviteit van het opleidingsprogramma en de aanpak ervan af te toetsen werd een enquête opgesteld en verdeeld bij de betrokken producenten van salami en “soppresse” in de Veneto regio (Italië) (HOOFDSTUK 3). De enquête was gericht op het onderzoeken van de algemene kennis en de goede werkpraktijken rond voorkomen van microbiologische besmetting en algemene hygiëne van de hierboven genoemde producenten. De enquête bestond uit 24 vragen (vnl. meerkeuzevragen) verdeeld over 4 delen (deel I – algemene informatie, deel II – basiskennis over voedselveiligheid; deel III – grondstoffen, infrastructuur en controlemaatregelen, deel IV: opinie en toekomstige noden). Vierendertig van de 70 betrokken kleinschalige producenten beantwoordden de enquête. In het algemeen hadden deze producenten een goede kennis van de basisprincipes van voedselveiligheid (vb. correcte kennis van belangrijkste voedselpathogenen, temperatuurbereik voor bacteriële groei en inactivatie, persoonlijke gezondheidscondities die niet toelaten om met voedsel te werken, belang van handen wassen, het verschil tussen de werking van detergenten en desinfectantia). Bovendien gebruikten het merendeel van de producenten dagelijks de richtlijnen en tools aangereikt door de regionale overheden om de veiligheid van hun producten te controleren en te garanderen. Bijna alle producenten waren tevreden met de financiële middelen die hen ter ondersteuning aangereikt werden en gaven aan een goede samenwerking te hebben met de bevoegde overheid. Ook zouden de deelnemers graag de nog beschikbare financiële middelen verder investeren in training en opleiding. Desondanks blijft de registratie en correctie documentatie van a handelingen een punt van aandacht. Deze studie toonde aan dat de geleverde inspanningen om de kennis over voedingshygiëne van salami en “soppresse” bij kleinschalige artisanale producenten te verbeteren, leidden tot het dagelijks toepassen van deze kennis, en dus een antwoord bieden op de effectieve

noden. De opleiding en training van afgelopen jaren bleek de producenten te engageren om effectief een voedselveiligheidsbeheersysteem te implementeren.

**2. Effect van consumentengedrag op de voedselveiligheid en het in rekening brengen van te verwachten consumentengedrag tot op moment van consumptie bij de inschatting van voedselveiligheid van het marktklaar product door de exploitant van een levensmiddelenbedrijf**

Eens levensmiddelen de productiehal verlaten en marktklaar zijn en dus in detailhandel en nadien in de keuken en op het bord van consument belanden, moet de voedselveiligheid ook nog steeds gegarandeerd worden in dit na-traject post-productie. Zowel de exploitanten van levensmiddelenbedrijven (FBO) als de eindconsument hebben hier een verantwoordelijkheid in. Temperatuur-misbruik tijdens bewaring, het overschrijden van de maximale bewaartermijn of het onvoldoende verhitten tijdens bereiding door de consument kunnen een belangrijk effect hebben op groei of overleving van bacteriën in het voedsel. Het is de verantwoordelijkheid van de FBO om op een realistische manier het te voorziene tijd- en temperatuurmisbruik in rekening te brengen bij het bepalen van de houdbaarheid van een levensmiddel of de risico-inschatting voor de volksgezondheid.

Het effect van temperatuur-misbruik op de groei of overleving *L. monocytogenes* werd bestudeerd in verschillende types rauwmelkse kazen door gebruik te maken van artificieel besmette kazen (‘provocatietesten’) maar ook met van nature besmette kaas (‘houdbaarheidstesten’). In HOOFDSTUK 4 worden de methodologische details over het uitvoeren van deze provocatietesten en houdbaarheidstesten in de verschillende types rauwmelkse kazen (zacht tot halfhard) toegelicht. De condities van tijd- en temperatuur-misbruik (7, 9 en 14 dagen; 7, 12 en 14°C), toegepast op de artificieel geïnoculeerde kazen, zorgden voor uitgroei van *L. monocytogenes* op twee van de vijf types kazen betrokken in deze studie. De twee kazen die de groei van *L. monocytogenes* ondersteunden, waren zachte tot halfharde witte schimmelkazen, bewaard gedurende 9 dagen bij 7 en 12°C en met fysicochemische eigenschappen (pH en wateractiviteit) waarvan verwacht werd dat ze de groei van *L. monocytogenes* zouden faciliteren. Anderzijds werd de afwezigheid van groei van *L. monocytogenes* vastgesteld in 3 halfharde kazen. Meer concreet, er werd geen groei van de pathogeen vastgesteld in de Belgische halfharde roodbacterie kaas, niet in de provocatietest en ook niet in de houdbaarheidstest uitgevoerd op dit type kaas. Aldus is de roodbacterie kaas niet ‘per definitie’ een risicoproduct dat de groei van *L. monocytogenes* tot hogere aantallen ondersteunt, groei kan en is in het verleden vastgesteld indien de

roodbacterie kaas een zachte kaas was, maar in dit geval van een halfharde roodbacteriekaas werd geen groei genoteerd. Echter, de houdbaarheidstests toonden wel aan dat *L. monocytogenes*, indien aanwezig - en in dit geval bij de van nature besmette halfharde roodbacterie kaas vrij homogeen aanwezig in lage aantallen (< 10 CFU/g) – de verdere rijping en bewaring overleefden (en dus de pathogeen niet afnam in aantal). Daarom vormt dit type kaas, ook indien laag besmet, gedurende de volledige houdbaarheidstermijn toch nog steeds een risicoproduct voor zwangere vrouwen en andere kwetsbare groepen voor listeriosis. Het is dan ook aanbevolen om te streven naar de afwezigheid van de pathogeen per 25 g rauwmelkse kaas onafhankelijke van het type kaas (en dit na productie te verifiëren zoals wettelijk is voorzien via een n = 5 monsternameplan).

Ondanks het feit dat provocatie- en houdbaarheidstesten belangrijke technieken zijn om de voedselveiligheid te beoordelen, is de gehanteerde methode gebaseerd op een enkele puntschatting van temperatuur en bewaartijd (conservatief, worst case waarden) in plaats van distributies van realistisch voorkomende (gemeten) waarden. Dit kan leiden tot een overschatting van de blootstelling aan verhoogde aantallen van *L. monocytogenes.* Daarom werden in HOOFDSTUK 5 gegevens over koelkasttemperaturen en bewaartijden van gekoelde voeding in huishoudelijke omgeving geanalyseerd zoals beschikbaar in de wetenschappelijke literatuur, om zo via modellering algemene meer realistische richtlijnen te formuleren voor houdbaarheidstests of risicobepalingen. De globale variabiliteit in temperatuur van koelkasten in huishoudelijke omgeving over Europa vertoonde een normaalverdeling: N (7.0, 2.7)°C voor de zuidelijke landen, en N (6.1, 2.8)°C voor de noordelijke landen. De bewaartijd vertoonde een exponentiële verdeling, overeenstemmend met de houdbaarheidsperiode gedeeld door vier. Wetende dat dergelijke individuele dataverzameling per regio geld- en tijdrovend is, kan dan bij de afwezigheid van regio-gebonden data, de geformuleerde richtlijn voor temperatuur- en tijdsdistributie als valide beschouwd worden voor gekoelde producten op de Europese markt.

Tenslotte werd het effect van consumentengedrag in de huishoudelijke omgeving op microbiële groei, overleving of inactivatie onderzocht. In HOOFDSTUK 6 werden in een experimentele keuken verschillende types hittebehandelingen gereproduceerd voor het bereiden van vijf types gevogeltevlees (burgers, worsten, kebabs, roulades van intact vlees en bewerkte vleesroulades). Sommige hittebehandelingen waren in overeenstemming met de instructies op het etiket zoals aangegeven door de fabrikant, andere betroffen onvoldoende verhitting. De residuele aanwezigheid - en schatting van (lage) aantallen – van artificieel geïnoculeerde *Salmonella* Typhimurium werd nagegaan. Drie

contaminatieniveaus (10 kve/g, 100 kve/g en 1000 kve/g) en drie verhittingstechnieken (grillen, bakken in de pan, en hete lucht oven bakken) werden toegepast. Bij hittebehandelingen uitgevoerd volgens de instructies van het etiket kon *Salmonella* Typhimurium geëlimineerd worden (afwezigheid in 25 g) in geval van contaminatieniveaus van 10 en 100 kve/g, maar niet voor een contaminatieniveau van 1000 kve/g. Na de onvoldoende hittebehandeling bleken 26 van 78 stalen positief te zijn voor *Salmonella* (23 van deze 26 stalen werden voor de hittebehandeling artificieel gecontamineerd met aantallen tussen 100 en 1000 kve/g). Aldus, bij het volgen van bereidingsinstructies aangegeven op het etiket worden meestal, maar niet altijd, veilig verhitte gevogeltebereidingen bekomen. Echter het niet opvolgen van de instructies op het etiket leidde tot occasionele overleving van *Salmonella* (i.e. aanwezigheid in 25 g), zelfs in geval van een laag initieel contaminatieniveau (10 kve/g). Van alle types vlees, werd *Salmonella* nog vaakst teruggevonden in kebab, en van alle hittebehandeling, liet bakken in de pan het vaakst residuele *Salmonella* optekenen.

De impact van temperatuur-misbruik hetzij gedurende bewaring thuis in de koelkast, hetzij gedurende ontdooien van voedsel (op kamertemperatuur) op de overleving of groei van *Salmonella* Typhimurium werd onderzocht in verschillende types gevogeltebereidingen (HOOFDSTUK 7). Natuurlijk of artificieel gecontamineerde burgers, worsten en kebabs werd bewaard bij verschillende koelkasttemperaturen (4°C versus 8°C of 12°C), met of zonder voorafgaand temperatuur-misbruik (25°C gedurende 2 uur) om transport van de winkel naar huis te simuleren. Ontdooien van het vlees gebeurde in het lab of overnacht in een koelkast bij 8°C of op het keukenaanrecht bij 23°C. Bij de artificieel gecontamineerde producten bleek significante groei ($p < 0.05$) op te treden van *Salmonella* Typhimurium bij 12°C (i.e. van ca. 8 ‘meest waarschijnlijk aantal’ [MWA]/g tot > 710 MWA/g) in kebabs na 7 en 10 dagen, maar meer gematigde groei in worsten (i.e. van ca. 14 MWA/g tot een maximum van 96 MWA/g na 9 dagen bewaring). Bewaring van natuurlijk gecontamineerde burgers of worsten (contaminatie gelijk aan of lager dan 1 MWA/g) bij 4, 8 of 12°C en een kort temperatuur- misbruik (2 uur bij 25°C) bewerkstelligde geen verhoging in het terugvinden van *Salmonella.* Ontdooien gedurende de nacht in de koelkast resulteerde in ofwel een matige reductie ofwel geen verandering in aantallen van *Salmonella* Typhimurium in burgers, worsten en kebabs.

De bovengenoemde resultaten (i.e. kookpraktijken thuis en temperatuur-misbruik tijdens bewaring of ontdooien) benadrukken de impact die een consument heeft met zijn gedrag op de residuele blootstelling en dus risico’s voor voedselinfectie na consumptie van,

in dit geval, *Salmonella* besmette vleesbereidingen op basis van gevogelte. Er werd in deze studie ook vastgesteld dat *Salmonella* occasioneel van nature aanwezig is (in lage aantallen) in deze rauwe - te verhitten – vleesbereidingen en dus residuele overleving van de pathogeen dus effectief reëel is in het geval van niet respecteren van de koele keten en verhitting in overstemming met de instructies op het etiket. Deze resultaten kunnen informatie verschaffen aan bedrijven en bevoegde overheden om de informatie op labels te optimaliseren en benadrukken het belang van duidelijke indicaties of informatieverstrekking aan de consument omtrent adequate bewaring, ontdooien indien van toepassing, en verhitting van dergelijke (rauwe) vleesbereidingen die toch erg gegeerd zijn bij consumenten. Er dient nog meer onderzoek uitgevoerd te worden om na te gaan hoe communicatie naar de consumenten met betrekking tot eigen (veilige) werkpraktijken in de keuken best verloopt en welke communicatiemiddelen de consument overtuigen om dergelijke richtlijnen ook effectief dagdagelijks te implementeren.

Dit doctoraal onderzoek heeft als zodanig bijgedragen aan de beheersing van voedselveiligheid bij kleinschalige voedselproductie en ambachtelijk voedsel:

1) Aan de hand van de casestudy van de ambachtelijke productie van salami en soppresse in de Veneto regio in Italië is aangetoond dat een samenwerking tussen de voedselproducent en de bevoegde overheid, gesteund door wetenschappelijk onderzoek, een waardevolle strategie is om tegemoet te komen aan de behoeften van kleinschalige exploitanten van levensmiddelenbedrijven om een vereenvoudigd voedselveiligheidsbeheersysteem te ontwikkelen, gebaseerd op de effectieve toepassing van goede hygiënepraktijken en de monitoring van enkele belangrijke punten in het productieproces mits gebruik van op maat gemaakte hulpmiddelen en procedures.

2) Uit experimenteel onderzoek in het laboratorium waarbij bewaring en bereiding van vleesproducten op basis van gevogelte in het huishouden werd gesimuleerd bleek dat het consumentengedrag en de manier van voedselbereiding thuis de voedselveiligheid sterk kan beïnvloeden. Daarom moeten verbeteringen worden nagestreefd van de informatie die voedselproducenten aan consumenten geven (bijvoorbeeld duidelijke en passende instructies voor bewaring en bereiding op het etiket) en moet de ontwikkeling van voorlichtingscampagnes door de bevoegde overheid die gericht zijn op passende praktijken voor bewaring en bereiding van voedsel door de consumenten worden voortgezet.

3) Er loopt nog steeds een discussie over de omstandigheden ‘voor redelijkerwijs voorzien misbruik op consumentenniveau’ die door voedselproducenten moet in rekening gebracht

worden bij bijvoorbeeld het bepalen van de houdbaarheid of het uitvoeren van een microbiologische risicobeoordeling van langdurige gekoelde bewaring van kant-en-klaar levensmiddelen. Verdere technische details en experimenteel werk dat werd uitgevoerd in dit doctoraal onderzoek met betrekking tot provocatietesten en houdbaarheidstesten voor het bepalen van het groeipotentieel van *L. monocytogenes* tijdens bewaring van rauwe melkkazen en benaderingen voor het omgaan met distributies van tijd en temperatuur in plaats van puntschattingen voor het schatten van het risico op aantreffen van verhoogde aantallen van *L. monocytogenes* in gekoelde levensmiddelen kan de voedselproducent en bevoegde overheid ondersteunen bij het uitwerken van protocollen wanneer rekening moet worden gehouden met het gedrag van de consument bij het beoordelen van voedselveiligheid.

# CHAPTER 1. Introduction to the research questions

## 1.1 Food-borne diseases: trends and sources

Food-borne illness is still a major health issue worldwide. The global burden of food-borne disease caused by 31 hazards in 2010 was 33 million Disability Adjusted Life Years (DALYs[1]) (Havelaar et al., 2015). In the United States, each year food-borne pathogens cause an estimated 9.4 million illnesses, 56,961 hospitalizations, and 1,351 deaths through contaminated foods. Overall, 90% of domestically acquired food-borne illnesses, hospitalizations, and deaths caused by known pathogens were attributed to seven pathogens: *Campylobacter*, *Clostridium perfringens*, *Escherichia coli O157*, *Listeria monocytogenes*, non-typhoidal *Salmonella*, Norovirus, and *Toxoplasma gondii*. These pathogens caused about 112,000 DALYs annually due to food-borne illnesses acquired in the United States. Years of life lost was the main driver of DALYs for *Listeria monocytogenes* and *E. coli* O157 (Scallan, Hoekstra, Mahon, Jones, & Griffin, 2015). Annually in EU, 13 million cases of microbial food-borne illnesses are estimated to occur, corresponding with 165,000 Disability Adjusted Life Years (DALYs) and 2,500 deaths (Kirk et al., 2015). Food-related pathogens cause annual costs to the society, in both economic and public health terms. The total cost of illness in 2011 of fourteen food-related pathogens and associated sequelae was estimated at € 468 million/year, in the Netherlands (Mangen et al., 2015).

According to the report on "zoonoses, zoonotic agents and food-borne outbreaks" of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), food-borne diseases (including waterborne) caused in 2015, 342,652 cases of illness, 34,412 hospitalisations and 470 deaths in 26 European Member States (EFSA & ECDC, 2016). In addition, the 'zoonosis' monitoring activities of 32 European countries showed that in 2015, the first five foodborne agents were *Campylobacter*, *Salmonella*, *Yersinia*, STEC and *Listeria monocytogenes*. *Salmonella* caused the second most reported number of infections (94,625 cases) with a fatality rate of 0.24% (the number one pathogen in terms of causing food-borne infections was *Campylobacter*). There was a statistically significant decreasing trend of salmonellosis in the 8-year period between 2008 and 2015, probably related to the control plans at farm level that each European Member State has to apply. *Salmonella* was most frequently detected in food of animal origin, primarily in broiler meat (6.5%) and turkey meat (4.6%), whereas a lower number of positive samples were found in pig meat (1.7%) and bovine meat (0.2%)

[1] DALY: a measure developed by the World Health Organization that combines data on premature mortality and on morbidity from acute illness and sequelae into a single statistic summarizing years of healthy life lost. The DALY aggregates the loss of life and health due to illness compared with 'perfect' health, using time as the common metric.

(EFSA & ECDC, 2016). Moreover, concerning food-borne outbreaks, *Salmonella* was the causative agent of 21.8% of all food-borne outbreaks and the first causative agent of strong-evidence food-borne outbreaks in 2015. *Salmonella* was strongly associated with households. Compared with the other places of exposure, the outbreak reporting rate for household outbreaks caused by *Salmonella* was more than four times higher.

Shiga-toxin producing *Escherichia coli* (STEC), is associated with more severe disease and increased complications compared to *Salmonella* and other bacterial causes of gastroenteritis. After the large outbreak of 2011, since 2014 the EU notification rate stabilized. In 2015, 5,901 confirmed cases of STEC infections were reported in the EU (EFSA & ECDC, 2016). Strains of *E. coli* serotype O157 caused the majority of disease, but in recent years, improved diagnostic assay for non-O157 serogroups have contributed to an increased appreciation of the incidence of disease caused by non-O157 STEC. Consumption of undercooked/raw meat and contact with animals or their environment were the transmission pathways most commonly associated with sporadic STEC infections (Kintz, Brainard, Hooper, & Hunter, 2017). The highest proportion of STEC-positive food samples in 2015 was detected in meat from ruminants (12.2%) (primarily sheep and goat, but also wild ruminants and cattle), followed by raw milk and dairy products (4.4%) whereas the proportion of positive samples in fruit and vegetables was very low (< 1%) (EFSA & ECDC, 2016).

*L. monocytogenes* is reported to be responsible for more deaths than *Salmonella* and *E. coli* O157 combined, with data suggesting that up to 90% of individuals with listeriosis are hospitalized (Gilliss et al., 2013). In fact, in 2015 the EU case fatality was 17.7% among the 1,524 confirmed cases with known outcome, according to the EU report on zoonosis and zoonotic agents (EFSA & ECDC, 2016). In addition, there was a statistically significant increasing trend of listeriosis over the period 2008 to 2015.

Listeriosis infections were most commonly reported in the elderly population in the age group over 64 years old and particularly in the age group over 84 years. Concerning foodstuffs, in EU in 2015, the highest non-compliance of samples collected at processing level was in fishery products (mainly smoked fish) (3.5 %), dairy products (other than cheeses) (1%) and heat-treated meat products (2.1%). At retail, non-compliance was highest in batches of fishery products (1.4%) and soft and semi-soft cheese (1%) (EFSA & ECDC, 2016).

### 1.1.1 Description of *Salmonella* spp.

The genus *Salmonella* includes Gram-negative, non-spore forming, facultative anaerobic bacteria. They are usually motile, rod shaped (0.7-1.5 x 2.0-5.0 μm) and able to ferment glucose, thus producing acids and gas. According to the Kauffmann- White- LeMinor scheme, which is based on the immune reactivation with two surface antigens (flagellar (H) and somatic (O) antigens), there are more than 2,600 known *Salmonella* serotypes. The genus *Salmonella* has two species: *Salmonella enterica* and *Salmonella bongori*. *S. bongori* is generally isolated from non-mammalian hosts. *S. enterica* itself is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. The majority of the serotypes belongs to *S. enterica* subspecies *enterica*; they are mainly associated with warm-blooded vertebrates and are usually transmitted by ingestion of food or water contaminated by infected faeces (Stevens, Humphrey, & Maskell, 2009).

Most *Salmonella* serotypes can grow at a temperature range between 7 and 48°C, with optimum growth temperature between 35 and 37°C. They are killed by pasteurization treatment, sensitive to low pH (4.5 or below), and do not multiply at $a_w$ below 0.94, especially in combination with a pH of 5.5 and below (Jay, 2000). Salmonellosis, the illness from *Salmonella* infection, is one of the most frequently occurring food-borne disease worldwide. Salmonellosis is characterized by fever, stomach cramps, and diarrhoea. Symptoms develop 8 hours to 3 days after ingestion and last 4 to 7 days. Most cases are self-limiting (Bollaerts et al., 2008).

*Salmonella* spp. is widely distributed in nature, with humans and animals being the primary reservoir. Its primary habitat is the intestinal tract of hosts; however, they may be found in other parts of the body from time to time. Being mainly intestinal, they are excreted in faeces (of animals and humans) and may be found in faecal polluted water. Food of animal origin (eggs, poultry, meat and meat products) is the most common food vehicle of salmonellosis to human. *S*. Enteritidis and *S*. Typhimurium were the most frequently isolated serotypes in Europe. *Salmonella* in eggs and *Salmonella* in pig meat and products thereof were among the top five combinations of causative agents and food vehicles in strong-evidence food-borne outbreaks in EU in 2015 (EFSA & ECDC, 2016).

The European policy has set down regulations in order to reduce the prevalence of *Salmonella* at farm and at processing level. In fact, at farm the monitoring of *Salmonella* in animals is mainly conducted through active routine monitoring of breeding flocks and production poultry species in different age groups. Regulation (EC) No 2160/2003 with subsequent amendments prescribes a harmonised sampling plan at farm level in the poultry

and pigs populations for the control of all *Salmonella* serotypes with public health significance. *S*. Enteritidis, *S*. Typhimurium and its monophasic variant, *S.* Infantis, *S.* Virchow and *S.* Hadar, are considered relevant serotypes for the poultry population (breeding flocks of *Gallus gallus*, laying hens, broilers, breeding and fattening turkeys) from a public health perspective (EC, 2003b). Moreover, Commission Regulation (EC) No 2073/2005 on microbiological criteria for food lays down food safety criteria for *Salmonella* in several specific food categories. According to this Regulation, *Salmonella* must be absent in food products when placed on the market, during their shelf-life. The absence is defined by testing five or, depending on the food category, 30 sampling units of 10 or 25 g per batch (EC, 2005). In addition with Regulation (EC) No 1086/2011, in fresh poultry meat it is required the absence of *S*. Enteritidis and *S*. Typhimurium (including monophasic *S*. Typhimurium strains) that are the regulated serovars in the context of the EU control programmes for poultry populations (EC, 2011).

### 1.1.2 Description of Shiga Toxin-Producing *Escherichia coli* (STEC)

*E. coli* is a Gram-negative, facultative anaerobe, within the family *Enterobacteriaceae*, and is normally found in the lower intestinal tract of healthy humans and animals. For the most part, *E. coli* is a group of harmless bacteria that are most often used as indicator organisms for faecal contamination and inadequate hygiene. However, several *E. coli* clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious intestinal and extra-intestinal diseases (Farrokh et al., 2013).
There are six categories of pathogenic *E. coli* that affect the intestines of humans: Shiga-toxin-producing *E. coli* (STEC; also called verocytotoxin-producing *E. coli* or VTEC), of which enterohaemorrhagic *E. coli* (EHEC) are a pathogenic sub-group; enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAEC); enteroinvasive *E. coli* (EIEC); and diffusely adherent *E. coli* (DAEC).

According to the “technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food”, the major STEC serotypes or serogroups of concern are *E. coli* O157:H7, O26, O103, O145, O111, and O91 (EFSA, 2009). These serogroups are a small number in the entire family of STEC, which comprises over 400 serotypes that differ greatly in both their physiological characteristics and their pathogenic potential to humans (Kintz et al., 2017). All STECs have the same morphology. They are Gram-negative bacilli belonging to the *Enterobacteriaceae* family and the *Escherichia* genus. STECs are characterised by their serogroup, virulence genes, toxins

and associated disease symptoms. STECs are so named because they produce one or more cytotoxins, called Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2).

Concerning the environmental conditions, most *E. coli* grow between 10 and 46°C, with some strains growing at 8°C. A recent publication has also shown that *E. coli* O157 strains possess inherent genetic mechanisms which enable better growth at low temperatures (< 15°C), compared to non-pathogenic *E. coli* (Vidovic, Mangalappalli-Illathu, & Korber, 2011). STECs, like other Gram-negative bacteria, do not exhibit unusual heat resistance under conditions of neutral pH and moderately high water activity (e.g. 0.95) and usually the pasteurisation treatment is effective for its inactivation. STEC strains show acid resistance which is mainly mediated by the RpoS mechanism. This feature helps STEC to survive the acidity of the stomach and to colonise the gastrointestinal tract. Furthermore, it also increases the survival of STEC in acidic foods, enabling survival for extended periods, particularly at refrigeration temperature (Farrokh et al., 2013; Vidovic et al., 2011).

Transmission pathways for the human infection include faecal-oral, food-borne, environmental, and person-to-person (Caprioli, Morabito, Brugère, & Oswald, 2005). The symptoms associated with STEC infection in humans vary according to the strain of *E. coli* encountered and the resistance of the individual to such illness. Cases typically present with abdominal cramps, vomiting, and/or diarrhoea, which may progress to haemorrhagic colitis. STEC infections can result in HUS (Haemolytic Uremic Syndrome), which is characterised by acute renal failure, anaemia and lowered platelet counts. HUS develops in up to 10% of patients infected with STEC O157 and is the leading cause of acute renal failure in young children (Gould et al., 2009).

The food-borne route often involves consumption of undercooked meat, water, dairy products made from raw milk or post-processing contamination of raw or undercooked vegetables and ready-to-eat food (Kintz et al., 2017).

Although ruminants, primarily cattle, represent a primary reservoir of STEC, many food products of other origins, including pork products, have been confirmed as vehicles for STEC transmission (Conedera et al., 2007; Ercoli et al., 2015).

Only in rare cases, pork consumption is associated with severe clinical symptoms caused by high pathogenic STEC strains. However, in these outbreaks, it is unknown whether the contamination of food products occurs during swine processing or via cross-contamination from foodstuffs of different sources (Tseng, Fratamico, Manning, & Funk, 2014). In swine, STEC plays an important role in the pathogenesis of oedema disease. In particular, a Shiga toxin subtype, named stx2e, is considered as a key factor involved in the damage of swine

endothelial cells (Gyles, 2007). On the contrary, stx2e-producing *Escherichia coli* has rarely been isolated in humans, and usually only from asymptomatic carriers or from patients with mild symptoms, such as uncomplicated diarrhoea. In fact, the presence of gene stx2e, encoding for stx2e, has rarely been reported in STEC strains that cause HUS. Moreover, stx2e-producing STEC isolated from humans and pigs were found to differ in serogroup, their virulence profile and interaction with intestinal epithelial cells (Sonntag et al., 2005). Because of the limited epidemiologic data of STEC in swine and the increasing role of non-O157 STEC in human illnesses, the relationship between swine STEC and human disease needs to be further investigated.

Following the large and deadly outbreak of *E. coli* O104:H4 food poisoning in Germany, related to the consumption of contaminated Greek fennel sprouts, a food safety criterion related to STEC was introduced. According to EU Regulation 209/2013 amending Regulation (EC) No 2073/2005, the absence in 25 grams of STEC O157, O26, O111, O103, O145 and O104:H4, is required in sprouts and sprout seeds placed on the market during their shelf-life (EC, 2013).

### 1.1.3 Description of *Listeria monocytogenes*

The genus *Listeria* includes six species: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. *Listeria monocytogens* is the only species of *Listeria* that is pathogenic to humans and is the causative agent of listeriosis (McLauchlin, Mitchell, Smerdon, & Jewell, 2004). *L. monocytogenes* infection can result in two types of human illness: non-invasive listeriosis affects the digestive system resulting in symptoms that include fever, muscle aches and sometimes gastrointestinal symptoms (nausea or diarrhoea) and the more serious invasive listeriosis is associated with clinical presentations of central nervous system infection, sepsis, and bacteremia. Because of the invasiveness of *L. monocytogenes*, listeriosis fatalities is particularly associated with high-risk populations, e.g. individuals with compromised immune systems such as persons with hematological malignancies (e.g. leukemia), persons suffering from liver cancer, older adults (> 74 years of age), pregnant women, and new born babies (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017; Goulet et al., 2012; McLauchlin et al., 2004).

*L. monocytogenes* is a Gram-positive non-spore forming bacterium, rod shaped (0.5 µm wide and 1 to 2 µm long), facultative anaerobic. Although it has an optimal temperature range of 30 to 37°C, it is able to grow over a wide temperature range, between 1 and 45°C (Junttila, Niemelä, & Hirn, 1998; Ray, 2004). As a psychrotolerant bacterium (0.4-45°C), it

can survive and even grow at refrigeration temperatures. The organism is particularly resistant to environmental stress and it is able to survive or multiply under a wide range of unfavourable conditions of pH (4.6-9.4, optimum 7.0) and $a_w$ (minimum 0.92) (McLauchlin et al., 2004).

The species *L. monocytogenes* is divided into 13 serovars based on somatic and flagellar antigens. Since 2005, these serovars have been replaced by five genoserogroups determined by PCR: IIa (serovars 1/2a and 3a), IIb (serovars 1/2b and 3b), IIc (serovars 1/2c and 3c), IVb (serovars 4b, 4d and 4e) and L (other serovars). Of these, IVb followed by IIa and IIb are the genoserogroups most frequently implicated in human cases (EURL-Lm, 2014).

*L. monocytogenes* was initially described in 1926 (Murray et al.) as an animal pathogen (in rabbit) and named *Bacterium monocytogenes* (because infecting monocytes). In 1940, Sir Joseph Lister a British surgeon renamed it to *Listeria monocytogenes*. *L. monocytogenes* has been considered for long time an animal pathogen. It is only in 1980-90 that *L. monocytogenes* has been associated to food and listeriosis in humans (Seeliger, 1988). Although still considered a zoonotic pathogen, *L. monocytogenes* is widely distributed in nature and food processing environments. It has been isolated from soil, vegetation, sewage, water, animal feed, fresh and frozen meat, slaughterhouse wastes and in the faeces of healthy animals including humans (McLauchlin et al., 2004). Therefore, it is not surprising that *L. monocytogenes* was isolated from a variety of foodstuffs (e.g. chicken meat, beef, fish, milk, cheese, vegetable, ice cream, sandwiches, fried rice, etc.) (Uyttendaele et al., 2004).

Because of the ability of *Listeria monocytogenes* to grow in a wide range of environmental conditions and due to the fact that ready-to-eat food (RTE) with prolonged shelf-life is considered to be the major vehicle for listeriosis, the European Regulation 2073/2005 setting microbiological criteria in food, distinguishes between food products able to support the growth and products not able to support the growth of *L. monocytogenes* (Table 1). Considering that for a healthy human population, foods contaminated with levels that do not exceed 100 CFU/g are considered to pose a negligible risk (Scientific Opinion of the Panel on Biological Hazards, 2007), the EU threshold limit is set at ≤ 100 CFU/g for RTE products on the market during their shelf-life. However, it is important that the above-mentioned limit is associated with a scientific basis (e.g. scientific literature, challenge tests, predictive microbiology) for the growth potential of *L. monocytogenes* in the food (EC, 2005).

**Table 1**. Food safety criteria for the presence of *L. monocytogenes* in different food products in European Union (EU Regulation 2073/2005 and subsequent amendments).

| RTE food products category | Sampling plan | | Limits | Stages where criterion applies |
|---|---|---|---|---|
| | n[1] | c [2] | | |
| I: Food for infants and for special medical purposes | 10 | 0 | Absence in 25 g | During the shelf-life |
| II: Able to support the growth of *L. monocytogenes* but not category I | 5 | 0 | ≤ 100 CFU/g | During the shelf-life (for the products that the manufacturer is able to demonstrate that the *L. monocytogenes* will not exceed the limit throughout the shelf-life) |
| | 5 | 0 | Absence in 25 g | For the products that the manufacturer is not able to demonstrate that the *L. monocytogenes* will not exceed the limit throughout the shelf-life |
| III: Unable to support the growth of *L. monocytogenes* but not category I [3] | 5 | 0 | ≤ 100 CFU/g | During the shelf-life |

[1] n: number of units comprising the sample.

[2] c: number of sample units giving values in the limits.

[3] Products with pH ≤ 4.4 or water activity ($a_w$) ≤ 0.92, products with pH ≤ 5.0 and $a_w$ ≤ 0.94, products with a shelf-life of less than 5 days belong to this category. Other categories of RTE products can also belong to this category, subject to scientific justification.

## 1.2 Consumer knowledge and behaviour in the domestic environment

Safe food handling by the consumer in the domestic kitchen is considered to be “the final line of defence” against food-borne illnesses. In fact, consumers have to be aware that they are responsible for the proper storage, handling and cooking of food (chapter 2, point 9, White paper) (Commission of the European Communities, 2000). However, while the farm-to-retail part of the food chain is covered by legislation and management systems to ensure the quality and safety of food products, the retail-to-fork part of the food chain is in the private consumer setting and is therefore difficult to regulate. Moreover, epidemiological studies indicated that the majority of food poisoning incidents are represented by sporadic cases or outbreaks associated with food prepared or consumed in the home (EFSA & ECDC, 2016; Redmond & Griffith, 2003).

Several factors contribute to outbreaks of food-borne illness in the home. Most food eaten is prepared at home, thereby contributing to the likelihood for food handling mistakes to occur in this setting. In addition, most consumers consider the domestic environment a safe place (Byrd-Bredbenner, Berning, Martin-Biggers, & Quick, 2013; Taché & Carpentier, 2014), thus underestimating the role of personal handling of products in contamination in the domestic environment. Moreover, home kitchens are multipurpose areas and are much

more than just food preparation and storage places (Scott, 2003; Wills, Meah, Dickinson, & Short, 2015). The implementation of proper food-handling practices can prevent cases of food-borne disease and the way in which consumers handle food in the kitchen affect the risk of pathogen multiplication, cross-contamination to other products and the inactivation of pathogens through cooking procedures.

Much consumer food safety research has been undertaken during the last 20 years; consequently, large amounts of consumer food safety data are available. Most of the studies evaluated consumer food safety knowledge and included self-reported data. Actual behavioural research based on observation and collation of data on attitudes toward food safety practices in the domestic kitchen are scarce. Redmond & Griffith (2003) performed an extensive review on consumer-based research studies dealing with food handling practices in the home. According to the review, from 1975 until 2002, 88 consumer food safety studies were retrieved and 83% of them were carried out since 1995. Moreover, the majority of the studies were conducted in the United Kingdom, Northern Ireland and the United States. Data on consumer food safety were collected through surveys (interviews and self-completion questionnaires) in 75% of the reviewed studies. The above-mentioned review investigated the consumer's knowledge, attitudes, perceptions, self-reported and observed practices in relation to microbial risks, cross-contamination (hand washing, separation of raw and cooked foods), and temperature control (cooking and storage of food). One of the most notable conclusions was that consumers' knowledge, attitudes, intentions and self-reported practices determined by surveys did not correspond well with the actual observed behaviours. Therefore, it seems that survey data appear to predict the occurrence of much safer consumer behaviour than is actually observed. In fact, the available observational studies highlighted that substantial numbers of consumers still implement unsafe food handling practices.

After 2002, more studies have been performed on food safety practices in the domestic environment. In particular, more observational studies were conducted. In the present paragraph, the results of studies regarding consumer's food handling in the home in the timeframe from 2003 to 2016 are presented and discussed. The retrieved studies were those examining consumer's knowledge and self-reported behaviour related to safe food handling practices within the home environment and the consumer's observed behaviour when preparing a meal.

The collected data have been summarised according to several aspects of safe food handling. In particular, studies have been organised according to "five keys to safer food"

(WHO, 2006) which core principles are: keep clean; separate raw and cooked; cook thoroughly; keep food at safe temperatures and use safe water and raw materials (Figure 2).

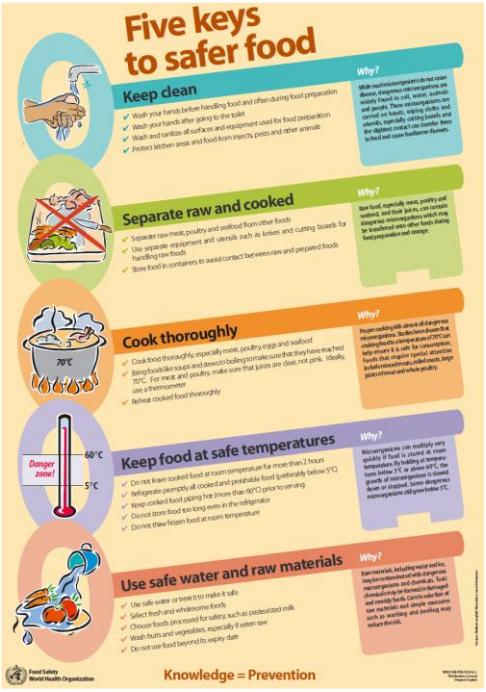


**Figure 2.** Five keys to safer food (WHO, 2006)

### 1.2.1 Keep clean and separate raw and cooked food: avoid cross-contamination

Cleaning hands and utensils and separation between raw and cooked food are important practices in order to prevent cross-contamination and therefore the transfer of microorganisms which can cause disease, from one food, object or surface to another food. For example, a questionnaire investigating Swiss consumer's behaviour related to campylobacteriosis and the domestic environment, reported that the major violations of food safety behaviour were related to avoiding cross-contamination during poultry preparations (Bearth, Cousin, & Siegrist, 2014).

The extensive review of Redmond & Griffith (2003) on food safety at home, reported that 75% of respondents (British and American) knew that handwashing is essential to the prevention of contamination, although one fifth of the respondents were not familiar with the most effective methods for washing and drying hands. In addition, the observational studies included in the above-mentioned review, showed that up to 100% of participants failed to wash and dry their hands adequately after handling raw chicken, and more than half of the participants failed to use separate or adequately washed and dried utensils for the preparation of raw meat and poultry and the preparation of RTE foods.

In Table 2 the results of the most recent studies investigating several aspects of cross-contamination are shown. Overall, the retrieved studies confirmed that cross-contamination events are happening quite frequently in the domestic environment (Anderson et al., 2004; Fischer et al., 2007; Langiano et al., 2012).

One of the most common adopted measure to avoid cross-contamination is hand washing. However, despite consumer's awareness of the importance of hand washing (Carbas, Cardoso, & Coelho, 2013; Gilbert et al., 2007; Jevšnik, Hlebec, & Raspor, 2008a; Kennedy et al., 2005; Langiano et al., 2012; Nesbitt et al., 2014; Sampers et al., 2012), effective hand washing is not practised by a significant proportion (from 60 to 70%) of respondents during food preparation (Anderson et al., 2004; Fischer et al., 2007; Kennedy et al., 2011; Phang & Bruhn, 2011; Van Asselt, Fischer, De Jong, Nauta, & De Jonge, 2009). The study of Van Asselt, De Jong, De Jonge, & Nauta (2008) investigating cross-contamination in the kitchen, observed a high decrease of bacterial transfer when hands washing was performed during meal preparation, thus highlighting the importance of proper hand washing in order to reduce cross-contamination.

Kitchen utensils and cutting boards are also key cross-contamination routes. However, most of the retrieved studies highlighted that the vast majority of consumers do not clean cutting boards and utensils sufficiently to prevent cross-contamination (Anderson et al., 2004; Fischer et al., 2007; Kennedy et al., 2005; Kennedy et al., 2011; Phang & Bruhn, 2011; Van Asselt et al., 2009).

Another important tool to prevent cross-contamination is the separation between raw and cooked food. Studies based on questionnaires and interviews indicated that consumers seemingly know how to avoid contacts between raw and cooked food (Carbas et al., 2013; Nesbitt et al., 2014). However, observational studies highlighted that direct and indirect contacts between raw and cooked food happens frequently in the domestic environment (Anderson et al., 2004; Van Asselt et al., 2009).

**Table 2.** Consumer knowledge, self-reported practices and observed behaviours associated with ***cross-contamination*** in the reviewed studies.

| CROSS-CONTAMINATION Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Handwashing** | Of the 433 observed hand washes, 34% (n = 127) were with soap, and 3.5% (n = 15) were for the recommended length of 20 seconds or longer. | OB | Anderson et al., 2004 (USA) |
| | 92% of consumers answered to wash hands before preparing raw meat. | SR-questionnaire | Carbas et al., 2013 (Portugal) |
| | Whereas most consumers washed their hands several times, 64% of participants did not use the soap. | OB | Fischer et al., 2007 (Netherlands) |
| | 52% of respondents selected a hand washing sequence that would help prevent cross-contamination. | SR-questionnaire | Gilbert et al., 2007 (New Zealand) |
| | After handling raw food, 57% of respondents wash their hands properly with soap and hot water. 34% washed hands with water only or did not wash at all (1.6%). 42% of respondents dry hands with a kitchen cloth; 27.5% use a disposable paper kitchen towel. 20% dry hands with a kitchen cloth used for wiping the dishes. | Face to face questionnaire | Jevsnik et al., 2008a (Slovenia) |
| | 65% of respondents need to wash hands after handling raw meat. 50% of respondents reported the importance of washing hands after using the toilet. | Face to face interview-questionnaire | Kennedy et al., 2005 (Northern Ireland) |
| | 70% of participants did not wash thoroughly their hands after handling the raw chicken when preparing the warm chicken salad. | OB | Kennedy et al., 2011 (Northern Ireland) |
| | After handling raw meat, 64.5% reported to always cleanse hands with soap and water, 32% with water alone and 2% with only a hand towel. | Face to face interview-questionnaire | Langiano et al., 2012 (Italy) |
| | 98-99% of consumers wash their hands frequently. 56 to 83% reported always washing their hands before preparing food and 75 to 87% reported always washing their hands after preparing food or handling raw meat. Use of soap and water (61%), water and a disinfectant soap (30%), water only (13%). | RB | Nesbitt et al., 2014 (Canada) - *review* |
| | 43% of volunteers washed their hands before beginning food preparation. 7% of all hand washing events lasted 20 s or longer. 41% of hand washing events involved the use of soap. Drying methods: shaking hands (47%), cloth towels (31%), clean paper towels (17%). | OB | Phang & Bruhn, 2011 (USA) |
| | 10% use cold water, 9.5% warm water, 40% cold water and soap, 41% warm water and soap. 64% washed their hands before and after handling the raw poultry meat. | SR-questionnaire | Sampers et al., 2012 (Belgium) |
| | Insufficient hand washing 66%. 25% of the participants washed their hands with soap after touching the raw chicken. | OB | van Asselt et al., 2009 (Netherlands) |

OB: observed behaviour; RB: reported behaviour; SR: self-reported

**Table 2 (continued).** Consumer knowledge, self-reported practices and observed behaviours associated with ***cross-contamination*** in the reviewed studies.

| CROSS-CONTAMINATION Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Surface/equipment cleaning** | 70 of 99 subjects attempted to clean the food preparation surfaces. Of the 228 cases in which raw meat directly contacted a surface, only 29% (n = 66) of the surfaces were rated as adequately cleaned after food preparation. | OB | Anderson et al., 2004 (USA) |
| | Rinsing the cutting board (24%) or the cutlery (12%) without detergent and/or with only cold water. | OB | Fischer et al., 2007 (Netherlands) |
| | A considerable percentage of respondents did not use effective means of properly cleaning cutting boards (23%) and knives (24%) after cutting raw meat. | Face to face interview-questionnaire | Kennedy et al., 2005 (Northern Ireland) |
| | 72% of participants failed to wash thoroughly the knife used in preparing raw chicken before its reuse on raw salad vegetables. 67% did not wash the chopping board after use with raw chicken and 27% prepared vegetables for the chicken salad on the board that was contaminated from raw chicken. | OB | Kennedy et al., 2011 (Northern Ireland) |
| | During food preparation, 79% affirmed using the same cutting board for raw and cooked foods. | Face to face interview-questionnaire | Langiano et al., 2012 (Italy) |
| | 83% always cleans the surfaces. 56% always washes and disinfects the cutting surface after handling raw meat and before using the surface to prepare other food. 59 to 96% uses soap and water to clean kitchen surfaces and utensils. | RB | Nesbitt et al., 2014 (Canada) - *review* |
| | 48% of volunteers washed their knives before reuse: 88% use plain water, 11% use water and soap. Drying knives: shaking off the water (88%), paper towel or dish towel (6%). | OB | Phang & Bruhn, 2011 (USA) |
| | 29% of participants managed to prevent cross contamination. Failure to adequately wash or change cutting boards (29%) and knives (33%). | OB | van Asselt et al., 2009 (Netherlands) |

OB: observed behaviour; RB: reported behaviour

**Table 2 (continued).** Consumer knowledge, self-reported practices and observed behaviours associated with ***cross-contamination*** in the reviewed studies.

| CROSS-CONTAMINATION Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Separation of raw meats from other food items** | 85% of consumers knew that raw and cooked foods should be separated in order to prevent bacterial transference. 66% of consumers said that the methods of detection of food contamination are mainly visual inspection, smell or taste. | SR-questionnaire | Carbas et al., 2013 (Portugal) |
| | 92% always, 5% sometimes and 3% never switched to a clean plate for the cooked meat after putting raw meat from a plate to the barbeque or cooking pan. 61% strongly agreed/agreed with the statement: "I have separate cutting boards for raw meats and vegetables". | RB | Nesbitt et al., 2014 (Canada) - *review* |
| **Avoid cross-contamination** | Nearly all subjects handled food in a manner that caused cross-contamination. The most common indirect transfer agents were hands (51%), counters (18%), and utensils (16%). | OB | Anderson et al., 2004 (USA) |
| | Cupboards opened with correctly cleaned or non-contaminated hands (46%); Cupboards opened with less cleaned hands (45%); cupboards opened with hands not at all been cleaned after touching raw chicken (9%). | OB | Fischer et al., 2007 (Netherlands) |
| | 41% and 28% of respondents would use knives and kitchen surfaces respectively in a manner that could allow cross contamination. | SR-questionnaire | Gilbert et al., 2007 (New Zealand) |
| | Direct contact between cooked and raw foods was only avoided in 36.5%. | Face to face interview-questionnaire | Langiano et al., 2012 (Italy) |
| | Potential cross-contamination was common, with an average of 43 events noted per household. Hands were the most commonly observed vehicle of potential cross-contamination (93%). | OB | Phang & Bruhn, 2011 (USA) |

OB: observed behaviour; RB: reported behaviour; SR: self-reported

### 1.2.2 Keep food at safe temperatures: storage practices

The storage temperature of food is a critical point in controlling the growth of bacteria and therefore the safety of food products. In particular, this is of paramount importance in case of psychrotrophic bacteria such as *L. monocytogenes*, which is able to grow at refrigeration temperature of 5°C and below.

Overall findings have revealed positive consumer attitudes toward the need for correct refrigerated storage. However, studies also revealed that large proportions of consumers (up to 80%) did not know the recommended refrigerator operating temperature (Table 3) (Jevsnik, Hlebec, & Raspor, 2008a; Kennedy et al., 2005; Marklinder, Lindblad, Eriksson, Finnson, & Lindqvist, 2004; Nesbitt et al., 2014; Ovca & Jevšnik, 2009). Several studies have been conducted on the temperature performance of domestic refrigerators, demonstrating that the majority of the refrigerators throughout the world are running at higher than recommended temperatures (0 to 5°C) and that the temperature can vary significantly according to the internal position measured (James, Evans, & James, 2008).

In addition, several studies have highlighted other consumer's storage practices, which are unsafe. In fact, up to 50% of consumers would thaw meat at room temperature instead of using the refrigerator (Table 3), thus increasing the risk of microbial growth (Gilbert et al., 2007; Jevsnik, Hlebec, & Raspor, 2008a; Langiano et al., 2012; Nesbitt et al., 2014).

Moreover, overall findings indicate that consumer understanding of use-by dates is lacking. Studies dealing with knowledge and attitudes report the awareness of the consumer on the importance of use-by dates as one of the best indicators of whether food was safe to eat and of the importance not to eat foods that are beyond their expiration dates. On the other hand, self-reported practices and actual behaviour studies show that consumers would eat food beyond the use-by date and keep in the fridge food with expired use-by date (Table 3) (Daelman, Jacxsens, Membré et al., 2013; Evans & Redmond, 2014; Marklinder et al., 2004; Van Boxstael, Devlieghere, Berkvens, Vermeulen, & Uyttendaele, 2014).

Once food is open, it is of paramount importance to store it properly. Consumer food safety studies concerning storage of opened RTE and leftover foods indicated that most consumers are aware of the importance of proper storage practices to guarantee food safety. However, 20 to 50% of consumers thought that to store food at room temperature was an acceptable practice (Evans & Redmond, 2014; Jevsnik, Hlebec, & Raspor, 2008a; Langiano et al., 2012; Nesbitt et al., 2014).

**TABLE 3.** Consumer knowledge, self-reported practices and observed behaviours associated with ***storage*** in the reviewed studies.

| STORAGE Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Thawing/Defrosting** | Forty-five of 99 subjects reported thawing frozen meat in the microwave, and 36 in the refrigerator. | RB | Anderson et al., 2004 (USA) |
| | 12 of 20 respondents defrosted the meat in the refrigerator. Six respondents defrost the meat on the kitchen worktop. | Face to face interview | Damen and Steenbekkers, 2007 (Netherlands) |
| | 60% of respondents would thaw meat at room temperature for up to 12 h. | SR-questionnaire | Gilbert et al., 2007 (New Zealand) |
| | The majority of respondents thawed meat on a kitchen counter (50%) or in hot water (13%). | Face to face questionnaire | Jevsnik et al., 2008a (Slovenia) |
| | 63% defrost meat and fish at room temperature; 27% defrost in the refrigerator. | Face to face interview-questionnaire | Langiano et al., 2012 (Italy) |
| | 51% defrosted frozen meat in the refrigerator, 31% in the microwave, 26% at room temperature and 8% in water. However, 43% sometimes to always defrosted frozen meat/poultry on the counter at room temperature. | Participants' reported knowledge/behaviour | Nesbitt et al., 2014 (Canada) - *review* |
| | 39% in the fridge, 37% room temperature, 23% microwave treatment. | Questionnaire | Sampers et al., 2102 (Belgium) |
| **Knowledge of refrigerator temperature** | Refrigerator should be set at 38°F (3°C) (average response) with a range from 0°F/°C to 55°F (13°C). Thirty subjects reported not knowing the appropriate temperature setting for the refrigerator, with 12 reporting recommended temperatures greater than 40°F (4°C). | Reported Knowledge | Anderson et al., 2004 (USA) |
| | 69% correctly answered that the temperature of food in a refrigerator should be at or below 8°C. | SR-Questionnaire | Carbas et al., 2013 (Portugal) |
| | Almost 40% of respondents did not know the temperature in their refrigerator. | Face to face questionnaire | Jevsnik et al., 2008a (Slovenia) |
| | 22% of respondents were aware of the correct refrigeration temperature of 0 to 5°C. 23% of surveyed refrigerators reportedly contained a thermometer. | Face to face interview-questionnaire | Kennedy et al., 2005 (Northern Ireland) |
| | 85% of respondents answered that 8°C should not be exceeded. 76% did not know the temperature of their refrigerator. | Interview | Marklinder et al., 2004 |
| | 60% did not know the recommended refrigerator temperature. 80% did not use a thermometer to determine if their refrigerator is cold enough. | Participants' reported knowledge/behaviour | Nesbitt et al., 2014 (Canada) - *review* |
| | 55% did not know the temperature of their domestic refrigerator or did not measure the refrigerator temperature (9.5%). | Face to face questionnaire | Ovca et al., 2009 (Slovenia) |
| | The temperature of the home refrigerator was not known by 20% of respondents. | SR-Questionnaire | Sampers et al., 2102 (Belgium) |

RB: reported behaviour; SR: self-reported

**TABLE 3 (continued).** Consumer knowledge, self-reported practices and observed behaviours associated with ***storage*** in the reviewed studies.

| STORAGE Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Handling of leftovers** | 96% of consumers knew that inadequate storage practices could result in illness. 16 to 33% of consumers thought it is acceptable to store cooked meat at ambient temperature. Observation data revealed that up to 58% of consumers failed to store food according to recommended practices. | SR-practices | Evans & Redmond, 2014 (UK) - *review* |
| | 54% of the respondents cool leftovers to room temperature and then put them in the refrigerator. 13% of respondents leave a meal that is not immediately consumed on the stove, until eaten sometime later. | Face to face questionnaire | Jevsnik et al., 2008a (Slovenia) |
| | 24% of cooked foods were stored in the refrigerator after cooling to room temperature, 28% were stored in the refrigerator within 24 hours if not consumed. | Face to face interview-questionnaire | Langiano et al., 2012 (Italy) |
| | 95% refrigerated or frozen leftovers within 2 h of cooking. 74% consumed leftovers within one to two days of initial preparation, 22% within 3 to 4 days, and 3% beyond 4 days. | Participants' reported knowledge/behaviour | Nesbitt et al., 2014 (Canada) - *review* |
| **Best-before date/ Use-by date** | 53% of the consumers fully respected the use-by date; 40% of consumers would consume the product until three days past the use-by date; 2.5% of the consumers would still consume the product after more than three days past the use-by date and 4.6% did not consider the use-by date. | SR-questionnaire | Daelman, Jacxsens, Membré, et al., 2013 (Belgium) |
| | Although three quarters of consumers indicated that it was very important to avoid consumption of foods that are beyond their expiration dates, up to 44%of consumers in another study reported they would eat food beyond the use-by date, and 60% of consumers did not know that food eaten beyond the use-by date could be unsafe. | SR-practices | Evans & Redmond, 2014 (UK) - *review* |
| | 18% percent of the respondents would not eat food beyond the best-before date; 70% said that they first smell and taste the food before discarding it.<br>46% of the respondents would not consume a food product after the consume-by date, whereas 30% would first smell and taste the food before discarding it. | Interview | Marklinder et al., 2004 (Sweden) |

SR: self-reported

### 1.2.3 Cook thoroughly

Among different food handling practices (e.g. cooling, separate raw and cooked food, cleaning and cooking), adequate heat treatment is an important factor in controlling food-borne disease (Kennedy et al., 2011; Luber, 2009; Medeiros, Kendall, Hillers, Chen, & Dimascola, 2001; Taché & Carpentier, 2014). However, undercooking is still a frequent event recorded in the domestic environment. Several observational studies reported that 30 to 70% of consumers undercooked meat (chicken or beef burgers or meatloaf) (Table 4) (Anderson et al., 2004; Kennedy et al., 2011; Phang & Bruhn, 2011; Sampers et al., 2012; Van Asselt et al., 2009).

Moreover, 50% to 88% of consumers think that subjective evaluation (visual inspection) is acceptable to determine the end of the cooking process (Lazou, Georgiadis, Pentieva, McKevitt, & Iossifidou, 2012; Redmond & Griffith, 2003). Observational studies in Europe reported up to 93% of consumers rely on visual indicators to determine the doneness of meat products (Table 4) (Anderson et al., 2004; Bergsma, Fischer, Van Asselt, Zwietering, & De Jong, 2007; Kennedy et al., 2005; Kennedy et al., 2011; Nesbitt et al., 2014; Phang & Bruhn, 2011; Sampers et al., 2012). Therefore, most of the consumers do not use thermometers in order to assess the doneness of the meat despite knowing the importance of proper cooking in eliminating bacteria (Table 4) (Anderson et al., 2004; Phang & Bruhn, 2011). The observational study of Phang & Bruhn (2011) showed that American consumers who possess a kitchen thermometer are reluctant to use it, even if they are aware of the importance of thorough internal cooking.

Finally, there is still space for improvement when handling leftovers or ready-to-reheat food before consumption. Studies highlighted that most of the consumers did not follow the reheating instructions eventually present on the label and most of the times meals are just warmed up according to their own sensory satisfaction for immediate consumption (Table 4) (Daelman, Jacxsens, Membrè, et al., 2013; Jevsnik, Hlebec, & Raspor, 2008a).

**TABLE 4.** Consumer knowledge, self-reported practices and observed behaviours associated with ***cooking/heating*** of food in the reviewed studies.

| COOK<br>Knowledge, self-reported practices, observed behaviours | Results | Type of information | Reference |
|---|---|---|---|
| **Undercooking** | 46% (17 of 46) of subjects undercooked the meat. | OB | Anderson et al., 2004 (USA) |
| | 69% of the cooked chicken samples did not achieve the optimum cook temperature (74°C). | OB | Kennedy et al., 2011 (Northern Ireland) |
| | 16% of the respondents would eat undercooked barbecued poultry meat if offered to them on a plate. | SR-questionnaire | Sampers et al., 2012 (Belgium) |
| | 30% of participants undercooked their meat. | OB | Van Asselt et al., 2009 (Netherlands); Phang & Bruhn, 2011 (USA) |
| **Check doneness of meat** | 76 of 94 subjects used a knife or another utensil to cut or poke the entree to evaluate changes in colour. Five of 94 subjects used a food thermometer. | OB | Anderson et al., 2004 (USA) |
| | The most frequently used method was cutting open fillets to check the colour of the inside of the meat. The use of thermometer was at the last place. | RB | Bergsma et al., 2007 (Netherlands) |
| | Visible inspection (40%), until the juices run clear (28%), when brown inside (12.5%), when cooked for a specified period (7.5%), until the meat fell from the bone (5%). | Face to face interview-questionnaire | Kennedy et al., 2005 (Northern Ireland) |
| | 39% of participants were observed cutting the chicken and examining the internal colour of the chicken. | OB | Kennedy et al., 2011 (Northern Ireland) |
| | 14% used a thermometer, 64% used visual cues, 33% used time, 10% used taste. | RB | Nesbitt et al., 2014 (Canada) - *review* |
| | Checking for brown interior (51%), juice clarity (38%). | OB | Phang and Bruhn, 2011, (USA) |
| | 9% used visual inspection of the exterior, 81% cut the poultry meat portion and checked the interior, 9.2% did not test or judge at all, 0.8% used a thermometer. | SR-Questionnaire | Sampers et al., 2012 (Belgium) |
| **Use of Thermometers** | 30 subjects stated to own a food thermometer and 48% reported being confident in using a food thermometer; 6 of 30 reported to use thermometer often/always in cooking. | RB | Anderson et al., 2004 (USA) |
| | 53% indicated that they owned meat thermometers; 33% said that they knew how to use a thermometer to test the doneness of burgers; 4% of households use a thermometer to check doneness of meat. | OB | Phang and Bruhn, 2011, (USA) |
| **Reheating a meal** | 50% of the consumers fully complied with the reheating instructions on the label, 36.5% only partially followed these instructions and 13% did not follow them at all. | SR-questionnaire | Daelman, Jacxsens, Membré, et al., 2013 (Belgium) |
| | 9% of respondents reported that they did not re-heat leftovers. 42% of respondents heat a meal so that it becomes warm and suitable for immediate consumption. 18% leave the meal to boil for some time. | Face to face questionnaire | Jevsnik et al., 2008a (Slovenia) |

OB: observed behaviour; RB: reported behaviour; SR: self-reported

## 1.3 Consumer preferences towards food products

In recent times, supply chains have had to become more demand-oriented and food production more responsive to feedback signals from the retail market and from consumers, who are now regarded as active agents in the food supply chain. Nowadays people consume not only to fulfil their basic biological needs, but also to express a sense of cultural identity and improve psychological wellbeing. Moreover, the worries about food safety, as consequence of food safety scandals, are moving consumers to organic and local food while mistrusting new food technologies (i.e. GMO, irradiation) (Falguera et al., 2012).

Therefore, there is an expanding demand for natural (free from chemical fertilizers or pesticides) foods and ingredients that are fresh or minimally processed and readily available. In addition, consumers are increasingly interested in foods that are linked with the place/region of origin as the local food and foods with a traditional image (Feldmann & Hamm, 2015). This change of food preferences towards traditional food products with attributes such as organic, local and artisanal, is due to the manifest consumer interest in the various food quality attributes associated with these types of food, which are perceived as higher quality, tasty and more sustainable food (environmental and climate friendly) that also provide a mean for cultural identity (Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009). In addition, cultivation of local raw materials and ingredients, which are mostly used in the production of traditional/artisanal foods, contributes to a more sustainable environment and it allows employing local people in rural areas. Therefore, consumers are willing to buy local food also in order to support the local community (Dodds et al., 2014).

According to Almli, Verbeke, Vanhonacker, Næs, & Hersleth (2011), European consumers define traditional foods as ‘‘frequently consumed or associated to specific celebrations and/or seasons, transmitted from one generation to another, made in a specific way according to the gastronomic heritage, naturally processed, distinguished and known because of their sensory properties and associated to a certain local area, region or country”. Despite the absence of a unanimous and official definition of what is intended for local, artisanal and traditional food, it is possible to find common features associated to this type of production system. In fact, such food is often produced by small/micro-scale farms and mostly sold directly at local food markets; therefore, it belongs to the so-called Short Food Supply Chains or Local Food Systems. ‘Local Food Systems’ (LFS) is defined as those where the production, processing, trade and consumption of food occur in a defined reduced geographical area (depending on the sources and reflections, of about 20 to 100 km radius).

'Short Food Supply Chains' (SFSC) on the other hand are where the number of intermediaries is minimised, the ideal being a direct contact between the producer and the consumer. Therefore these two concepts obviously overlapping (Kneafsey et al., 2013).

Moreover, concerning the size of the food enterprise, the EU recommendation 2003/361 provides the definition of micro, small and medium enterprises (SME). The main factors determining whether an enterprise is a micro or small one are: the staff headcount and either turnover or balance sheet total. Within the SME category, a small enterprise is defined as an enterprise which employs fewer than 50 persons and whose annual turnover and/or annual balance sheet total does not exceed EUR 10 million while a microenterprise is defined as an enterprise which employs fewer than 10 persons and whose annual turnover and/or annual balance sheet total does not exceed EUR 2 million (EC, 2003a).

On the policy side, the European Commission has promoted and sustained the development of such types of food chains and several EU Member States have developed legal frameworks and incentives to support them. For example, France has developed a nationally recognised definition of the SFSC, which is "characterised by no more than one intermediary between producer and consumer" and it has supported this production system with actions of training and education towards short food chain producers. Moreover, Italy has established legislative decrees for the regulation of the farmers markets and has promoted artisanal food products through regional legislation and training and education activities too (Kneafsey et al., 2013).

Besides the positive attributes associated to this type of production system, as previously stated, the safety of this type of food has to be guaranteed. The European hygiene legislation (Regulation (EC) No. 852/2004; Regulation (EC) No. 853/2004; Regulation (EC) No. 854/2004) (EC, 2004a, 2004b, 2004c) and the microbiological criteria for food stuffs laid down in Regulation (EC) No. 2073/2005 (EC, 2005) is valid for all kinds of food production systems. However, European Regulations allow a certain degree of flexibility and do not apply in case of direct supply by the producer, of small quantities of products to the final consumer or local retail establishments. In this case, each Member State establishes national rules that guarantee the achievement of the objectives of the Regulations. Therefore, EU Regulations do not strictly apply in case of supplying food products to consumers or other retail establishments on the condition that the supply is a marginal, localised and restricted activity. National legislation will define the notion of small quantities and the conditions to be fulfilled assuring that food business operators will apply Good Manufacturing and Hygienic Practices (GMP/GHP) and a simplified HACCP system

necessary to ensure food safety. In the specific case of artisanal Italian fermented sausages (salami and soppresse) a regional legislation, which can differ from region to region, states that the producer can rear and process maximum 30 pigs each year. The derived products can be sold directly to the consumer or at local markets located in the province of productions or in the nearby provinces.

However, even if the legislation allows a certain degree of flexibility, food safety assurance requires the availability of enough resources (economical and expertise) in order to build up an effective food safety management system (FSMS) among small-scale food business operators of local food system. In fact, because food products of the local-short supply chain are mainly produced in small-scale enterprises and thus in a non-standardized environment, deviations of microbiological safety can occur. Moreover, the lack of the available resources makes it difficult for the producers to have access to the needed knowledge, which can be useful in order to build up and implement an effective FSMS within the enterprise (Verraes, Uyttendaele, et al., 2015).

Literature data shows how artisanal food products harbour specific autochthonous microbial communities, which provides the specific sensory characteristics. Moreover, several researches demonstrated that the autochthonous microflora present in many traditional food products, especially fermented food products such as cheese or salami, carries out an inhibition activity towards spoilage and pathogenic bacteria (Dal Bello et al., 2010; Ferreira et al., 2009; Fontana, Cocconcelli, & Vignolo, 2005; Johnson, 2016). This antibacterial activity may often be due to the production of organic acids (lactic and acetic acids), with a consequent reduction in the pH, or to the production of a number of antimicrobial substances such as hydrogen peroxide, diacetyl and bacteriocins (Dal Bello et al., 2010; Montel et al., 2014).

On the other side, the occasional presence of food-borne pathogens in several types of artisanal food products is reported. Pathogens as *Listeria monocytogenes*, *Salmonella* and STEC have been commonly detected in artisanal fermented sausages (Ferreira et al., 2009; Lücke & Zangerl, 2014; Panagou, Nychas, & Sofos, 2013). Moreover, a Belgian study on the microbiological safety of dairy products belonging to the short food supply chain showed that *L. monocytogenes* was detected in 15% of sampled dairy products and that generic *E. coli* counts had a greater variability in products from the short supply chain compared to the conventional chain (Verraes, Uyttendaele, et al., 2015). In addition, food-borne outbreaks have been associated with the consumption of fermented meat and dairy

products produced in small-scale enterprises and belonging to the short chain market (Conedera et al., 2007; Montel et al., 2014).

However, currently the available information on the characteristics of the production process and the safety of food products put on the short-chain market are still scarce. Therefore, a better knowledge of the production process of this kind of food products highlighting the strength and weak points is needed. This could help in identifying the most critical steps of the production process that should be put under control by the producer in order to assure the safety of the products.

Even though lack of knowledge and expertise has been identified as one of the major gaps of this kind of production system (Conter et al., 2007), on the other hand, the restricted number of processing and transaction steps and employees involved facilitates efficient communication and control (Verraes, Uyttendaele, et al., 2015). Therefore, the investigation of these types of artisanal small-scale production system, focusing on the microbiological aspects as well as on the technical aspects, the knowledge, practices and expectations of producers could be helpful in order to identify gaps and better address the educational and training efforts spent by the public health authority or the food business operator itself to deliver high quality and safe foods to the consumer.

### 1.4 The Food Business Operator and the Competent Authority: role and responsibility

The request for tasty and healthy food, food as identity coupled at the same time with time scarcity of many consumers has been implicated in changes in food consumption patterns leading to an increase in the consumption of ready-to-eat or ready-to-reheat foods but also at the same time of local/artisanal food products. Therefore, the food supply system is a complex scenario in which the constantly changing of consumer's preferences for food, the diversity of food business operators being part of this system, the globalization of food trade and the consumer's expectation about food safety and food quality challenge the control and assurance of food safety. Ensuring food safety in such a complex framework is only possible with a concerted effort of all stakeholders involved including governmental agencies, food industry and consumers as well (a concept that the WHO describes as *shared responsibility*) (FAO & WHO, 1997).

The main aim of the European food policy is to assure a high level of protection of human health and consumers' interest in relation to food (EC, 2002). Therefore, since 2002, the European food safety policy is based on the farm-to-fork approach, where each step of the food chain has to deal with its own responsibility in the framework of food safety. As

mentioned above, managing food safety is a shared responsibility for all involved in the food supply chain (i.e. primary production, processing, distribution and sale), but especially for governments whose role is to develop and enforce policy and for food business operators who have the ultimate responsibility of producing safe products (EC, 2002). Food safety assurance is based on the establishment of appropriate control measures and operational food safety management systems (FSMS) throughout the food supply chain, which according to Regulation (EC) 178/2002 should be founded on science using the risk analysis framework (EC, 2002; Gkogka, Reij, Gorris, & Zwietering, 2013).

### 1.4.1 Food Business Operators

Food Business Operators are responsible of the food products they put on the market and shall ensure that all stages of production, processing and distribution of food under their control satisfy the relevant legislation requirements (EC, 2004a, 2004b). In order to fulfil this duty food business operators should develop a risk-based FSMS, which is built on the application of Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP), HACCP principles and self-checking system. In order to effectively design and implement a FSMS, several resources are needed. In fact, human resources with appropriate level of knowledge and expertise as well as enough financial resources are required. Therefore, an ongoing training and education and transfer of information on food hygiene, a risk-based approach to identify points of attention or critical control points (HACCP-based system) is needed and required.

Larger facilities consider systems related to food safety and quality as an effective investment and in most cases adequate resources to build an effective management system are available (Mari, Saija, & Janne, 2013). On the other side, small business operators may find regulations difficult to understand, lacking both the time to interpret requirements and the resources to hire outside expertise (Buckley, 2015). Therefore, small producers might experience technical and financial difficulties in complying with official food safety regulations. However, several guidance documents are available from the side of EU (https://ec.europa.eu/food/safety/biosafety/food_hygiene/guidance_en) and EU regulations allow a certain degree of flexibility that can be applied in case of selling small quantities of food products directly from the producer to the consumer in a restricted geographical area (EC, 2004a, 2004b). Thus, in order not to compromise food hygiene objectives, a simplified food safety management system should be developed and used. The use of manuals on good manufacturing and hygienic practices, guidelines and educational and training efforts

could be valuable tools in order to develop a simplified food safety system appropriate for small/micro-scale enterprises.

Moreover, under Article 3 of Regulation (EC) No 2073/2005, FBOs are obliged to ensure that the food safety criteria applicable throughout the shelf-life of the products can be met under reasonably foreseeable conditions of distribution, storage and use (EC, 2005). This means that FBO has to consider the effect of consumer's storage, cooking and handling practices in the domestic environment on microbial growth, death or survival in food products. In particular, the above-mentioned regulation states: "the food business operators responsible for the manufacture of the product shall conduct studies in order to investigate compliance with the criteria throughout the shelf-life. In particular, this applies to ready-to-eat foods that are able to support the growth of *Listeria monocytogenes* and that may pose a *Listeria monocytogenes* risk for public health". The types of studies that can be used in order to assess the growth or survival of *L. monocytogenes* in foods include: consultation of available scientific literature; predictive mathematical modelling; tests (e.g. challenge test or durability tests) to investigate the ability of the microorganism to grow or survive in the product under different reasonably foreseeable conditions of distribution, storage and use (Annex II of Regulation 2073/2005) (EC, 2005).

Since 2008, the EURL (European Union Reference Laboratory) for *L. monocytogenes* published a technical guidance document on the challenge test protocol. This document which has been updated in 2014, describes the practical aspects on the execution of challenge tests in order to comply with the EU Commission Regulation N° 2073/2005 on microbiological criteria for foodstuff (EURL-Lm, 2014). However, even if these guidelines can be useful for FBOs, details concerning the different food matrices and technical specifications on how to conduct tests according to the aim of the study are still partial and need further improvements. In addition, the so-called "reasonable foreseeable conditions" are often difficult to define due to the large variability. Temperature, for example, is identified as one of the crucial factors controlling growth of *L. monocytogenes* in RTE (ready-to-eat) food products during the shelf-life. Besides the temperature of refrigerators at retail level, which mean values ranged from 3.7 to 5.6°C (Scientific Opinion of the Panel on Biological Hazards, 2007), most of RTE products spend a considerable part of their shelf-life in domestic refrigerators. Studies show that 20 to 35% of domestic refrigeration temperature in Europe was above 8°C (James et al., 2008).

Thus, when building its FSMS, FBO need to collect and put together a lot of information, that are not always easy to gather and understand. Therefore, there is the need

to clearly summarize the information available on the farm-to-fork chain and make them easily available for the different stakeholders of the farm-to-fork chain. Moreover, concerning small-scale FBO, a simplified FSMS based on the deep knowledge of the production process and the control points, which are monitored by tools easily available, could match the objectives stated in the European food safety legislation (EFSA Panel on Biological Hazards et al., 2017; European Commission, 2016).

Finally, in the perspective of a shared responsibility of food safety, as stated in the EU food safety policy, essential and accurate information has to be provided to consumers so that they can make informed choices (Commission of the European Communities, 2000). Therefore, FBOs should provide appropriate and sufficient information on the packaging of food products, reporting details not only on the composition of the products but also on the correct storage and use of a product.

### 1.4.2 Competent Authorities

On the other side, the Competent Authority has the responsibility to develop policies that assure food safety. In particular, its key responsibility is to articulate the level of control that they expect the food industry to achieve. Therefore, in order to reach an appropriate level of protection (ALOP[2]) food metrics such as food safety objectives, threshold limits or criteria for microbiological contaminations in food have been developed and applied. Overall these set criteria or targets at the end or any other defined point of the food chain leaves flexibility to individual food chains or food business operators in the way this is achieved and how they set-up their individual food safety management system and define their control measures (Gorris, 2005).

Once defined the food safety metrics, the competent authority is responsible for carrying out official controls to verify FBOs' compliance with food safety requirements (EC, 2004c). Collection of samples at different points of the production process until the retail level as well as facility inspections and audits by public food safety agencies' personnel represent primary means of evaluating compliance with food safety requirements and therefore assess and manage food safety risks. The educational background of the inspectors should be wide enough in order to be familiar with food safety legislation, food microbiology, HACCP, risk analysis but also with the production process under examination.

[2] ALOP is defined as: "the level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal and plant life or health within its territory" (WTO, 1995).

This in fact, could help inspectors to identify the non-compliances relevant in terms of food safety.

Moreover, inspections and controls represent not only means to verify food safety legislation requirements but in addition a way to interact with FBO. Inspections represent valuable occasions for the public agency personnel to go through the points of attention or critical control points in the production process together with the FBO and give adequate guidance on legislation, thus helping the FBOs understand the reasons behind non-compliances and the relevance of correcting the non-compliances for the safety of the products (Mari et al., 2013). Therefore, in sharing information and knowledge, the inspector may influence the FBOs' understanding of the importance of product safety thus helping the FBO to be committed to food safety issues and be aware of food safety risks. In order to carry out official controls and inspections adequate resources should be allocated, thus allowing an enough frequency of inspections. In this way, the inspector and the FBO can become familiar to each other, and develop a positive and collaborative approach. On the other side, inadequate resourcing of food control agencies may lead to less frequent inspections, which may weaken the efficacy of controls (Buckley, 2015; Mari et al., 2013).

Despite the requirements of the legislation and the development of quality management systems to ensure quality and safety of the products produced by food processors, food-borne outbreaks and disease are still occurring and the trend is not decreasing, as illness and disability may result from improper consumer food handling practices. In fact, according to the European Summary Report on zoonosis and zoonotic agents (EFSA & ECDC, 2016), in case of food-borne strong-evidence outbreaks, household was the most frequent place of exposure in 2015. Therefore, there is the need to develop a responsible use of food products, which asks action from all stakeholders in the food chain, government, producers and also consumers.

Thus, another important task of the competent authority, here represented by food safety agencies, the ministry of public health or the ministry of agriculture depending on each member state, is the development and dissemination of educational campaigns on food safety addressing consumers in order to promote public health. The hazards of incorrect food preparation and storage practices as well as the tools to mitigate food safety risks should be explained in order to create awareness of responsibility among consumers. The findings of studies on consumer's knowledge and behaviour in the domestic environment should be incorporated in order to develop mitigation and communication strategies to be included in educational campaigns (Fischer, de Jong, de Jonge, Frewer, &

Nauta, 2005; Nauta et al., 2008). In US this type of campaigns are quite heavily focused on and various initiatives are taken (https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/teach-others/fsis-educational-campaigns). At European level, several governmental, national or international, programmes to create awareness about food safety at home have already been initiated. These are programmes such as the Chicken Challenge by the Food Standards Agency in the UK, and the 'ziekmakers zie je niet' (you cannot see sick makers) campaign initiated by The Netherlands Nutrition Centre Foundation, whereas in Switzerland the Federal Office for Food Safety and Veterinary Office initiated a small campaign during holiday season in December and grill season in summer period. The French Agency for Food, Environmental and Occupational Health & Safety is now working on the consumer information to prevent biological hazards, with a first inventory of hazard-food combinations (or hazard-food-vulnerable population combinations) for which better consumer information may have an impact on risk reduction (Anses, 2014).

In summary, the safety of food products and the promotion of public health are the core principles of the European food safety policy. The knowledge of the features of the different actors of the food chain is of paramount importance in order to develop effective food safety policies. In fact, the mutual understanding of the production process of a food product and of the different characteristics of large and small/micro-scale enterprises between the various food business operators being part of the food supply chain as well as between the FBO and the official inspector represent the basis in order to develop a collaborative approach.

At the same time, the knowledge of consumer's behaviour in the domestic environment and the effect of this behaviour on microbial growth, survival or death in food products, as well as the consumer's preferences for different kinds of food products and production systems are essential in order to direct the educational intervention both by the FBO and the competent authority and built a fit for purpose message.

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# CHAPTER 2. Artisanal Italian salami and soppresse: identification of control strategies to manage microbiological hazards

**Redrafted from:**

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## ABSTRACT

Traditionally fermented pork products such as salami and soppresse are increasingly interesting to consumers due to the embedded regional identity, social values and specific taste. These products are produced using traditional methods in small processing units, in uncontrolled environments and without starter cultures. Consequently, variability in the fermentation process and pH/$a_w$ decrease can occur in critical steps of production, which means that if pathogens are present the environmental conditions can allow their growth/survival. The presence of food-borne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157, and *Salmonella* spp. in fermented sausages has been reported. Therefore, this study aimed to identify control strategies that can be easily applicable and implemented by producers themselves with the supervision and control of the Competent Authority in order to manage the hazards associated with traditional salami and soppresse.

Since 2008, samples have been collected along the farm-to-fork chain in order to study the production process of salami and soppresse, estimate the prevalence of several food-borne pathogens (*Salmonella* spp., *Campylobacter* spp., *E. coli* O157, *L. monocytogenes*) and obtain data on the physical-chemical parameters (water activity ($a_w$), pH) of the products. In addition, during the production season 2009-2010, an intensive sampling plan was set up in order to determine if there was a possible correlation between the observed weight loss and $a_w$ in relation to the presence/ survival/ growth of pathogens throughout the duration of the natural fermentation process. The applied sampling plans allowed the definition of a control strategy to manage microbiological hazards associated with salami and soppresse, which was applied in the subsequent production seasons (2010-2013).

The data collected from 2008 to 2010 showed that the fermentation process was characterized by considerable variability between producers in relation to the ripening conditions, which was reflected in a wide range of pH and $a_w$ values along the different steps of the production process. Moreover, microbiological analyses performed during the 2009-2010 season showed that the main pathogen of concern was *L. monocytogenes*, which prevalence in sausage batter and in salami-soppresse was 13% (17/131) and 9% (11/124), respectively. During the 2010-2013 production seasons, *L. monocytogenes* was detected in 11% (66/620) of sausage batter and in 30% (22/72) of salami-soppresse samples. The pathogen was detected at levels above 10 CFU/g in 23% (5/22) of salami and soppresse at the end of the ripening period.

Additionally, analysis of data collected allowed us to determine that, generally, a weight loss value of at least 25% would result in an $a_w$ equal or below 0.92 for salami-soppresse,

and thus multiplication of *L. monocytogenes* to elevated numbers would be avoided, in the case of accidental contamination. Therefore, a control strategy coupled with the application of an annual monitoring plan (including environmental sampling) was defined: salami and soppresse weight loss must be controlled by producers during ripening. Salami and soppresse are allowed to be marketed when: 1) no pathogens are detected in the batter (sampled and analysed by the CA); 2) a weight loss of at least 25% is reached, and; 3) the $a_w$ value is 0.92 or less.

In conclusion, in order to avoid the marketing of potentially hazardous salami-soppresse and to improve the perception of good quality of traditional food attributed by consumers, a winning strategy is represented by the combination of several tools and expertise such as: continuous education and training of the producers; support from food scientists in order to provide new simple tools such as the one proposed in this study to manage microbiological hazards in relation to the measurement of weight loss during salami-soppresse production, and close professional collaboration between the producers and the CA.

## 2.1 Introduction

Consumer's perception of "traditional or artisanal" food is at the basis of the increasing interest towards this type of food. In fact, European consumers trade off the relative expense and time required for preparation of traditional food for its specific taste, quality, appearance, nutritional value, healthiness and safety (Almli et al., 2011; Guerrero et al., 2009). Such food is often produced by small farms, and so the rural economy benefits from the increase in activity and profits through direct sales at local food markets (Berlin, Lockeretz, & Bell, 2009; Carey, Bell, Duff, Sheridan, & Shields, 2011).

Although the term "traditional foods" is widely used, the concept of traditional food products embraces different dimensions and there are hardly any definitions that clearly define traditional foods. In order to identify "traditional" foods, the EU legislation (EC, 2006a; EC, 2006b; EC, 2012) has defined criteria based on product designations that are linked to geographical origin or traditional production methods. In addition, the EuroFIR FP6 Network of Excellence provided a definition of traditional foods which includes statements about traditional ingredients, traditional composition and traditional type of production and/or processing method (Weichselbaum, Benelam, & Soares Costa, 2009).

Among European countries, Italy is the lead producer of traditional foods and products such as foods with Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI), followed by France, Spain, Portugal and Greece (ISMEA, 2013). Additionally, it is estimated that Italy has around 5,000 traditional local food products without any certification (CIA, 2015), which could represent an important resource contributing to the development and sustainability of rural areas, providing ample variety in food choice for the consumer and a remarkable income for the economy. With its 371 typical products, Veneto region is the fourth Italian region according to number of traditional food products after Toscana, Campania and Lazio (Mipaaf, 2014). In addition, since 2007, Veneto region has implemented regional legislation which defines a simplified procedure to sell small quantities of traditional food products at local level directly from the producer to the consumer (DGR, 2007; DGR, 2008). In Veneto region, many typical fermented sausages such as salami and soppresse are produced with traditional technologies. Therefore, regional legislation has been focused firstly on these products and subsequently on other types of meat products (poultry and rabbit meat) and products of non-animal origin (canned food; fruit juices; flour and dried vegetables; bread and bakery products; extra virgin olive oil). In relation to fermented sausages, the legislation defines the production season, the maximum number of animals that can be reared and the minimum rearing period for pigs on

the production farm as well as the minimum hygienic pre-requisites of the working areas used for processing pork meat into fermented sausages. Since these sausages are mainly produced following traditional practices in small processing units, starter cultures are not added to the minced pork meat and ripening is carried out in rooms with less temperature and relative humidity control than that used by industrial manufacturers. Therefore, deviations in temperature and/or humidity can result in insufficient fermentation-drying processes, meaning the absence of pathogens in the final products is not assured. The presence of food-borne pathogens such as *Listeria monocytogenes*, *Escherichia coli* O157, and *Salmonella* spp. in fermented sausages has been reported.

Concerning *L. monocytogenes*, the pathogen was detected at the end of ripening in 40% of "Salsiccia Sarda" (a traditional southern Italian fermented sausage) with contamination levels always lower than 100 CFU/g (Meloni et al., 2012). Another study conducted in the north of Italy reported a prevalence of 15% in fermented sausages (De Cesare, Mioni, & Manfreda, 2007). Other studies investigating traditional fermented sausages at the end of the ripening period showed a *L. monocytogenes* prevalence of 10% in France (Thevenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005), 16% in Spain (Martin, Garriga, & Aymerich, 2011), 42% in Greece (Gounadaki, Skandamis, Drosinos, & Nychas, 2008) and 60% in Portugal (Ferreira et al., 2007). The prevalence of *Salmonella* spp. in traditional fermented sausages is lower than *Listeria monocytogenes*: the presence of *Salmonella* was reported in two out of 38 batches of traditional Portuguese sausages (*alheiras*) (Ferreira et al., 2007) and in three out of 21 (14%) batter samples of traditional Greek fermented sausages but not in the final products (ready to be sold) (Gounadaki et al., 2008). In relation to Shiga Toxin-Producing *Escherichia coli* (STEC), including *E. coli* serotype O157:H7, for which meat and meat products are considered the main source of infection for humans, an overall STEC prevalence of 16% was found in fresh pork sausages collected in the southern part of Italy (Villani, Russo, Blaiotta, Moschetti, & Ercolini, 2005).

In addition, food-borne outbreaks associated with the consumption of fermented meats are reported in the literature. In Veneto region of Italy, in January 2004, a family outbreak of *E. coli* O157 infection caused by a dry-fermented traditional salami made with pork meat and produced in a local plant occurred (Conedera et al., 2007). In Norway, an outbreak caused by *E. coli* O103:H25 involving 17 patients was attributed to the consumption of fermented sausages (Sekse et al., 2009). Concerning *Salmonella*, an outbreak of *Salmonella* Typhimurium DT104A involving 63 cases associated with the consumption of

traditional pork salami was reported in Lazio region of Italy (Luzzi et al., 2007). Another outbreak of *Salmonella* Typhimurium associated with the consumption of unriped salami was reported in Lombardia region of Italy (Pontello et al., 1998). *L. monocytogenes* outbreaks associated with the consumption of fermented sausages have not been reported, to our knowledge, even though *L. monocytogenes* has been implicated in several listeriosis outbreaks linked to the consumption of pre-sliced ready-to-eat deli meats (Thevenot, Dernburg, & Vernozy-Rozand, 2006). The infective doses of the above-mentioned microorganisms can vary widely according to several factors such as the strain, the susceptibility of the host, and the food matrix involved. In case of *L. monocytogenes* in susceptible individuals, it is unlikely that fewer than 1,000 cells may cause disease (Scientific Opinion of the Panel on Biological Hazards, 2007). Concerning *Salmonella* the infective dose is variable but often low numbers of cells (between 10 to 1,000) are sufficient to cause disease, the same for STEC which is known for its low infective dose (Strachan, Doyle, Kasuga, Rotariu, & Ogden, 2005; Teunis et al., 2010). The difference in dose-response relationship between the three pathogens may also, to some extent, explain the difference in stringency in surveillance. In EU Regulation, tolerance of up to 100 CFU/g of *L. monocytogenes* in ready-to-eat meat products is accepted at the end of shelf-life, whereas usually action limits of absence of *Salmonella* and STEC per 25 gram are applicable.

In order to avoid the marketing of potentially hazardous traditional fermented pork sausages (Italian salami and soppresse) produced within the Veneto region, this study was initiated by the regional competent authorities in collaboration with the small-scale producers with the following aims: a) investigate the production process of traditional salami and soppresse in Veneto region of Italy; b) identify the microbiological hazards associated with this type of food, and finally; c) identify control measures easily applicable directly by the FBO with the supervision and control of the regional CA in order to manage the hazards associated with this type of traditional fermented meat products.

## 2.2 Materials and methods

### *2.2.1 The artisanal salami and soppresse production process in the Veneto region of Italy*

With the word *salami* we intended the traditional fermented pork sausages, characterized by a weight of 600 to 800 grams, a diameter of 6 to 8 cm and a length of 20 to 30 cm, while with the name *soppresse* the traditional fermented pork sausages of 1.5 to 2 kg of weight and a diameter of 10 to 12 cm is addressed.

The different steps in the salami and soppresse production process and its technical features are described in Figure 3. Each producer can rear a maximum number of 30 pigs annually, as defined by the regional legislation. Pigs between 50 and 90 kg are bought during the summer season (June-August) and reared for approximately 18 months until they reach a weight of approximately 230 kg. Next, during the autumn-winter season (from November until March), on average every 15 days, two pigs are sent to the slaughterhouse. Thus, each production season encompasses two sequential years. After slaughter, carcasses are transported back to the producer and are usually processed immediately or are kept in a cooling room at 4°C for a maximum of one day.

The following steps can be recognised in the salami and soppresse production process: sorting meat cuts; mincing the meat; manually mixing the minced meat with the ingredients (salt-NaCl, spices) according to the recipe; stuffing the sausage batter into natural collagen gut casing; labelling the product. Next, salami and soppresse move to the fermentation phase, which can be divided into two periods: the drying and the ripening. On average, the drying period lasts 7 days for salami and 20 days for soppresse while the ripening period is usually 30-40 days for salami and at least 120 days for soppresse. On average, 200 kg of minced meat is obtained from two pigs, which in terms of production, means 40 salami, each approximately 1 kg and 15 soppresse, each approximately 2.5 kg.

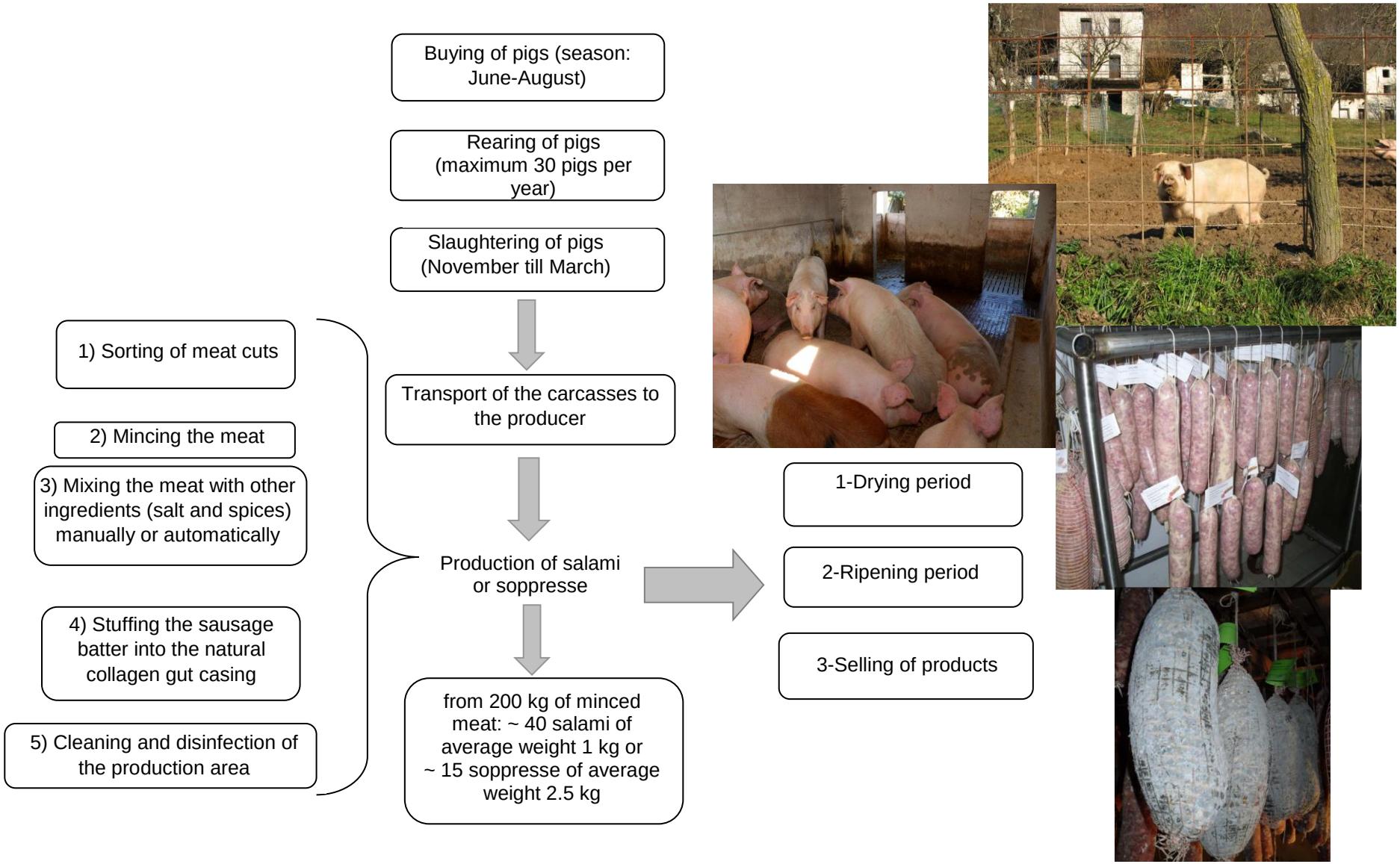



**Figure 3**. Production process of artisanal salami and soppresse.

### *2.2.2 An explorative pilot study: the production season 2008-2009*

In order to collect information on the variability of the production process, the microbiological quality and the occurrence of pathogens in traditional salami and soppresse, a *pilot study* was performed during the production season 2008-2009. The study involved 21 producers, all located in Veneto region in the north-east of Italy. Samples of at least one salami-soppresse batch (identified by the production date) per producer were collected by the local veterinary authorities. Sampling took place at primary production (one faecal sample from pigs on farm), at slaughter (one lymph node sample from pigs), at processing on the day batter was stuffed into casing (one sausage batter sample), and then during the ripening period (one salami or one soppresse) (Table 5).

The collected samples were analysed according to the ISO reference methods for the following food-borne pathogens: *Campylobacter* spp. (ISO 10272-1:2006) (ISO, 2006), *E. coli* O157 (ISO 16654:2001) (ISO, 2001a), *Salmonella* spp. (ISO 6579:2002/Cor 1:2004 (E)) (ISO, 2004c) and *L. monocytogenes* (ISO 11290-1:1996/Amd 1 2004 (detection) and ISO 11290-2:1998/Amd 1 2004 (enumeration)) (ISO, 1996, 1998a). In relation to the above-mentioned methods, the limit of detection was presence/absence in 25 grams while the limit of enumeration was equal to <10 CFU/g or $cm^2$.

In addition, in the case of batter samples and salami or soppresse samples, $a_w$ and pH were measured. The $a_w$ was measured, on samples of 5 to 10 grams, with an electric hygrometer (AquaLab) according to the ISO 21807:2004 (ISO, 2004a). The pH was measured by immersing a pH probe of a digital pH meter (Crison Basic 20) in a diluted and homogenized sample containing 3 g of sausage batter or salami-soppresse and 30 ml of KCl 0.1 M according to the ISO 2917:1999 (ISO, 1999).

### *2.2.3 The elaboration of a food safety control strategy: the production season 2009-2010*

Building further on the results of the pilot study, a general sampling plan (including 32 producers) and an intensive sampling plan (including four selected producers) was elaborated and applied during the salami and soppresse production season 2009-2010. The *general sampling plan* was aimed mainly at collecting more microbiological data and physical-chemical parameters ($a_w$, pH) of the products (either freshly stuffed sausage batter or (partially) ripened salami or soppresse), as had been done prior in the pilot study (Table 5), but now encompassing a greater number of producers (21 producers in the pilot study

and 32 in this general sampling plan). In addition, with regard to salami and soppresse, two units of one batch (identified by the production date) were identified and weighed on the first day of ripening and also on two different days nearer to or at the end of the ripening period. This enabled us to determine the weight loss, which occurred during the drying-ripening period.

Moreover, on each sampling day, $a_w$ and pH of the sausage batter or the (drying-ripening) salami or soppresse were recorded, as well as the temperature and humidity of the drying and ripening rooms. Water activity and pH were measured as described above, while the temperature and the relative humidity were measured with a digital thermometer and hygrometer (TFA, Germany).

Furthermore, an *intensive sampling plan* was developed involving two producers (A and B) for soppresse and two producers (C and D) for salami (Table 5). This intensive sampling plan was set up in order to determine if there was any correlation between the observed weight loss and the $a_w$ in relation to the presence or survival or growth of pathogens throughout the natural fermentation process. The intensive sampling plan enabled us to explore whether weight loss could be monitored and if setting a minimum threshold value for weight loss could be used as a simple tool for producers to assess the safety of the product at the end of drying-ripening period. Data collected throughout the general sampling plan were used to support the applicability of this monitoring tool.

**Table 5.** Overview of the sampling plans to collect data on microbiological and physical-chemical parameters of production of traditional salami and soppresse in Veneto region, northern Italy as applied during the production seasons 2008-2009 (n=21 producers) and 2009-2010 (n=32 producers).

| **Sample type** | **Sampling scheme** | **Microbiological parameters** |
|---|---|---|
| FAECES | 1 pooled[a] faecal sample (200 g) | *Campylobacter* spp.; *E. co*li O157 |
| LYMPH NODES | 1 pooled[a] of lymph nodes (all pigs per batch) | *Salmonella* spp. |
| SAUSAGE BATTER | 200-250 g of batter ready for stuffing or collected maximum 3 days after stuffing | *Campylobacter* spp.; *Salmonella* spp.; *E. co*li O157; *Listeria monocytogenes*; pH and $a_w$ |
| SALAMI | (2[b]) salami per batch[c]/producer, identified and weighted the first day of ripening, sampled after approximately 20 and 40 days of ripening | |
| | (4[d]) salami every Monday: $a_w$, pH, age, weigh + microbiological analysis Every Thursday: $a_w$, pH, age and weight | |
| SOPPRESSE | (2[b]) soppresse per batch[c]/producer, identified and weighted the first day of ripening, sampled after approximately 90 and 130 days of ripening | |
| | (4[d]) soppresse, once per week: $a_w$, pH, age, weight + microbiological analysis | |

[a] Pooled: faecal samples from all pigs raised on farm pooled to one batch.

[b] 2 samples in production season 2009-2010.

[c] Batch: the total number of pigs sent to the slaughterhouse on one day and the carcasses thereof received to make sausage batter and fermented sausages. If positive batches (meaning presence of one of the pathogens in at least one salami or soppresse sample) sampling of one salami or soppresse every 15 days, up to two consecutive negative results for the pathogen under consideration.

[d] Intensive sampling plan (producers A and B for soppresse, producers C and D for salami).

### *2.2.4 Making the control strategy operational: seasons 2010-2011, 2011-2012 and 2012-2013*

The sampling plans applied during the season 2009-2010 allowed the development of a control strategy to manage microbiological hazards in the production and marketing of artisanal Italian salami and soppresse in Veneto region. Figure 4 shows the decision tool which was developed and used to determine, for each step of the production process, the type and number of samples to collect and the analyses to perform according to the results of the previous sampling point. This approach was executed by the local veterinary authorities, with the limitation that resources would be available for this purpose, and with voluntary collaboration of the producers during the production seasons 2010-2011, 2011-2012 and 2012-2013. In total, 48 producers of Veneto region were involved in the monitoring plans, which were based on sampling individual batches of salami and soppresse in combination with environmental sampling and monitoring of the production environment. In addition, since 2010 a dedicated information and training program on good hygienic practices was provided for these producers, as a holistic approach to raise awareness and commitment of the producers in managing the safety and quality of the artisanal production process of salami and soppresse.

In summary, it was recommended that before slaughter, faeces and boot swabs were collected at the farm by the local veterinary authorities in order to be analysed for *Salmonella* and *E. coli* O157. Then, after pigs were slaughtered, one sausage batter sample of about 200-250 grams was collected before or after stuffing for the detection of pathogens and $a_w$ measurement. Concerning *L. monocytogenes*, the batch was considered to be satisfactory for use even if the presence of *L. monocytogenes* was detected, on the condition that the quantity of the pathogen was below 10 CFU/g. Once a 25% weight decrease of salami or soppresse was achieved - 25% weight loss being the minimum threshold set, based upon data obtained during the intensive sampling scheme - the local veterinary authorities collected one unit of salami and soppresse, which, in the case of a prior negative result for the sausage batter sample, was analysed for $a_w$, or in the case of a prior positive result for the sausage batter sample, was analysed for the pathogen previously detected and $a_w$ (Figure 4). When the measured $a_w$ of salami or soppresse was > 0.92 or the pathogen was detected, one more salami or soppresse was sampled at a later time point in the ripening period until results were satisfactory, meaning absence of the pathogen in question and $a_w$ < 0.92. The release of the production batch for sale was then allowed.

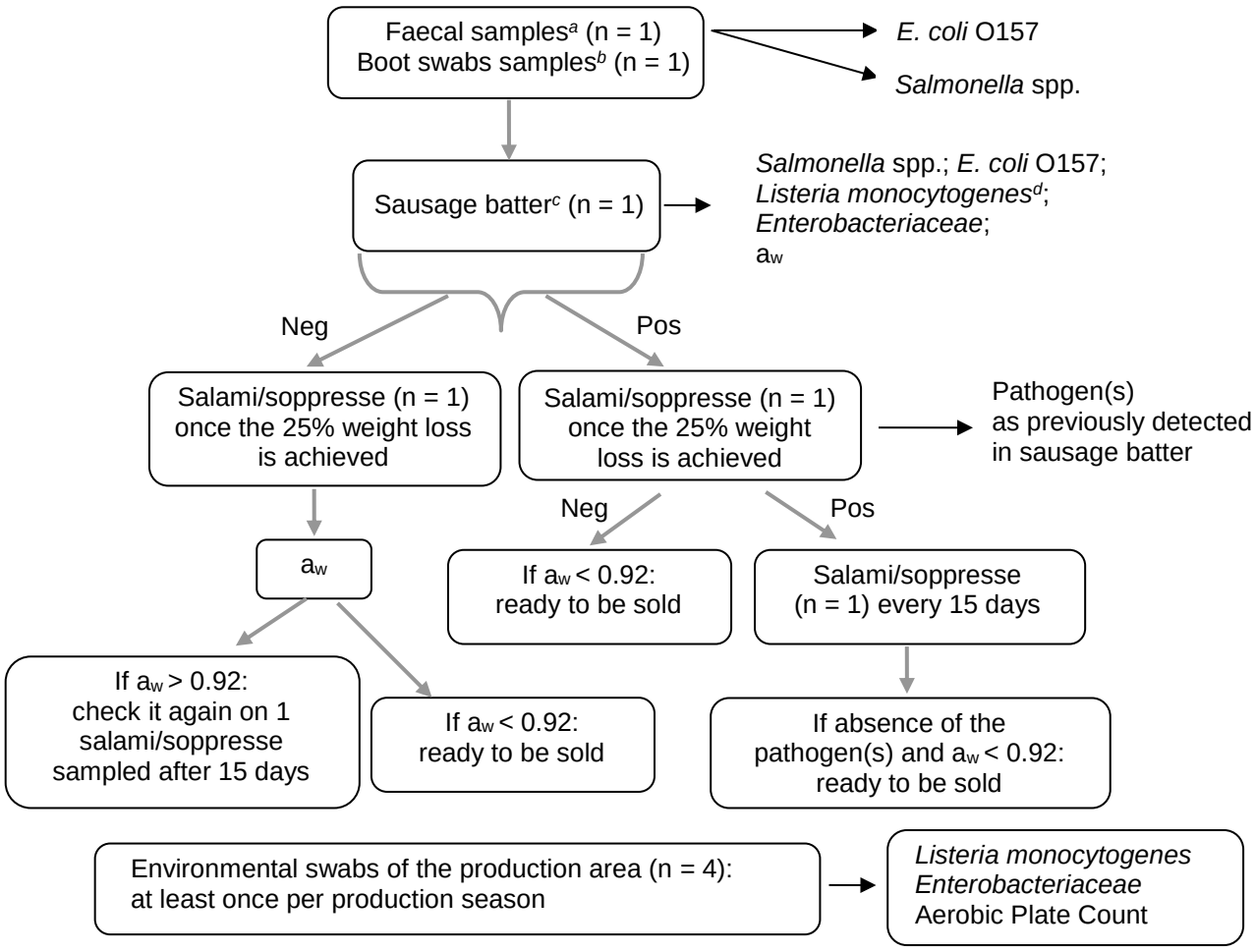



**Figure 4.** Decision tree showing the type of samples and analysis to perform in the case of positive or negative results for the investigated pathogens (n = number of samples; Neg: negative; Pos: positive) as applied during the seasons 2010-2011, 2011-2012 and 2012-2013.

[a] 1 pooled faecal sample of 200 g; [b] 1 pair per batch; [c] 200-250 g per batch; [d] If *L. monocytogenes* is detected but < 10 CFU/g, the batch is considered satisfactory for use.

Moreover, in order to have an indication of the hygienic status of the working area after cleaning and disinfection, four environmental swabs (delimited area: 100 $cm^2$) of the production area (mincing machine, mixing and stuffing machine, working tables, knives) were collected by the local veterinarians at least once per production season. The collected samples were analysed for detection of *L. monocytogenes* in 25 grams and enumeration in 25 grams of *Enterobacteriaceae* (EB) (ISO 21528-2:2004) (ISO, 2004b) and Aerobic Plate Count (APC) (ISO 4833-1:2013) (ISO, 2013) (Figure 4). In addition, as an indication of overall good manufacturing practices applied by the producers, *Enterobacteriaceae* (EB)

were enumerated (in 25 grams; LOD: <10 CFU/g) in the sausage batter samples collected for the detection of pathogens.

A traffic light approach was defined in order to easily identify situations needing increased attention in the salami-soppresse production process. A red colour indicated the presence of the tested pathogen(s) in the batch and/or of *L. monocytogenes* in the environmental samples and/or an $a_w$ value above 0.92: thus an additional ripening period, cleaning and disinfection of the production area and collection of further samples until they produced negative findings were mandatory. A yellow colour indicated that in a sausage batter sample, EB count was above 5,000 CFU/g or that in an environmental swab, APC was above 200 CFU/cm$^2$ or EB count was above 50 CFU/cm$^2$. Finally, a green colour indicated absence of the tested pathogens in the sampled units of the batch, an $a_w$ value of salami/ soppresse < 0.92 and absence of *L. monocytogenes* in the environmental samples.

## 2.3 Results

### *2.3.1 An explorative pilot study: the season 2008-2009*

The data collected during the pilot study were useful in order to study the overall variability of the production process, providing insight into the prevalence of microbiological hazards in the pigs, raw sausage batter and (nearly) final product (salami or soppresse), and the physical-chemical characteristics such as pH and $a_w$. The overall aim was to obtain an indication of control of the production process and in particular the effectiveness of the drying-ripening period during the natural fermentation process, which is important in ensuring a safe and microbiologically shelf-stable end product.

The measured $a_w$ and pH values of salami (n = 20) and soppresse (n = 34) monitored during the ripening period (on day 87 for salami and on day 162 for soppresse), collected from 21 production sites, showed a great variability between producers. For example, the $a_w$ values ranged from $a_w$ 0.83 to 0.92 on day 43 for salami and from $a_w$ 0.88 to 0.94 on day 86 for soppresse. The pH values seemed to be more uniform, although still showed substantial variation, from pH 5.3 to 6.3 for salami on day 43, and somewhat less variation for soppresse, i.e. from pH 5.5 to 5.8 on day 86.

With regard to microbiological analysis, a complete set of samples (from the pigs to the ripened salami or soppresse) was examined for 11 of the 21 producers in the pilot study. *Campylobacter* spp. and *Salmonella* spp. were not detected in any of the 75 samples analysed. *L. monocytogenes*was detected in three of 45 samples, namely one soppresse

sampled on day 130 and two salami sampled on days 43 and 88, but on all occasions numbers were below 10 CFU/g. *E. coli* O157 was detected in six of 75 samples: two pig faeces, two sausage batter, one salami and one soppresse, with the latter both sampled after 87 days of drying-ripening. Five of the six *E. coli* O157 positive samples were linked to the same producer and the same batch.

### *2.3.2 The elaboration of a control strategy for monitoring production: the season 2009-2010*

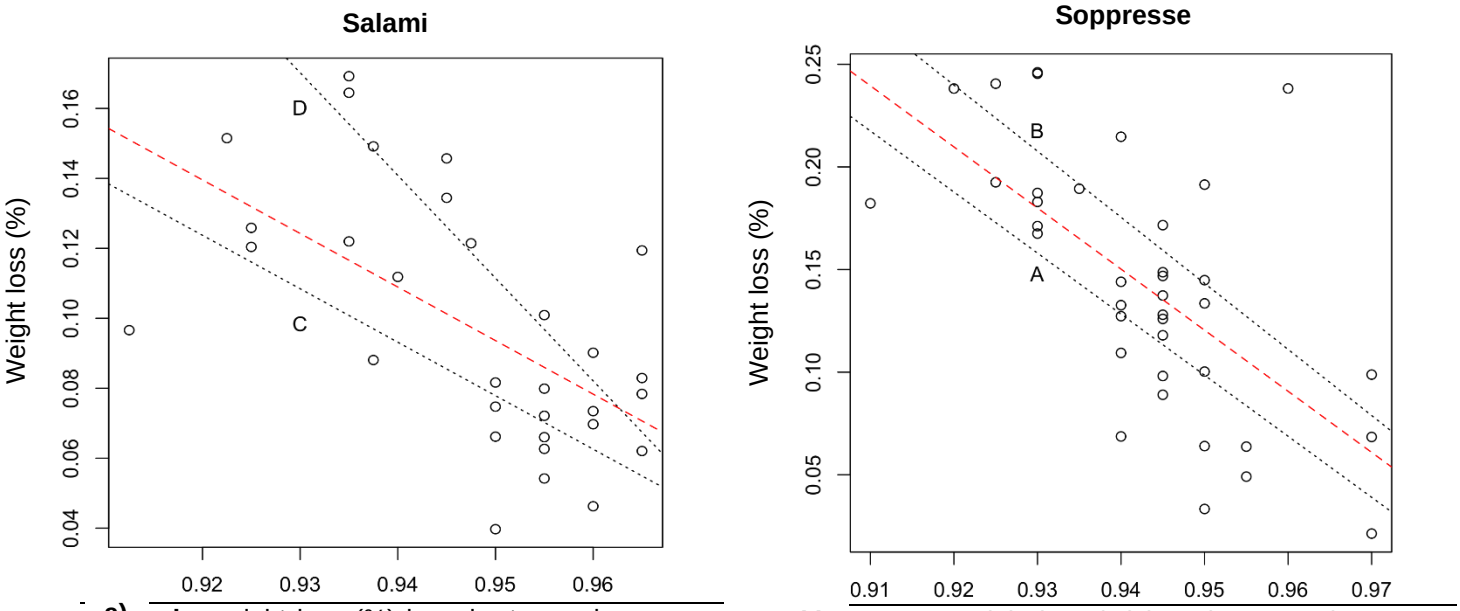
The microbiological analyses performed on samples collected during the general sampling scheme (n = 32 producers) showed that the main microbiological hazard detected was *L. monocytogenes*, with a prevalence of 13% in the sausage batter samples and 9% in the salami and soppresse during or near the end of the ripening period (Table 6). At the farm, *E. coli* O157 was never detected, not in the pig faecal samples nor in the sausage batter or fermented sausages, whereas *Salmonella* spp. and *Campylobacter* spp. were detected in 3% and 5% of samples taken from the live animals, respectively, but *Campylobacter* was not detected after slaughter in the derived batter or in the salami and soppresse ready for sale.

**Table 6**. The general sampling plan (season 2009-2010): results of the microbiological analyses (n = 32 producers).

| Sample | *Campylobacter* spp. (N. of positive samples/N. examined) | *E. coli* O157 (N. of positive samples/N. examined) | *Listeria monocytogenes* (N. of positive samples/N. examined) | *Salmonella* spp. (N. of positive samples/N. of examined) |
|---|---|---|---|---|
| Faeces | 7/138 | 0/138 | - | 6/138 |
| Lymph nodes | - | - | - | 2/137 |
| Subtotal | 7/138 (5%) | 0/138 | - | 8/275 (3%) |
| Sausage batter (until 6 days) | 0/131 | 0/131 | 17/131 | 1/131 |
| Subtotal | 0/131 | 0/131 | 17/131 (13%) | 1/131 (0.76%) |
| Salami (18-28 days) | 0/28 | 0/28 | 2/28 | 0/28 |
| Salami (31-53 days) | 0/30 | 0/30 | 4/30 | 0/30 |
| Soppresse (91-129 days) | 0/36 | 0/36 | 3/36 | 0/36 |
| Soppresse (130-161 days) | 0/30 | 0/30 | 2/30 | 0/30 |
| Subtotal | 0/124 | 0/124 | 11/124 (9%) | 0/124 |
| Total | 7/393 (1.7%) | 0/393 | 28/255 (11%) | 9/530 (1.6%) |

Figures 5 shows the correlation between $a_w$ and weight loss in salami or soppresse: a high variability of $a_w$ and weight loss values was noted. Taking into account that *L. monocytogenes* was the main pathogen of concern in this type of meat product, as confirmed by the results of microbiological analysis mentioned above, and given the fact

that according to European Regulation CE 2073/2005 (EC, 2005), products with an $a_w \leq 0.92$ are considered unable to support the growth of *L. monocytogenes*, a linear regression model of weight loss in function of $a_w$ was developed. The model estimated that a weight loss of at least 14% (Figure 5a) and 21% (Figure 5b) would be needed for salami and soppresse, respectively, in order to obtain a fermented product with an $a_w \leq 0.92$.




**a)** **mi** - weight loss (%) in order to reach an $a_w$ =

| | |
|---|---|
| Producers C+D | 14% |
| Producer C | 12.4% |
| Producer D | 19.9% |

**b)** **ɔresse** - weight loss (%) in order to reach an $a_w$ =

| | |
|---|---|
| Producers A+B | 20.9% |
| Producer A | 18.8% |
| Producer B | 23.9% |

**Figure 5.** Data collected on weight loss versus $a_w$ achieved for a) salami and b) soppresse. The internal dotted line is the estimated regression line based on all the observations and the external dotted lines are the estimated regression lines of each sampled unit.

However, the correlation between weight loss and reduction of $a_w$ was equal to 0.35 for salami and 0.40 for soppresse, thus the correlation was moderate and it did not provide an accurate predictive value of the $a_w$ content. On the other hand, it could give to the producer some indication on the goodness of the production process which can be verified later with the actual $a_w$ measurement. In fact, several factors such as the loss of water but also the production of, for example, lactic acid during natural fermentation, will affect the reduction of $a_w$.

Furthermore, during the general sampling plan (involving 32 producers) additional data on weight loss and $a_w$ were collected even if with a lower frequency (2 samples per batch/producer) compared to the intensive sampling plan (weekly collection of 4 salami/ soppresse for each of the 4 involved producers). These data were analysed with the linear regression model applied to the data of the intensive sampling plan. Table 7 shows the weight loss percentage of salami and soppresse in order to reach an $a_w \leq 0.92$ and the days needed to reach it, comparing the data set obtained during the general sampling plan (initial data set) and the data set of the intensive sampling plan (validation data set).

**Table 7**. Weight loss percentage of salami and soppresse in order to reach an $a_w \leq 0.92$ and days needed to reach it according to the validation data set (results of the general sampling plan) and the initial data set (results of the intensive sampling plan).

| Fermented sausages | Data set | Weight loss (%) | Days |
|---|---|---|---|
| Salami | Initial test data | 14% | 60 (69*) |
| | Validation data | 25% | 52 (58*) |
| Soppresse | Initial test data | 21% | 116 (137*) |
| | Validation data | 25% | 123 (124*) |

* estimated days based on the model

Taking into account the estimated weight loss percentages of the initial test data and the validation data and to be fail-safe, on the basis of these results, we established that a weight loss value of at least 25% would result in $a_w \leq 0.92$ for salami and soppresse, and thus multiplication of *L. monocytogenes* to elevated numbers would be avoided, in the case of accidental contamination. This recommendation, that a minimum 25% weight loss during the drying-ripening period must be achieved, is a tool easily and immediately applicable by the producers in order to monitor the artisanal production process and be of help to some extent to indirectly also manage the main microbiological hazard associated with salami and soppresse fermentation.

Based on this, the following control strategy was elaborated. The producer must monitor the weight loss of each batch during the fermentation process: five salami or soppresse representative of the batch (for shape and size) are chosen and weighed twice per week during the whole drying-ripening period. Once the 25% weight loss is achieved in each monitored sausage product, the producer must inform the local veterinary unit, which subsequently collects an official sample to determine the $a_w$ value and the microbial status of the product, as previously described (Figure 4).

### *2.3.3 Making the control strategy operational: seasons 2010-2011, 2011-2012 and 2012-2013*

The identification of the control strategy as mentioned above combined with the application of an annual monitoring plan including environmental sampling of the production environment provided an appropriate methodology for both CAs and this sector composed of small producers of traditional fermented sausages to control microbiological hazards associated with this type of meat product.

Moreover, during each sampling year a verification of the tool (weight loss of 25% to result in $a_w \leq 0.92$) previously identified in order to control *Listeria monocytogenes* outgrowth was performed. As an example, we reported in Figure 6 the results of the $a_w$ values as a function of the age in the sausage batter samples (in blue), salami (in pink) and soppresse (in yellow) collected during the monitoring year 2010-2011.

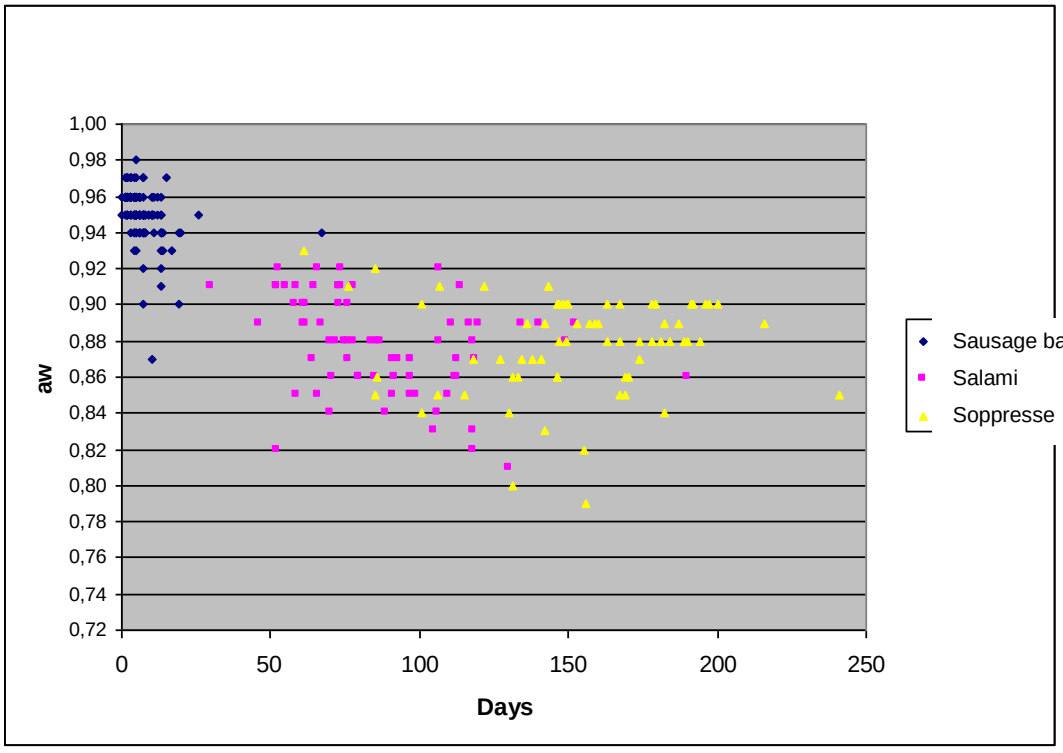



**Figure 6**. $a_w$ values of batter samples, salami and soppresse in function of the age (days) and the weight loss of 25%. ***Note***: two samples collected at days 26 and 67 were recorder as batter samples.

The age of salami collected once the weight loss was equal to 25%, ranged from 30 to 190 days, with a mean value of 88 days. The recorded $a_w$ values were from minimum 0.81 to maximum 0.92, with a mean of 0.87. In relation to soppresse, the ones sampled once the 25% weight loss was achieved, had an age ranging from 61 to 241 days, with a mean value

of 152 days. The recorded $a_w$ values were from minimum 0.79 to maximum 0.93 with a mean of 0.87.

The control strategy identified in 2009-2010 was implemented in the production seasons 2010-2011, 2011-2012 and 2012-2013, when 48 producers were involved and 654 batches of salami-soppresse were analysed, of which 42 were found positive for at least one of the investigated pathogens.

With regard to *E. coli* O157, sausage batter samples collected from two batches - they were from two different producers - were positive on day 3 and 7 after stuffing as soppresse, respectively. When units of soppresse from these batches were later analysed on days 142 and 169, *E. coli* O157 was not detected.

*Salmonella* spp. were detected in six batches from five producers: *Salmonella* spp. were detected in batter samples collected after stuffing on days 1, 2, 6, 12 and 26. Four batches were assigned to soppresse, one batch to salami and another batch to production of both salami and soppresse. Concerning salami, *Salmonella* spp. initially detected in stuffed batter on days 2 and 12, were still detected in another unit of these two positive batches analysed at days 26 and 33. Later, *Salmonella* spp. were not detected in one batch (unit analysed on day 55) but were still detected in the other batch (unit analysed on day 40). Regarding soppresse, four of five positive batches were negative when sampled between days 146 and 191, but one batch was still *Salmonella* positive on day 144.

*L. monocytogenes* was detected in batter samples from 34 batches belonging to 17 producers: 27 of 34 positive batches contained *L. monocytogenes* at levels less than 10 CFU/g while the remaining 7 positive batches had levels above 10 CFU/g. Among the 34 positive batches, 17 batches were assigned to soppresse production, 6 to salami and 11 to both types of fermented meat products.

Concerning the seven batches (sausage batter) in which *L. monocytogenes* was detected at levels > 10 CFU/g, four of the seven were assigned to the production of soppresse, one was used for salami while two were used for both salami and soppresse production. Five of the seven positive sausage batters were contaminated with *L. monocytogenes* at levels of more than 100 CFU/g (160, 190, 350, 790 and 3,300 CFU/g), one contained very high levels of *L. monocytogenes* (5,000,000 CFU/g), while another batch contained 70 CFU/g (Table 8). All of these batches were re-sampled near to the end of the ripening process.

In case of the six positive batches with low or moderate levels of contamination, *L. monocytogenes* was either not detected anymore (absence per 25 gram) in 3 of 6 salami-

soppresse batches, in other two batches it was shown to be < 10 CFU/g (detection by enumeration only) while in in one batch (soppresse on day 103) it was detected with levels of 60 CFU/g.

**Table 8.** Detection and quantification of *Listeria monocytogenes* in salami-soppresse batches (Prs: presence; Abs: absence; T1: sampling time 1; T2: sampling time 2).

| Producer | Batch | Sausage batter | T1 | T2 | Decision/ Note |
|---|---|---|---|---|---|
| **E** | Batch 1 salame | n = 1 at day 3: Prs/25 g, 3,300 CFU/g | n = 4 at day 136: 3/4 Abs/25 g and 1/4 3,000 CFU/g | - | Discarded batch |
| | Batch 3 soppressa | n = 1 at day 11: Prs/25 g, 190 CFU/g | n = 3 at day 157: Abs/25 g | - | OK |
| **L** | Batch 1 soppressa | n = 1 at day 0: Prs/25 g, 70 CFU/g | n = 1 at day 146: Abs/25 g | - | OK |
| **N** | Batch 1 soppressa | n = 1 at day 9: Prs/25 g, 160 CFU/g | n = 1 at day 99: Prs/25 g < 10 CFU/g | - | OK |
| **P** | Batch 1 soppressa | n = 1 at day 5: Prs/25 g, 350 CFU/g | n = 1 at day 117: Prs/25 g < 10 CFU/g | - | OK |
| | Batch 2 soppressa | n = 1 at day 8: Prs/25 g, $5*10^6$ CFU/g | n = 1 at day 113: Prs/25 g, $2.1*10^6$ CFU/g | n = 1 at day 180: Abs/25 g | Discarded batch |
| | Batch 2 salame | | n = 2 at day 33: Prs/25 g, $9.9*10^4$ and $2.9*10^5$ CFU/g | n = 1 at day 99: Prs/25 g < 10 CFU/g | |
| **S** | Batch 1 salame | n = 1 at day 4: Prs/25 g, 790 CFU/g | n = 1 at day 103: Abs/25 g | - | OK |
| | Batch 1 soppressa | | n = 1 at day 103: Prs/25 g, 60 CFU/g | - | Discarded batch |

In the sausage batter highly contaminated (producer P-batch 2), *L. monocytogenes* was detected in the salami and soppresse, respectively sampled on days 33 and 113 (T1), at 290,000 and 2,100,000 CFU/g. *L. monocytogenes* was not detected or it was detected with numbers < 10 CFU/g in one salami and one soppresse from the same batches, sampled on days 99 and 180 (T2). However, this result has to be considered with caution, due to the fact that on each sampling day, a different salami and/or soppresse was collected.

During the three years of the monitoring plans, three of the seven positive batches have been rejected due to presence of *L. monocytogenes* at level > 10 CFU/g in the derived fermented meat products. Thus, in cases where the numbers of *L. monocytogenes* were in the order of two log units (approximately 100 CFU/g) in the initial sausage batter, the ripening process was able to reduce those levels of one to two log units until either negative results (or < 10 CFU/g) were found, and the salami and soppresse was able to be declared as ready to be sold. On the other hand, numbers of *L. monocytogenes* in the order of six log units in batter samples were clearly unacceptable to make the batch suitable for sale.

Concerning the non-pathogenic bacterial groups analysed as an indication of good manufacturing and hygienic practices, figures 7a and 7b show the results of EB enumeration in meat samples and environmental swabs, respectively and figure 7c reports the results of APC enumeration on environmental swabs. With regard to EB, 34% of batter samples recorded EB levels above the set threshold value of 5,000 CFU/g, with some samples even containing higher levels of contamination (e.g. 16% with > 50,000 CFU/g) indicating that improvements of the cleaning and disinfection procedures for the working areas were needed (Figure 7a).

The analysis performed on environmental swabs of the working area and utensils used during the production process revealed that 24% of swabs had EB counts above the threshold value of 50 CFU/cm$^2$ and in 4% of swabs, counts between 50,000 and 500,000 CFU/cm$^2$ were determined (Figure 7b).

In relation to APC, 56% of swabs contained levels above the threshold value of 200 CFU/cm$^2$ and in 6% of swabs, counts between 200,000 and 2,000,000 were determined (Figure 7c). These results highlighted that the reinforcement of training of personnel, hygienic handling of meat and equipment, and revision of procedures are on-going requirements in the industry.

Finally, 197 environmental swabs were also collected and analysed for L. monocytogenes during the monitoring plans of three production seasons: the pathogen was detected in only one swab, which showed that environmental contamination of this pathogen was under very good control.

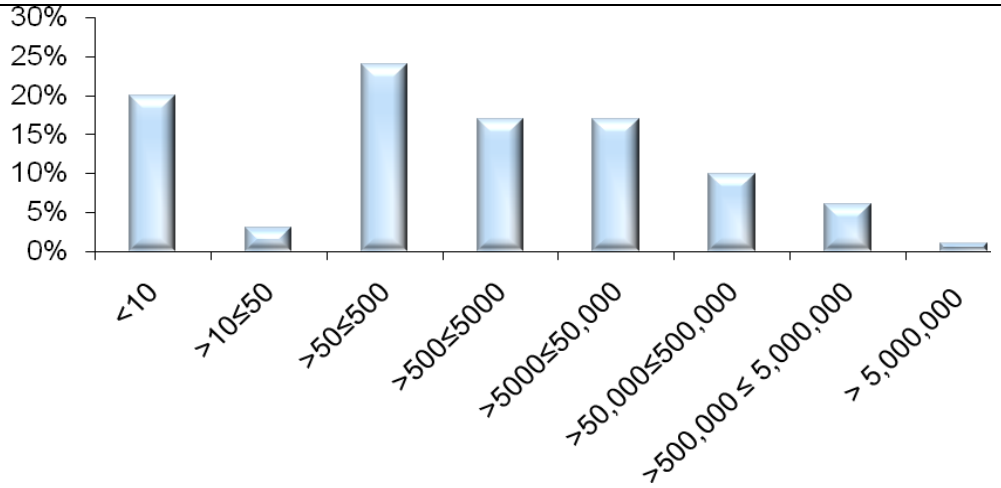



**Figure 7a.** Enumeration (n=549) of *Enterobacteriaceae* (CFU/g) in meat samples (sausage batter and salami or soppresse) collected during 2010 to 2013

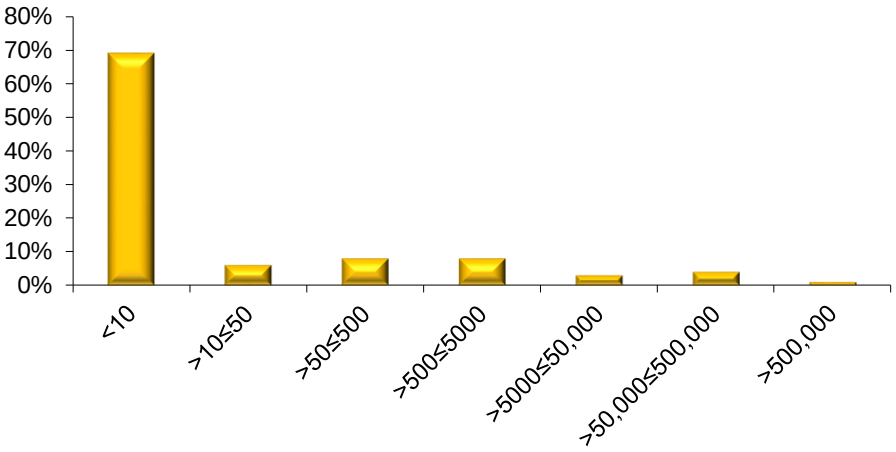



**Figure 7b**. Enumeration (n=185) of *Enterobacteriaceae* (CFU/cm$^2$) in environmental swabs from the production environment of salami or soppresse, collected during 2010 to 2013.

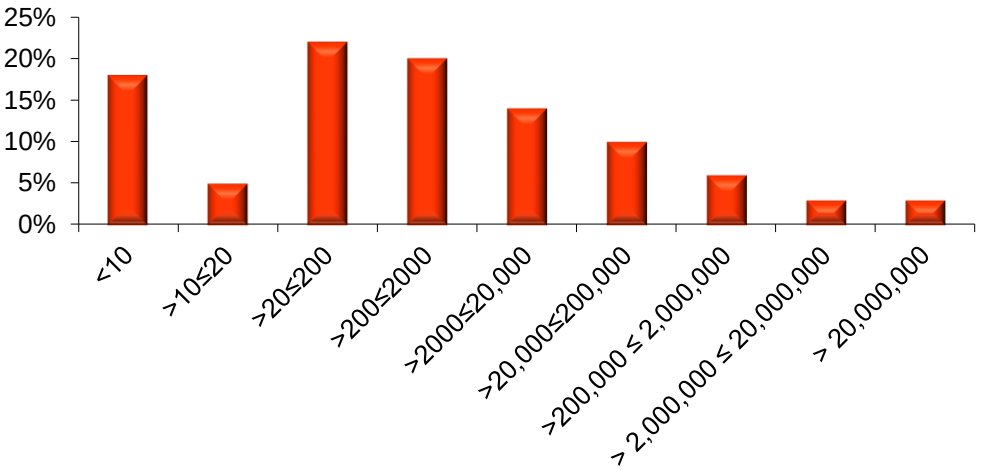



**Figure 7c**. Enumeration (n=240) of aerobic plate count (CFU/cm$^2$) in environmental swabs from the production environment of salami or soppresse, collected during 2010 to 2013.

## 2.4 Discussion

The production of traditional salami and soppresse in Veneto region of Italy has grown in the last eight years, increasing from an estimated production of 4,600 salami and 1,725 soppresse in 2008 to 21,846 salami and 8,192 in 2012 (*personal communication*). The safety of this type of traditional fermented product is mainly associated with the efficacy of hurdle technology: the combination of the use of salt, the in-house microbiota responsible for natural fermentation and the length of the drying-ripening period to cause the decreases in redox potential, $a_w$ and pH that inhibit many aerobic bacteria in the early stages of production (Fontana et al., 2005; Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006). Nevertheless, the presence of pathogens such as *Salmonella* spp., *L. monocytogenes* and STEC in fermented sausages has been previously reported (Ferreira et al., 2009; Lücke & Zangerl, 2014; Panagou et al., 2013).

This study has investigated the production process of traditional salami and soppresse in Veneto region, Italy, the microbiological hazards associated with these products, and has produced an additional useful tool in order to monitor the artisanal production process and manage the microbiological hazards. The study of the production process has highlighted that the manufacturing of traditional fermented sausages is characterized by considerable variability between producers in relation to the drying-ripening process. This, in turn, is reflected in a wide range of pH and $a_w$ values along the different steps of the production process of traditional salami and soppresse, as was also previously reported by several authors (Chevallier et al., 2006; Gounadaki et al., 2008; Lebert et al., 2007).

In relation to the microbiological analysis of foods, in the current study, food-borne pathogens were isolated from the sausage batter and the ready-to-sell meat products. In particular, the pathogen most commonly detected in the final products was *L. monocytogenes*, followed by *Salmonella* and *E. coli* O157. Concerning *L. monocytogenes*, 18 out of 51 samples (salami and soppresse) were positive, of which four contained pathogen numbers above 10 CFU/g. Literature data demonstrates that *L. monocytogenes* has a high tolerance to environmental stress factors, such as low pH conditions and high salt concentrations, and may thus be particularly likely to survive the steps involved in the manufacture of fermented meat products. A study (Thevenot et al., 2005) on artificially contaminated pork sausages has shown that *L. monocytogenes* tends to decrease substantially during the ripening period (from day 5 to day 20), probably because of the exposure to a series of hurdles (low $a_w$, low pH, high salt content). Moreover, the reduction

rate was strain dependent and hurdle-adapted strains were significantly more difficult to eliminate from sausages than non-adapted strains. Total elimination of the inoculated strains was noted in all the sausages between day 35 and day 60 (Thevenot et al., 2005).

Our results showed that during the ripening period *L. monocytogenes* was not able to grow. In addition, the ripening process can lead to a reduction of the numbers of *L. monocytogenes* present, also depending on the initial level of contamination. In fact, in case of contamination levels of approximately 100 CFU/g, *L. monocytogenes* tends to decrease 1 to 2 log units at the end of the ripening period. On the other side, when *L. monocytogenes* is present in the sausage batter at levels > 3 log CFU/g the ripening period usually applied to salami (40 to 60 days) and soppresse (120 to 240 days) do not always ensure a substantial reduction of the pathogen and still levels of > 100 CFU/g were noted. Finally, numbers of *L. monocytogenes* in the order of 6 log units per gram as found in one sample of sausage batter, shows that occasionally the ripening process is not able to reduce *L. monocytogenes* and consequently the batch should be considered unacceptable for consumption.

Levels of contamination of *L. monocytogenes* in the order of 6 log units per gram are rarely detected in foods. In the present case of fermented sausages, it is not clear what is the origin of these high numbers of *L. monocytogenes* found. It is not likely to result only from outgrowth of *L. monocytogenes* during fermentation and ripening. It is more probably linked to a higher (unacceptable) initial number of *L. monocytogenes* present on the raw pork meat, for example due to the non-respect of cold storage temperature of the pork meat (thus a lack of application of PRPs), but this hypothesis could not be verified.

In relation to *Salmonella* spp., in our study, four out of nine salami and soppresse were positive at the end of ripening. Challenge tests performed on several Italian salami with ripening periods ranging from 20 to 40 days showed that *Salmonella enterica* may decrease by 1 log or more during the fermentation and ripening processes (Mataragas et al., 2015). In addition, *Salmonella* was more sensitive than *L. monocytogenes* (the latter achieved reduction of less than 1 log in similar challenge test) to the sequence of hurdles (pH, $a_w$ and fermentation temperature) encountered during the production process of fermented sausages (Mataragas et al., 2015). Finally, in the current study with regard to *E. coli* O157, the pathogen was detected in batter samples but not in the products ready to be sold. Literature studies indicated that the association between swine STEC and human illnesses needs further investigation (Ercoli et al., 2015; Tseng et al., 2014). However, our positive finding of *E. coli* O157 in sausage batter confirms that pork meat can be a source of the

main STEC serotype (O157). Nevertheless, several strain specific virulence characteristics should be determined before linking this finding to the presence of a potential pathogenic *E. coli* O157.

Still, this study showed that the *E. coli* O157, rarely detected in the present study, was not able to survive during the ripening process. A review discussing the studies that investigated the factors associated with the reduction of *E. coli* O157 in different types of fermented sausages concluded that large variations in reduction of *E. coli* exist, ranging from nearly zero to almost 5 log reduction, whilst most reduction values lie between 1.5 and 3.5 $\log_{10}$. No single parameter influenced the survival of *E. coli* to such an extent that it can assure the complete safety of sausages (Holck et al., 2011). Therefore, an optimal combination of different hurdles would increase the safety of the sausages. Moreover, the above-mentioned studies showed that $a_w$ decrease is one of the most important hurdles encountered in the sausage manufacturing process and a key factor in the survival of microorganisms (Chevallier et al., 2006; Mataragas et al., 2015; Thevenot et al., 2006).

Predictive modelling could be a useful tool in order to get information on the changes in pathogen counts in fermented sausages during manufacturing process (Lindqvist & Lindblad, 2009; Mataragas et al., 2015). However, modelling the behavior of pathogens in artisanal – less standardized - food products, is quite difficult compared to the more industrialized foods, due to the fact that pH and $a_w$ of traditional fermented sausages are changing throughout the production process and probably also at post-marketing level. In addition, some heterogeneity of pH and $a_w$ can be observed intra-batch and inter-batch and especially between batches from various small producers.

Consequently, challenge tests with pathogens inoculated into the sausage batter can provide more reliable data concerning their fate throughout the manufacturing process (Mataragas et al., 2015). However, the use of challenge test is a costly choice, especially in the case of the artisanal sausage manufacture, which is mainly conducted by small-scale producers. In this kind of production system, the whole production system from raw material until end product and direct selling or serving to consumers is usually managed by the same person or a small team. Even though lack of knowledge and expertise has been identified as one of the major gaps of this system (Conter et al., 2007), on the other hand, the restricted number of processing and transaction steps and employees involved facilitates efficient communication and control (Verraes, Uyttendaele, et al., 2015) Therefore, the development of a tailored-fit tool to be easily and directly used by the producer would be an effective measure contributing to the management of microbiological hazards. In fact, the collection

of data regarding the weight and $a_w$ of salami and soppresse during ripening has allowed us to establish some correlation between these parameters although the correlation is not good enough for accurate predictions of $a_w$ by measuring weight loss. Still, we have identified weight loss, including a fail-safe factor, as the control point to be used by the small-scale FBO to monitor its production process and initiate further analysis to help to manage food safety of its products. In fact, once the 25% weight loss was achieved, the $a_w$ value was measured on salami or soppresse. In addition, since 2010 a booklet (Figure 8) was made available to FBO, providing information on good manufacturing and hygienic practices and on how to use the weight loss tool (and keep record of it).


**Figure 8**. Booklet on good manufacturing and hygiene practices provided to FBO. a) cover of the manual; b) sheet on the critical steps of the drying process; c) sheet on the ripening process and the weight loss measurement; d) record sheet of weight loss

Besides, the booklet gave examples of filling forms that FBO can use in order to record other important parameters of the ripening process such as: the temperature of the drying

and ripening rooms (thermometer); the amount of salt and additives added to the minced meat (balance); the humidity of the drying and ripening rooms (hygrometer) and the duration of drying and ripening (calendar).

Furthermore, the effectiveness of the provided tool (i.e. weight loss) is continuously being verified during the yearly monitoring plans. In fact, according to the control strategy adopted, once the salami or soppresse reached the 25% weight loss, at least one sample of salami/soppresse for each batch has to be controlled for the $a_w$ value and eventually for the detection of pathogens if already present in the previously analysed sausage batter. Thus, the results collected during time showed that the use of weight loss percentage combined with the education and the support of the local competent authority was a feasible and effective approach, in order to improve the safety of artisanal salami and soppresse.

Finally, in order to assess the hygienic status of the traditional salami and soppresse facilities and the application of GMP/GHP practices, indicator organisms were monitored in batter samples and environmental swabs. Our study showed that 34% of batter samples had EB counts above 5,000 CFU/g, while in relation to environmental swabs, 24% and 56% had EB counts above 50 CFU/cm$^2$ and APC counts above 200 CFU/cm$^2$, respectively. A study investigating the microbial ecosystem of traditional fermented sausage processing environments found that most of the investigated Italian processing units were characterized by low levels of contamination, regardless of the microbial groups and the surface samples. When contaminated, the most highly contaminated surfaces were mainly the knives and the tables (Talon et al., 2007). Other literature data shows that the majority of food contact surfaces in small-scale facilities producing traditional sausages are highly contaminated (> 5 CFU/cm$^2$) by spoilage microbiota such as *Enterobacteriaceae*, and that knives, tables and mincing machines are the most heavily contaminated surfaces. Moreover, high levels (> $10^4$ CFU/g) of *Enterobacteriaceae* were enumerated also in the final products (Ferreira et al., 2006; Gounadaki et al., 2008; Talon et al., 2007). In general, EB levels decrease during fermented sausage maturation due to the strong competitive effect of lactic acid bacteria. However, high levels of these microorganisms indicate product of low microbiological quality (FSAI, 2001; Gilbert et al., 2000). Concerning pathogenic microorganisms, *L. monocytogenes* was isolated in our study from only one environmental swab. The presence of *L. monocytogenes* in the pork meat processing environment has been frequently reported and its persistence in the environment represents an important source of cross-contamination of meat products (Meloni et al., 2012; Thevenot et al., 2006).

Therefore, in order to produce safe products, the FBO needs to develop a high level of awareness of its responsibility in the manufacturing of these products and manage the hazards associated with them, plus have in-depth knowledge of the production process and any associated hazards. Tools as training and education coupled with a close collaboration with the CA could represent effective means in order to provide the required knowledge and support the development of an effective FSMS.

Thus, a study was set up in order to investigate how these artisanal small-scale producers perceive the tools described in this chapter as result of the collaboration between CA and FBO.

# CHAPTER 3. Food safety knowledge and practices of small-scale producers of artisanal dry-fermented sausages (salami and soppresse) in Veneto region, Italy

**Redrafted from:**

Roccato, A., Uyttendaele, M. and Ricci A. (2017). Food safety knowledge and practices of small-scale producers of artisanal dry-fermented sausages (salami and soppresse) in Veneto region, Italy. (*In preparation*)

## ABSTRACT

Small-scale producers may lack knowledge on microbiological hazards and good manufacturing and hygienic practices. Since 2009, the local authorities of Veneto region of Italy started to put some control points along the production process of small-scale producers of artisanal fermented meat products (salami and soppresse) and developed tailored made training programs and guidelines on good manufacturing and handling practices of salami and soppresse production.

In the present study, a questionnaire was developed in order to investigate the level of knowledge and practices of small-scale producers of artisanal salami and soppresse in the Veneto region of Italy. The questionnaire consisted of 24 questions (mainly multiple-choice questions) divided over 4 parts (Part I - general information; Part II – basic knowledge on food safety; Part III – resources, infrastructures and control measures; Part IV: opinions and future needs).

The final questionnaire was delivered and collected by the local official veterinarians to the small-scale producers as part of their routine inspections from October 2016 to January 2017. 42% (34/80) of small-scale producers located in Treviso province of Veneto region (Italy) returned the questionnaire. Overall, small-scale producers of artisanal salami or soppresse had a good knowledge of the main principles of food safety. In fact, most of the respondents could identify the most relevant food-borne pathogens (97% (33/34) and 85% (29/34) of respondents indicated *Salmonella* and *L. monocytogenes,* respectively) and were aware (68%; 23/34) of the basic temperature control requirements to prevent growth or to accomplish inactivation of microbial hazards. In addition, the majority of small-scale producers, had a good knowledge of the difference between cleaning (88%; 30/34) and disinfection (94%; 32/34), of the importance of hand washing (94%; 32/34) in preventing diseases and of the main health conditions (e.g. skin wounds, diarrhoea) which did not allow food handling (68%; 23/34).

The survey clearly highlighted that most of the respondents were daily using the tools and guidelines provided by the regional authorities in order to assess the safety of their products. Moreover, almost all respondents were satisfied with the financial resources allocated by the regional authorities in order to support them and 73% (25/34) of participants would like to invest further available resources in training and education. In addition, a good collaboration with the Competent Authority was present, the inspectors being perceived as persons to discuss with in case of food safety concerns. Still a point of attention seemed to

be the documentation and record keeping of the performed activities and the production process, this answer as an option being chosen by 5 producers.

Food hygiene knowledge of food business operators is fundamental in order to prevent the transmission of food-borne pathogens and thus, protects consumers from food-borne illnesses. In order to make food hygiene knowledge effective, the support and cooperation with local food control authorities coupled with fit for purpose training and education, answering to the effective needs of the food operators, could be the starting point for the development of the awareness and motivation among small-scale producers to implement food safety management systems.

## 3.1 Introduction

Traditional food products are an important part of European culture, identity, and heritage (Guerrero et al., 2009). One such type of food product manufactured in the north of Italy is artisanal dry-fermented sausages, namely salami and soppresse, usually produced in small-scale enterprises according to recipes and techniques derived from local traditions, as described in chapter 2. Many consumers consider these type of traditional products as an important part of their consumption pattern (Conter et al., 2008). In particular, 'taste' and 'quality' are often mentioned as an important dimension of the traditional food products (Guerrero et al., 2009). Still, also food safety needs to be ensured.

The persons in charge of traditional micro or small-scale business, most of the time have to deal with multiple tasks (various aspects of the production process as well as the commercial and administrative side of sales of food products) and they may have a lack of profound food safety knowledge to assess the risks associated with their products (Taylor & Kane, 2005; Verraes, Uyttendaele, et al., 2015). In addition to a lack of expertise, also human and financial resources may be limited to develop and implement an effective Food Safety Management System (FSMS) (Ball et al., 2010; Fotopoulos, Kafetzopoulos, & Psomas, 2009). Moreover, the complexity of EU legislation, may present a challenge to these small-scale producers in complying with the legislation and assuring a safe product. However, EU legislation provides flexibility in application of some of the more complex rules for these small-scale producers, with '"marginal, localised and restricted activity" of production and distribution of traditional food products. For example EC Regulation N° 853/2004 (general hygiene rules for food of animal origin) does not apply to small quantities of products of animal origin supplied directly by the producer to the final consumer or to local retail establishments directly supplying the final consumer (EC, 2004b). For this type of production and local sale, each EU Member State can lay down under national law the rules necessary to ensure food safety. As such, often it is allowed for this type of small-scale producers not to have a fully developed tailored made HACCP plan but a simplification of it that is represented by the application of good manufacturing and hygienic practices (European Commission, 2016). Regional authorities can provide guidance or take initiatives in order to help producers comply with the relevant legislation and to develop a sense of commitment and responsibility towards food safety.

In Veneto region of Italy, the production of dry-fermented sausages by small-scale producers is part of the local culture. Since 2009, the regional competent authority has been active to put in place some controls along the production process of artisanal salami and

soppresse in order to assure the safety of these meat products. In chapter 2, the elaboration of control measures for microbiological hazards associated with the production of traditional salami and soppresse in Veneto region were presented. Microbiological analysis combined with monitoring of the weight loss of fermented sausages during ripening was shown to be an effective but simple tool to be used by small-scale producer to ensure the safety of its production process. In addition, a dedicated manual on good practices in salami & soppresse production was elaborated and provided to these producers, along with a related training program on identification of food-borne hazards and implementation of good hygienic and manufacturing practices (http://www.pplveneto.it/documenti/manuali/manuale-ppl-veneto-carni-trasformate.pdf ).

Several studies pointed out that the level of food hygiene knowledge, attitudes and practices of small food business operators is still lacking and should be implemented trough appropriate training and educational activities (Gomes-Neves, Cardoso, Araújo, & Correia da Costa, 2011; Jevšnik, Hlebec, & Raspor, 2008b; Jianu & Chiş, 2012; Walker, Pritchard, & Forsythe, 2003a). A meta-analysis study has shown that food safety training increases knowledge and improves attitudes about good manufacturing and hygienic practices and that refresher training and recurrent emphasis on GMP/GHP may have ongoing positive effects among food producers (Soon, Baines, & Seaman, 2012). Therefore, education and training represent important tools in order to build knowledge among food business operators, thus creating the awareness of their responsibility about food safety in the framework of the farm to fork chain. However, the acquired knowledge on food hygienic practices will be implemented if adequate resources (e.g. infrastructure, sufficient staff and time) and a supportive management culture exist (Clayton, Griffith, Price, & Peters, 2002; Green & Selman, 2005). In addition, in order to be effective, education should answer to the needs of the food producers and thus be tailored made.

The competent authority of Veneto region together with the regional food safety research “Istituto Zooprofilattico Sperimentale delle Venezie” (IZSVe) started in 2010 to invest time and resources in order to improve food hygiene knowledge among artisanal producers of salami and soppresse (Chapter 2). In order to investigate the effectiveness of the educational programs and the assistive approach developed towards these producers, a questionnaire was developed and administered to small-scale salami and soppresse producers in the Veneto region of Italy.

The present study describes the outcome of the above-mentioned questionnaire focusing not only on the current acquired level of food hygiene knowledge but also on the

appreciation of the efforts made by the competent authorities and further needs for education and training in food hygiene for small-scale producers.

## 3.2 Materials and Methods

### *3.2.1 Questionnaire*

Based on literature review and expert interviews, a questionnaire was developed. The questionnaire was not built with a scoring system but rather designed as a self-assessment survey that small-scale producers of salami and soppresse can fill out in order to identify how they perceived food safety and hygiene. The questionnaire consisted of 24 questions: one open question and the remainder 23 being multiple-choice questions with the possibility to select between one up to three appropriate answers among 5 to 8 possible answers given. Among the possible answers, the answer ‘do not know’ and ‘other’ (open field) were included, in order to minimize the possibility of selecting the correct answer by chance.

The questionnaire consisted of four parts. The full questionnaire (in English) is provided in the Annex 1. However, for distribution to the small-scale producers, the questionnaire was translated in Italian. Part I of the questionnaire focused on general information on the type of meat products they produced and several features of the small-scale producers that participated to the survey (e.g. gender, age, level of education, etc.). Part II (10 questions) included questions on basic knowledge on food safety. Part III (9 questions) contained questions on the resources available and infrastructures or control measures in place by small-scale producers in order to prevent that microbiological contamination can occur or growth of microbiological hazards was possible. Finally, part IV (5 questions) was elaborated in order to collect the opinion of small-scale producers on the tools provided to them by the regional Competent Authority (as described in Chapter 2) and their further needs and expectations. The questionnaire took approximately 20 minutes to complete. The questionnaire was prepared, tested and discussed in relationship to its clarity and understanding together with the official veterinarians involved with these small-scale salami and soppresse producers.

### *3.2.2 Respondents and data collection*

The final questionnaire was delivered and collected by the official veterinarians to the small-scale producers of salami and soppresse in the province of Treviso (892,000 inhabitants; 2,477 km²), Veneto region of Italy, as part of their routine inspections in the period between October 2016 and January 2017. Treviso province is one of the Veneto region provinces, which counts the highest number of registered small-scale producers. In particular, concerning micro/small-scale producers of artisanal salami and soppresse, nowadays, 80 producers are registered in Treviso province of Veneto region (regional database: https://www.regione.veneto.it/web/sanita/piccole-produzioni-locali).

The questionnaire was filled in by participants in front of an official local vet at the time of collection. Therefore, it was not possible for participants to consult information sources (e.g., internet, manuals or guidelines). The participants to the survey remained anonymous and were informed of the objectives of the survey with the understanding that confidentiality would be assured.

## 3.3 Results

### *3.3.1 General information*

Among the 80 small-scale producers producing artisanal salami and soppresse in Treviso province of Veneto region, 34 producers provided answers to the questionnaire. Therefore, a success rate of 42.5% (34/80) was reported, which is a considerable result in relation to the total number of small-scale producers of artisanal salami and soppresse available.

Concerning the *general information* (Table 9), it can be noted that 44% (15/34) of producers reared approximately ten pigs, 26% (9/34) of producers between 11 and 20 pigs and the other 26% between 20 up to above 30 pigs, thus confirming the small-scale size for the majority of these enterprises. All the respondents produced both short and long ripened meat products, most (70.5%; 24/34) focusing mainly on long ripened ‘soppresse’ products. The main location for sale was at the premises of the small-scale producers (91%; 31/34) but some also sold these products to food service operations (18%; 6 of 34 producers) or at local markets (15%; 5 of 34 producers). Having available both long and short ripened products, many small-scale producers thus build some flexibility in having batches of dry-fermented sausages available, ready to be set to market. As such, almost half (47%; 16/34)

of these producers were able to sell traditional on-farm produced meat products throughout the entire year.

**Table 9.** Summary of the results from *Part I* of the questionnaire - *general information*. Total number of respondents: 34. (NR: not reported)

| **Number of reared pigs** | **N (%)** | **Type of products** | **N (%)** |
|---|---|---|---|
| 0-10 | 15 (44%) | 30% short ripening products; 70% long ripening products | 10 (29%) |
| 11-20 | 9 (26%) | 50% short ripening products; 50% long ripening products | 9 (26%) |
| 21-30 | 7 (20%) | 20% short ripening products; 80% long ripening products | 4 (12%) |
| >30 | 2 (6%) | 40% short ripening products; 60% long ripening products | 4 (12%) |
| | | NR | 4 (12%) |
| NR | 1 (3%) | 15% short ripening products; 85% long ripening products | 2 (6%) |
| | | 60% short ripening products; 40% long ripening products | 1 (3%) |
| **Selling season (number of months)** | **N (%)** | **Main location for sale** | **N (%)** |
| 12 | 16 (47%) | Factory | 24 (70%) |
| 6 | 6 (18%) | Factory; Food services | 3 (9%) |
| NR | 5 (15%) | Factory; Local market | 3 (9%) |
| 4 | 3 (9%) | Food services | 1 (3%) |
| 9 | 3 (9%) | Local market; Food services | 1 (3%) |
| | | Factory; Local market; Food services | 1 (3%) |
| 10 | 1 (3%) | NR | 1 (3%) |
| **Gender** | **N (%)** | **Age** | **N (%)** |
| Male | 26 (76%) | 51-60 | 10 (29%) |
| Female | 8 (23%) | 41-50 | 9 (26%) |
| **Education level** | **N (%)** | 31-40 | 6 (18%) |
| High school | 18 (53%) | 61-70 | 5 (15%) |
| Primary school | 12 (35%) | 20-30 | 3 (9%) |
| University | 2 (6%) | NR | 1 (3%) |
| NR | 2 (6%) | | |
| **Starting year of production** | **N (%)** | **Years of professional experience** | **N (%)** |
| 2001-2010 | 9 (26%) | 6-10 | 10 (29%) |
| 2011-2017 | 7 (20%) | 1-5 | 5 (15%) |
| 1991-2000 | 5 (15%) | 26-30 | 5 (15%) |
| 1971-1980 | 4 (12%) | NR | 5 (15%) |
| 1981-1990 | 4 (12%) | 16-20 | 3 (9%) |
| NR | 4 (12%) | 36-40 | 3 (9%) |
| 1946 | 1 (3%) | 11-15 | 2 (6%) |
| | | 31-35 | 1 (3%) |
| | | 21-25 | 0 |
| **Food safety training** | **N (%)** | | |
| Yes | 31 (91%) | | |
| No | 2 (6%) | | |
| NR | 1 (3%) | | |

*Short ripening products*: ripening period of 40 to 60 days (e.g., salami); *Long ripening products*: ripening period of at least 120 days (e.g., soppresse).

Seventy six percent (26/34) of respondents were males; for the remaining ones the owner was female, often this activity of on-farm production of fermented meat products probably representing a second income for the family next to another job. Most of the respondents (56%; 19/34) were between 40 and 60 years old, 26% (9/34) being younger

than 40 years and 15% (5/34) aged above 60. Respondents usually obtained at least a high school diploma, but some had only finished primary school (35%; 12/34) without further secondary education. Still, 91% of respondents (31/34) declared to have received food safety training, the latter being an outcome probably of the regional initiatives and legislation in Veneto region making it mandatory for producers to take part in food safety training. Already 41% (14/34) of respondents were producing artisanal salami and soppresse even before the year 2000, confirming that this type of artisanal production of fermented meat products has a long track record in the Region, before the occurrence of regional legislation.

### *3.3.2 Basic knowledge on food safety and good hygienic practices*

Small-scale producers of artisanal salami or soppresse had overall a good knowledge of the main principles of food safety and hygiene (Table 10). In fact, 97% (33/34), 85% (29/34) and 68% (23/34) of respondents identified *Salmonella*, *L. monocytogenes* and *E. coli*, as main microbiological hazards to control for their fermented meat production activities. In fact, 59% (20/34) selected the combination of *Salmonella* and *L. monocytogenes* as the dangerous bacteria to be considered and 23% (8/34) indicated *Salmonella, L. monocytogenes* and *E. coli*, thus acknowledging *E. coli* to be important, if not always being a pathogen, then at least serving as an indicator microorganism when monitoring the hygiene *status* of a food enterprise. Concerning the effect of temperature on bacterial survival, 68% (23/34) of producers considered temperatures between 70 and 80°C able to kill bacteria, and none of the respondents considered the temperatures between 30 and 40°C to serve effective inactivation.

In relation to temperatures, which allow bacteria to multiply, 25°C was the most selected option (68%; 23/34), often in combination with temperatures of 35 or 15°C. Among the possible options, indeed 4°C (as a wrong option) was never chosen.

Health conditions which were considered not acceptable in food handling were skin wounds and diarrhoea (68%; 23/34), followed by vomit (62%; 21/34), cold (41%; 14/34) and fever (35%; 12/34). None of the respondents selected the given option of ‘headache’ as inappropriate health condition. In case of skin wound, the most frequently reported behaviours were, “protect the wound and use gloves” (94%; 32/34), “disinfect the wound and continue to work” (88%; 30/34) and “be involved in activities other than handling of meat” (56%; 19/34). Moreover, almost half of the respondents (44%; 15/34) selected the combination “be involved in activities other than handling of meat” and “protect the wound

and use gloves", showing that among producers the reflection to avoid cross-contamination of potential hazards in case of skin wound was indeed present.

Most of the producers identified correctly the different functionality of detergents *versus* disinfectants. In fact, according to their opinion detergents were useful in order "to remove dirtiness and organic matter" (88%; 30/34) and "to make surfaces clean" (65%; 22/34) while disinfectants were "able to kill the bacteria" (94%; 32/34) and "act on bacterial cells" (76%; 26/34). Ninety four percent (32/34) of producers stated they performed hand washing, an important personal hygienic measure to be taken, after going to the toilet and 65% (22/34) of respondents stated to do this before starting the working activities. Only five small-scale producers selected as an additional option to wash hands after handling raw meat.

Furthermore, the responses to the questionnaire showed that according to the small-scale food business operators involved, the main factors that might affect the safety of salami and soppresse were related to the ripening room and in particular to the hygiene and the maintenance of the ripening room (59%; 20/34) and its temperature and humidity (50%; 17/34). However, other factors such as the sequence of the working operations, the quantity of added salt, the experience of the employee and the raw materials used were chosen by at least one third of the participants. Therefore, the answers on this topic were more widely distributed over the possible options provided, highlighting that the safety of salami/soppresse is indeed a multi-factorial issue and some factors may be perceived more important than others by individual participants. Moreover, it was noted that producers selected (usually in combination) as main tools to monitor the goodness of the ripening the following ones: checking the weight once per week (65%; 22/34) and monitoring the temperature and humidity of the ripening rooms (79%; 27/34). These tools have been actively promoted and communicated to the small-scale producers as the result of the collaborative effort to determine control measures, involving some of these artisanal salami and soppresse producers, with the scientific support of the regional institute IZSVe and the Veneto region's competent authority for food safety, as was described in chapter 2.

**Table 10.** Summary of the results from *Part 2* of the questionnaire - *basic knowledge on food safety*. Total number of respondents: 34. The percentages are reported in brackets. (*Salm: Salmonella; L. m: Listeria monocytogenes; E. coli: Escherichia coli; LB: Lactobacillus; PS: Pseudomonas; Y. Ent.: Yersinia enterocolitica*). (NR: not reported)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Question1 - Dangerous bacteria*** | *Salm.* 33 (97%) | *L. m* 29 (85%) | *E. coli* 23 (68%) | *LB* 0 | *PS* 0 | *Y. Ent.* 0 | Do not know 0 | Other 0 |
| ***Question 2 - Bacteria multiplication*** | 25°C 23 (68%) | 35°C 17 (50%) | 15°C 15 (44%) | 8°C 6 (18%) | 55°C 2 (6%) | NR 1 (3%) | 4°C 0 | Do not know 0 |
| ***Question 3 - Bacterial death*** | 70°C 23 (68%) | 80°C 23 (68%) | 60°C 10 (29%) | Do not know 2 (6%) | 50°C 1 (3%) | NR 1 (3%) | 30°C 0 | 40°C 0 |
| ***Question 4 - Health conditions not acceptable*** | Diarrhoea 23 (68%) | Skin wound 23 (68%) | Vomit 21 (62%) | Cold 14 (41%) | Fever 12 (35%) | Headache 0 | Do not know 0 | Other 0 |
| ***Question 5 - In case of wound skin*** | Protect the wound and use gloves 32 (94%) | Disinfect the wound, continue to work 30 (88%) | Activities other than handling of meat 19 (56%) | Protect the wound, continue to work 2 (6%) | NR 1 (3%) | Be actively involved in daily activities 0 | Not cover the wound, continue to work 0 | Do not know 0 |
| ***Question 6 - Detergents are used for*** | Remove dirtiness and organic matter 30 (88%) | Make surfaces clean 22 (65%) | Act on bacterial cells 5 (15%) | Kill the bacteria 5 (15%) | Reduce time for cleaning 1 (3%) | Avoid tools 0 | Do not know 0 | Other 0 |
| | Kill the bacteria 32 (94%) | Act on bacterial cells 26 (76%) | Remove dirtiness and organic matter 1 (3%) | Reduce time for cleaning 1 (3%) | Make surfaces clean 0 | Avoid tools 0 | Do not know 0 | Other 0 |
| ***Question 8 – Hands are washed*** | After going to the toilet 32 (94%) | Before starting the working activities 22 (65%) | Several times during the day 18 (53%) | At the beginning and at the end of the working operations 12 (35%) | When hands are dirty 7 (20%) | After handling raw meat 5 (15%) | Other* 1 (3%) | Once during the day 0 |
| ***Question 9 – Factors influencing food safety of meat products*** | The hygiene and maintenance of the ripening room 20 (59%) | The temperature and humidity of the ripening room 17 (50%) | The sequence of the working operations 14 (41%) | The quantity of added salt 14 (41%) | The experience of the employee 12 (35%) | The raw materials 11 (32%) | Do not know 0 | Other 0 |
| ***Question 10 – Tools used to monitor the ripening*** | Monitoring temperature and humidity 27 (79%) | Check weight once per week 22 (65%) | Weekly inspection 15 (44%) | Collecting samples for microbial analysis 14 (41%) | Measure weight beginning and end of ripening process 11 (32%) | Measure pH 2 (6%) | Do not know 0 | Other 0 |

**Each time there is a contact with different materials*

### *3.3.3 Resources available, infrastructures/ control measures in place*

Concerning part III of the questionnaire, dealing with *resources and capacity* (Table 11), almost all the respondents agreed that efforts were undertaken and the local competent authority invested enough financial resources in order to support the food safety of this type of small-scale enterprises. In addition, if further financial resources would become available, the respondents indicated they would prefer to use them mainly for further "training and education" (73%; 25/34), followed by "purchase of new equipment" (56%; 19/34) and "performing lab analyses" (47%; 16/34). According to the answers provided, it seems there was less priority to allocate resources to the maintenance of the equipment, the implementation of cleaning and disinfection and pest control, maybe due to the fact that these aspects were already well in place currently. Most of the producers stated they had bought new equipment between 2008 and 2014, this period coinciding also with the beginning of the regional legislation. In particular, purchase of a mincing machine, weighting scale, thermo-hygrometer and sausage maker were often stated to be bought. Probably this latter period of investment in equipment can explain when asking participants, which lack of infrastructure being present in the premise, that 82% (28/34) of small-scale producers did not identify any, except for four respondents who indicated the temperature monitoring as the main issue to be further invested in.

Above half of the respondents indicated the cleaning and disinfection of the working areas, the application of working procedures, the personal hygiene and the registrations, as the good practices being most frequently put in place in their premises. However, only 12% (4/34) of small-scale producers chose the answer "keep a systematic documentation of records of their production process and activities being executed" and just 23% (8/34) of producers responded to perform a systematic pest control (it was also stated by four respondents in question 12 that some financial resources were (mainly) used for pest control).

Eighty eight percent of the producers (30/34) considered food safety legislation "important and necessary in order to prevent the arising of problems affecting food safety". In addition, 38% (13/34) and 35% (12/34) of respondents indicated that food safety legislation was beneficial and "made their daily working activities well organised and under control" and it "increased the trust of consumer in the food sector", respectively. Twenty three percent (8/34) of producers stated that the application of food safety legislation was part of their responsibility and daily behaviour.

A good collaboration and trust seemed to be present between producers and the regional competent authority. In fact, in case of food safety concerns, a substantial number of producers indicated to notify this to the competent authority, and thought this to be their duty (76%; 26/34) or at least selected the option to discuss a food safety problem with the competent authority (53%; 18/34). In addition, 50% (17/34) of producers stated that in case of food safety concerns the food company would react to it and take initiatives to solve the problem and take corrective measures. On the other hand, only 15% (5/34) of respondents that filled out the questionnaire indicated that they would explicitly discuss on the food safety issue within their own company team.

In reply to the question on which objectives they mainly focus on in their own premises in order to ensure food safety, participating small-scale producers selected the following options: “to adhere to good working practices as part of the everyday employee behaviour” (82%; 28/34), and “to seek to comply with current food safety legislation” (65%; 22/34). In addition, 38% (13/34) of producers set as objective in their premise “a good organisation of tasks and responsibility for each employee”. Once the objectives defined, food safety was made operational and put into practice in the premise mainly through the “application of daily cleaning of the working room and utensils at the end of each working day” (97%; 33/34), followed by “a regular check of the maintenance of the equipment” (41%; 14/34) and “the respect of the working sequence” (32%; 11/34). On the other hand, only few producers selected the given options of “to control regularly the registrations” (9%; 3/34) and “to discuss the problems with the colleagues” (6%; 2/34) which is in line with other few responses related to filling out registrations and discussion of food safety problems within company for other similar questions mentioned above.

**Table 11.** Summary of the results from *Part 3* of the questionnaire - *resources and capacity*. Total number of respondents: 34. The percentages are reported in brackets. (NR: not reported)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Question 11 - Financial resources are enough*** | Agree | Totally agree | Disagree | Neutral | Totally disagree | | | |
| | 17 (50%) | 15 (44%) | 1 (3%) | 1 (3%) | 0 | | | |
| ***Question 12 – Financial resources are used for*** | Training/ education | Equipment purchasing | Lab analysis | Equipment maintenance | Implementation of cleaning/disinfection | Pest control | External consultants | Other |
| | 25 (73%) | 19 (56%) | 16 (47%) | 8 (23%) | 6 (18%) | 4 (12%) | 1 (3%) | 0 |
| ***Question 13 – Tools bought 2008-2014*** | Mincing machine | Weighting scale | Thermo hygrometer | Sausage maker | Mixing machine | Nothing | pH meter | Other |
| | 18 (53%) | 17 (50%) | 15 (44%) | 15 (44%) | 6 (18%) | 5 (15%) | 3 (9%) | 0 |
| ***Question 14 – Lacking infrastructures*** | None | The temperature monitoring | Other** | The working space is not enough | The available equipment | The drying/ripening rooms | The working area | The maintenance of working area |
| | 28 (82%) | 5 (15%) | 2 (6%) | 1 (3%) | 0 | 0 | 0 | 0 |
| ***Question 15 – Procedures, guidelines in place*** | Cleaning/disinfection of the working areas | Working hygiene and procedures | Personal hygiene | Registrations | Maintenance of the equipment | Pest control | Documentation and records | NR |
| | 20 (59%) | 18 (53%) | 16 (47%) | 16 (47%) | 12 (35%) | 8 (23%) | 4 (12%) | 1 (3%) |
| ***Question 16 – Food safety legislation*** | It is important and necessary | It increases the trust of consumer | Makes my daily work well organized/ controlled | It is my responsibility/ part of daily behaviour | It enables me to put products on the market | It helps to harmonize | It is mandatory but not essential | Other |
| | 30 (88%) | 13 (38%) | 12 (35%) | 8 (23%) | 7 (20%) | 1 (3%) | 0 | 0 |
| ***Question 17 – In case of food safety concerns*** | It is my duty to notify the CA | It is common to discuss it with the CA | The food company solve the problem/take measures | Discussion within the company team | Discussion with colleagues of other companies | Usually the CA is not notified/ consulted | Any action will be taken | Other |
| | 26 (76%) | 18 (53%) | 17 (50%) | 5 (15%) | 1 (3%) | 0 | 0 | 0 |
| ***Question 18 – These objectives are set*** | Good working practices are part of daily behaviour | Comply with food safety legislation | Good organisation | Obtain consumer's trust | Not to be involved in food safety illness/outbreak | Avoid complaints from the consumer | Sell more products | Other |
| | 28 (82%) | 22 (65%) | 13 (38%) | 8 (23%) | 7 (20%) | 6 (18%) | 1 (3%) | 0 |
| ***Question 19 – Food safety is put in practice with*** | Each day room and utensils are cleaned | Check regularly the maintenance of the equipment | The working sequence is defined and respected | A remark is done in case of failure | I stress the importance of good quality products | I regularly control registrations | I discuss the problems | Other |
| | 33 (97%) | 14 (41%) | 11 (32%) | 9 (26%) | 8 (23%) | 3 (9%) | 2 (6%) | 0 |

*** In case of financial support, I will improve the working environment; the equipment and the working space is not used from the middle of February until the middle of November (9 months).*

### *3.3.4 Opinion and expectations of small-scale producers*

Finally, part IV of the questionnaire (Table 12), allowed to collect some information on the small-scale producer's personal experience on the tools provided to this sector by the regional competent authority, and any further supportive activities that small-scale producers would appreciate. Overall, these salami and soppresse producers were (very) satisfied with the guidelines on good manufacturing and hygienic practices provided to them by the competent authority.

Moreover, during the production season 2011-2012, an *ad hoc* database (IT tool), enabling the recording and tracing of salami/soppresse production, was set available that could be accessed both by the producers and the official vets of the competent authority in order to allow a rapid identification of critical situations (e.g. a batch testing positive for food-borne pathogens). After several years of use of the IT tool, producers thought that it was helpful in order to detect in advance food safety problems (82%; 28/34). However, despite the other positive opinions ("it allows to understand problems and find solutions"; "it is an effective tool") chosen by almost half of the respondents, 18% (6/34) of respondents stated that the tool was not organised in an easy and user-friendly way.

Another topic covered by the provided guidelines was food labelling and thus, a question on this aspect was also presented in the questionnaire. Appropriate labelling is a legislative requirement but not always fully understood or correctly applied by these small-scale producers. Almost all respondents (91%; 31/34) answered that the main function of food labelling was to guarantee traceability.

At the end of the survey, when asking which topic should be better explained during training courses, the answers provided were distributed over the various options provided. The top four topics were noted to be related to routes of cross-contamination (35%; 12/34), the HACCP system (32%; 11/34), food legislation and food-borne illness (29%; 10/34). In addition, it seems that training and education activities are highly valued by small-scale producers, as many requested further initiatives for additional education and training in the open question at the end of this survey.

**Table 12.** Summary of the results from *Part IV* of the questionnaire - *other*. Total number of respondents: 34. The percentages are reported in brackets.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Question 20 – The available guidelines are useful*** | I agree<br>24 (70%) | I totally agree<br>10 (29%) | Totally disagree<br>0 | Disagree<br>0 | Neutral<br>0 | | | |
| ***Question 21 – Which topics should be better explain*** | Cross-contamination routes<br>12 (35%) | HACCP system<br>11 (32%) | Food legislation<br>10 (29%) | Food poisoning and food-borne illness<br>10 (29%) | GMP and GHP<br>7 (20%) | Cleaning/ disinfection<br>5 (15%) | Personal hygiene<br>4 (12%) | NR<br>3 (9%) |
| ***Question 22 – My opinion on IT tool*** | It helps to detect in advance food safety problems<br>28 (82%) | It allows to understand problems and find solutions<br>15 (44%) | It is an effective tool<br>15 (44%) | I used it regularly<br>10 (29%) | It is organized in an easy and user friendly way<br>6 (18%) | I don't get any advantage/ improvement<br>0 | It is complicated<br>0 | Other<br>0 |
| ***Question 23 – Food labelling*** | It guarantees the traceability<br>31 (91%) | It is a law requirement<br>13 (38%) | It provides several useful information<br>11 (32%) | It allows a synthetic but effective description of the product<br>9 (26%) | It identifies the producer<br>8 (23%) | It guarantees the safety<br>8 (23%) | It is an important tool but not easy<br>2 (6%) | Other<br>0 |
| ***Question 24 – Suggests further initiatives (open field)*** | Training courses<br>5 (15%) | Meetings<br>2 (6%) | Reduction of the selling time<br>1 (3%) | A higher collaboration between operators and authorities<br>1 (3%) | | | | |

## 3.4 Discussion

In the framework of food safety, EU Regulation 852/2004 indicates that food business operators and food handlers must be adequately trained in food hygiene matters and on the principles of the HACCP system (EC, 2004a). It is widely recognized that basic knowledge and understanding of good manufacturing and handling practices represent the starting point in order to establish and apply an effective Food Safety Management System (FSMS) in food businesses, thus protecting consumers from food-borne illnesses (Buccheri, Mammina, Giammanco, Giammanco, La Guardia, & Casuccio, 2010; Seaman, 2010). Therefore, training and education are essential to ensure that workers have the awareness and the knowledge necessary to comply with food hygiene demands and be actively involved in the prevention of food-borne illnesses (Gomes-Neves et al., 2011; McIntyre, Vallaster, Wilcott, Henderson, & Kosatsky, 2013; Yapp & Fairman, 2006).

The present study investigated the food hygiene knowledge and practices and the employees' attitude towards food safety among small-scale producers, producing the traditional fermented salami and soppresse in Treviso province of Veneto region, in the north of Italy. Among the 80 producers of Treviso province of Veneto region, local vets collected thirty-four questionnaires. It can be argued that a success rate of less than 50% is not satisfactory considering that these FBOs were already involved in educational programs by local vets. However, it has to be stated that this survey was managed by the local veterinary services that had to collect the questionnaire during the period of one year. During that time, one vet was employed for only three months in order to follow the activities of these small-scale producers (and thus collect the questionnaire). Therefore, due to the scarcity of resources, it was not possible for the local vets to organize systematically the collection of the questionnaire from all small-scale producers.

In relation to the general features of the investigated FBOs, most of the time, the producers had few people working there and could thus be identified as either 'micro-businesses' (less than 10 employees) or the remainder as 'small-scale producers' (less than 50 employees), according to EC Recommendation 2003/361 (EC, 2003a). In particular, almost half (15 of 34) of the producers reared maximum 10 pigs, the meat of it being used for dry-fermented sausage production. Working spaces of these small-scale premises were usually restricted to few rooms where several activities were subsequently or in parallel performed.

Due to the paucity of resources and the broad kind of activities to deal with, a lack of knowledge and specialization in this traditional micro or small-scale business could have

been expected. However, it seemed that these salami and soppresse producers had a good knowledge of the main principles of food safety. In fact, the most relevant food-borne pathogens (e.g. *Salmonella* and *L. monocytogenes*) were well known by most of the respondents. In addition, since the temperature control is frequently a critical control point of the production process, the issue of poor temperature understanding could be a major obstacle for food safety. In fact, several other surveys on food handlers in small-scale food production businesses and/or food service operations reported the lowest level of knowledge in particular on this topic of appropriate temperature control (Gomes-Neves, Araújo, Ramos, & Cardoso, 2007; Jianu & Chiş, 2012; Martins, Hogg, & Otero, 2012; Walker et al., 2003a). However, in the present study most of the respondents were aware of the basic temperature control requirements to prevent growth or to accomplish inactivation of microbial hazards. Concerning health conditions not acceptable in food handling, most employees correctly identified that they should not handle food when dealing with health problems as skin wounds, vomit, cold and fever. The majority of small-scale producers also recognized the importance of the sanitation program and had a good knowledge of the difference between cleaning and disinfection. This good knowledge on these aspects related to cleaning and sanitation of premises, surfaces and utensils, was usually also reported by other studies (Jianu & Chiş, 2012; Martins et al., 2012; Walker et al., 2003a). The importance of hand washing in preventing disease was well known among small-scale producers in the present study. Positive attitudes to hand washing among food handlers have been reported in other studies as well (Angelillo, Viggiani, Rizzo, & Bianco, 2000; Jianu & Chiş, 2012; Pichler, Ziegler, Aldrian, & Allerberger, 2014).

Most of the respondents indicated correctly that the safety of their products (salami and soppresse) was mainly depending on how the ripening process was conducted. Thus, the hygiene, the temperature and the relative humidity of the ripening room were indeed acknowledged to be among the main factors affecting the ripening process. Therefore, the correct application of standard operating procedures (SOPs), such as the measurement of weight loss and the measurement of temperature and humidity of the ripening room, coupled with the application of PRPs (Pre-Requisite Programs) were rightly stated by the respondents, to provide protection against the proliferation and survival of the main microbial hazards identified. As such, the survey clearly demonstrated that indeed most of the respondents understood and had implemented the guidelines provided by the regional competent authorities.

Participants indicated they were satisfied with the financial resources allocated by the regional authorities in order to support them, ensuring the safety of their traditional production system and thus the trust to consumers, which is important for positively influencing the perception of traditional foods, being high quality foods providing added value to the producers. In addition, respondents would use the available financial resources for investment in new equipment but it should be highlighted that still the majority of them would mainly like to invest further resources in training and education. This last statement was reaffirmed by small-scale producers in filling out the open question, showing a clear appreciation of food hygiene training.

Still, a point of attention seems to be the registration and documentation of actual implementation of activities related to good practices. In a survey by Walker, Pritchard, & Forsythe (2003b), only 65% of small-scale producers kept some form of records and the majority did not understand the importance of documentation other than the one requested from the health officer. Several studies highlighted that in companies where the verbal communication plays a major role in the management of their business (as the case for small-sized enterprises), documentation and record keeping is perceived by producers as time consuming with a lot of additional work, thus hindering the effective implementation of PRP or HACCP system (Dzwolak, 2014; Jevšnik, Hlebec, & Raspor, 2009; Taylor & Kane, 2005). Documentation and record keeping is a crucial part in self-checking system and in small-scale businesses and hence, they should be integrated into the existing practices with minimal disruption (Luning et al., 2009). Therefore, if simple registration forms are set available, as they were in this case study with examples of forms being part of the dedicated manual provided to salami and soppresse producers in the Veneto region, recording implementation of ‘good practices’ should not request too much administration. Another point of attention that was noted from the questionnaire was the sharing and discussion of the problems raised in the enterprise among employees, which was performed by only five respondents. Communication and involvement represent important tools in order to develop highly motivated employees, which will improve the teamwork and the efficiency of the FSMS (De Boeck, Jacxsens, Bollaerts, & Vlerick, 2015).

The opinion of small-scale producers on food control and the regional food safety authority was strongly positive. In fact, there was quite a lot of trust and confidence in the competent authority, the inspectors being perceived as persons to discuss with in case of food safety concerns. This is a positive result and probably it reflects the outcome of the allocated time and resources spent by the regional competent authority to act constructively

in providing guidelines and trying to improve this kind of traditional food production system. Therefore, this type of constructive cooperation with the competent authority, which should not be seen only as 'control' authority, could be beneficial for both sides. Other papers (Conter et al., 2007; Mari et al., 2013) have reported the positive effect of the cooperation between small-scale producers and competent authority. In particular, Mari et al., (2013) reported that 90% of several types of food business operators in Finland perceived local inspectors as the most important sources of information concerning food safety and that the discussion with them helped to understand the food safety risks of their processes.

The present study has some limitations. It was limited to small-scale producers of artisanal salami and soppresse in the province of Treviso and cannot be representative for this type of producers throughout Italy. However, considering that the majority of small-scale producers of artisanal salami and soppresse of Veneto region (Italy) are located in Treviso province and that the response rate was 42%, the results of this survey can be considered representative of small-scale producers of artisanal salami and soppresse of Veneto region (Italy). Furthermore, the respondents might have been inclined to react and answer positively to the questions as they filled out the questionnaire in the presence of an official vet and there was already a collaboration with the local veterinary services. Therefore, there might have been a conflict of interest in replying to the questionnaire. However, overall, the questionnaire was a final survey to measure the satisfaction and adoption of food hygiene and safety knowledge and practices by these small-scale producers participating in the training and education program.

This survey is also limited by the fact that the results were derived from self-reported practices and behaviours. It has been pointed out that acquired knowledge on food safety could be not supported by the 'observed' practice (McIntyre et al., 2013). In fact, several studies, have demonstrated a lack of correlation between food hygiene training and improvements in food hygiene/handling behaviour. Although training may increase knowledge on food safety, this does not always result in a positive change in food handling behaviour (Clayton et al., 2002; Roberts et al., 2008). Therefore, training should be based on the training needs and its effectiveness should be clearly assessed. The results of our study showed that training and education were really a need for these small-scale producers, which placed training and education at the first place among the possible answers on where to allocate available future financial resources. In addition, the provided guidelines on good manufacturing and hygienic practices were built after obtaining a profound knowledge on salami and soppresse production system, as described in chapter

2, therefore they were dedicated, science-based supported and fit for purpose which may explain the outcome of the questionnaire that recommendations on control measures were well adopted. Moreover, the effectiveness of a training program does not only depend on the capability of the trainers but also on the attitude of small-scale producers and the hygiene culture of an organisation (Jevšnik, Hlebec, & Raspor, 2008b). This survey highlighted that most of the respondents considered food safety legislation important and necessary to prevent food safety problems and trusted the competent authority, therefore showing a positive attitude towards education and training in order to improve the food safety of their products.

Knowledge is the starting point in order to build food safety awareness among small-scale producers, which is a pre-requisite in order to internalize and implement food safety practices in small-scale production site. In addition, it has to be clear that for small or micro-sized food enterprises a simplified approach of FSMS should be developed. A recent EFSA opinion (EFSA Panel on Biological Hazards et al., 2017), has suggested the application of a simplified approach of FSMS based mainly on PRPs. The retailer does not have to own specific knowledge of the hazard but should be aware that 'biological', 'chemical', 'physical' or 'allergen' hazards may be present and also of activities that contribute to increase or decrease the occurrence of the hazard.

It is evident that the safety of artisanal meat/food products is the result of the interplay of many factors and actors. Support of local and national food safety authorities, with scientific support of research institutes, can provide information, expertise and training to small food business operators. In addition, the responsibility and the active role of small-scale producers in order to assure food safety is of crucial importance (Violaris, Bridges, & Bridges, 2008). In this framework, food hygiene knowledge is a pre-requisite, which allows developing the awareness of small-scale producers. It was proposed however, that the food safety culture prevailing in the company can influence the human behavior (e.g. actual execution of procedures) and thus support the effective implementation of control measures put in place to ensure food safety (Powell, Jacob, &Chapman, 2011). The study of De Boeck, Jacxsens, Bollaerts, Uyttendaele, & Vlerick (2016) showed that despite a less elaborated/fit-for-purpose food safety management system (as the case of small-scale enterprises), some companies are able to achieve an appropriate microbiological output, if a favourable food safety climate is present in their organization.

In conclusion, this study showed that the surveyed salami and soppresse small-scale producers were aware of the main microbiological hazards related to their fermented meat

products, the good manufacturing and hygienic practices to put in place, the importance of training and education and the value of a positive and good cooperation with the competent authority. Therefore, the efforts spent during time by the local competent authority and the research institute IZSVe, in order to provide simple but effective control points along the production process of artisanal salami and soppresse and improve the knowledge of small-scale salami and soppresse producers was effectively up taken in their everyday practices, thus answering to the effective needs. In fact, the cooperation between small-scale producers and regional competent authorities has allowed the development and application of a simplified food safety management system, which is based on the application of PRPs (as reported in the provided manual on GMP/GHP) and simple control points as the measurement of the weight loss of salami and soppresse and the control of the temperature and humidity of the ripening rooms.

Therefore, the present study supports that knowledge coupled with the awareness and motivation, are the fundamental principles which could develop and maintain a simplified but effective FSMS within small-scale producers.

# ANNEX 1

Legnaro, October 2016

Dear Mr., Mrs.,

The Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in collaboration with the Italian local veterinary authority (ASL8) is interested to know your opinion on food safety knowledge, the resources and capacities needed to produce food in micro-sized enterprises.

**The questionnaire is addressing salami and soppresse producers.**

- Filling out the questionnaire will take approximately 20 minutes of your time
- The questionnaire consists of 24 multiple-choice questions that allow multiple answers
- There are no right or wrong answers. Please try to answer as honestly as possible
- All the data will be processed strictly confidential and your anonymity is guaranteed
- You can deliver the questionnaire to the vet Fabiano Carraro, ALS 8-Montebelluna

We would like to thank you for your cooperation in this research! The data collected with the questionnaire are part of a joint PhD between IZSVe and Ghent University (Belgium).
In case you are interested in the results of this study, you can contact us via the information mentioned below.

Your sincerely,

Anna Roccato (Veterinarian)
IZS delle Venezie
SCS1-Risk Analysis and Public Health Department
Viale dell'Università 10, 35020 Legnaro (PD), Italy
e-mail: aroccato@izsvenezie.it
Phone: 049 8084304

**PART 1: GENERAL INFORMATION**

Farm registration number: _____
Number or reared pigs (per production season): _____
Type of products: salami, soppresse, cotechino, salsiccia, coppa, pancetta, other
What is the "selling season" for salami, soppresse: _____
Main location /outlet for sales of salami and/or soppresse (local market? shops? the factory itself?)
Estimated number /production volumes of salami and/or soppresse marketed per week: _____

**Demographic characteristics of the food producers** (educational level; gender, age, number of years in the sector; etc...)
- Gender (male, female)
- Age
- Level of education (primary school; high school; University)
- Received professional food safety training (Yes, No)
- Years of Professional experience in the food sector: _____

**PART 2: BASIC KNOWLEDGE ON FOOD SAFETY**

**1) The most dangerous bacteria that can cause human illness through the consumption of fermented meat products are** (provide minimum 1 - maximum 3 answers)**:**
a) *E. coli*
b) *Lactobacillus*
c) *Listeria monocytogenes*
*d) Pseudomonas*
*e) Salmonella*
*f) Yersinia enterocolitica*
g) Do not know
h) Other

**2) The dangerous bacteria can multiply at the following temperatures** (provide minimum 1 - maximum 3 answers)**:**
a) 4°C
b) 8°C
c)15°C
d) 25°C
e) 35°C
f) 55°C
g) Do not know
h) Other

**3) The dangerous bacteria will die at the following temperatures** (provide minimum 1 - maximum 3 answers)**:**
a) 30°C
b) 40°C
c) 50°C
d) 60°C
e) 70°C
f) 80°C
g) Do not know
h) Other

**4) Health conditions, which are not acceptable in food handling** (provide minimum 1 - maximum 3 answers)**:**
a) Cold
b) Diarrhoea
c) Fever
d) Headache
e) Skin wound
f) Vomit
g) Do not know
h) Other

**5) In case of the presence of a wound on the skin I will** (provide minimum 1 - maximum 3 answers)**:**
a) Be actively involved in the daily working activities
b) Be involved in activities other than handling of meat (e.g., recordings)
c) Disinfect the wound and continue to work
d) Not covering the wound in order to let it dry and continue to work
e) Protect the wound and continue my working activities
f) Protect the wound and use gloves
g) Do not know
h) Other

**6) During the cleaning operations, detergents are used in order to** (provide minimum 1 - maximum 3 answers)**:**
a) Remove the dirtiness and the organic matter in order to improve the activity of disinfectants
b) Make surfaces clean
c) Act on bacterial cells, leading to their death
d) Kill the bacteria making the cleaning operations effective
e) Reduce the time spent for the cleaning operations
f) Avoid the use of tools in order to remove dirtiness mechanically
g) Do not know
h) Other

**7) During the cleaning operations, disinfectants are used in order to** (provide minimum 1 - maximum 3 answers)**:**
a) Remove the dirtiness and the organic matter in order to improve the activity of disinfectants
b) Make surfaces clean
c) Act on bacterial cells, leading to their death
d) Kill the bacteria making the cleaning operations effective
e) Reduce the time spent for the cleaning operations
f) Avoid the use of tools in order to remove dirtiness mechanically
g) Do not know
h) Other

**8) Hands are washed during the working day** (provide minimum 1 - maximum 3 answers)**:**
a) After going to the toilet
b) After handling raw meat
c) At the beginning and at the end of the working operations
d) Before starting the working activities
e) Once during the day
f) Several times during the day
g) When hands are dirty
h) Other

**9) Factors that can influence the safety of salami and soppresse** (provide minimum 1 - maximum 3 answers)**:**
a) The experience of the employee
b) The hygiene and the maintenance of the ripening room
c) The sequence of the working operations
d) The quantity of added salt
e) The raw materials
f) The temperature and humidity of the ripening room
g) Do not know
h) Other

**10) Which of the following tools do you use in order to monitor the goodness of the ripening process** (provide minimum 1 - maximum 3 answers)**:**
a) Check and reporting of salami/soppresse weight (at least once per week)
b) Monitoring the temperature and humidity of the drying/ripening room
c) Weekly visual, tactile and olfactory inspection of salami and soppresse
d) Measure pH
e) Collecting samples for microbial analysis
f) Weight measurement at the beginning and at the end of the ripening process
g) Do not know

h) Other

**PART 3: RESOURCES and CAPACITY**

**11) The available financial resources are sufficient to support hygiene and food safety** (provide only one answer).
a) Totally disagree
b) Disagree
c) Neutral
d) Agree
e) Totally agree

**12) The available financial resources are mostly used for** (provide minimum 1 - maximum 3 answers)**:**
a) External consultants
b) Implementation of cleaning and disinfection
c) Lab analysis
d) Maintenance of the equipment
e) Pest control
f) Purchase of equipment
g) Training and education
h) Other

**13) Which of the following tools did you buy between "2008-2014"?** (provide minimum 1 - maximum 3 answers)**:**
a) pH-meter
b) Thermo-hygrometer
c) Sausage maker
d) Mixing machine
e) Mincing machine
f) Weighting scale
g) Nothing
h) Other

**14) Indicate among the following infrastructures, which are lacking in the premise** (provide minimum 1 - maximum 3 answers)**:**
a) None
b) The temperature monitoring of the drying and ripening rooms
c) The available equipment (cutting tables, mincing and mixing, stuffing machines, knives) is not well maintained and regularly checked
d) The drying and/or ripening rooms are not well maintained and are used also as storing rooms
e) The working area is not clearly divided between the clean and the dirty operations
f) The working space is not enough
g) The maintenance and control of the working area
h) Other

**15) Indicate which good procedures, guidelines and instructions concerning hygiene and food safety are in place in the premise** (provide minimum 1 - maximum 3 answers)**:**
a) Checking the maintenance of the equipment
b) Personal hygiene
c) Pest control
d) Registrations (salami and sausages weight during ripening process and cleaning and disinfection procedures)
e) Systematic documentation and keeping records
f) Working hygiene and working procedures
g) Cleaning and disinfection of the working area
h) Other

**16) Among the following options your opinion on food safety legislation is** (provide minimum 1 - maximum 3 answers)**:**
a) I think it is important and necessary in order to prevent the arising of problems affecting food safety
b) It enables me to put my products on the market
c) It increases the trust of the consumer in the food sector

d) It is mandatory; however, it is not essential in order to have safe products
e) It is my responsibility and part of the daily behaviour for people involved in handling and production of food
f) It makes my daily working activity well organized and under control
g) It helps to harmonize the working practices between companies
h) Other

**17) In case of food safety concerns** (provide minimum 1 - maximum 3 answers)**:**
a) It is common to discuss it with the Competent Authority
b) It is my duty to notify the Competent Authority
c) The food company will solve the problem and take corrective measures
d) Usually the Competent Authority is not notified or not consulted on this
e) It will be discussed within the company team
f) It will be discussed with my colleagues of other companies
g) Any action will be taken
h) Other

**18) The following objectives are set in the premise** (provide minimum 1 - maximum 3 answers)**:**
a) Avoid having complaints from the consumer
b) Good organisation of the tasks and responsibilities of each employee
c) Make good working practices and food safety guidelines part of the everyday employee behaviour
d) Obtain consumer's trust
e) Seek to comply with food safety legislation
f) To sell more products
g) Not to be involved/banned for a food safety illness/outbreak/crisis
h) Other

**19) In my company food safety is put in practice through the following actions** (provide minimum 1 - maximum 3 answers)**:**
a) I regularly control the registrations
b) A remark is done in case of failure to follow good working practices
c) At the end of each working day, the room and the utensils are cleaned
d) I discuss the problems raised during the daily working activity together with my colleagues on a regular basis (e.g.: once per week)
e) I stress the importance of good quality products
f) I check regularly (e.g.: every day or once per week) the maintenance of the equipment
g) The working sequence is clearly defined and carefully respected
h) Other _____

**PART 4: OTHER**

**20) Do you think that the available guidelines (from the regional working group) on salami and soppresse production are useful for your daily working activity? (*provide only one answer*)**
a) Totally disagree
b) Disagree
c) Neutral
d) Agree
e) Totally agree

**21) In relation to education and training, indicate which among the following topics should be better explained** (provide minimum 1 - maximum 3 answers):
a) Cleaning and disinfection
b) Cross-contamination routes
c) Food legislation
d) Food poisoning and foodborne illness
e) GMP and GHP
f) HACCP system
g) Personal hygiene
h) Other

**22) My opinion on the IT-tool developed in order to enable the recording and tracing of sausage production is** (provide minimum 1 - maximum 3 answers)**:**

a) I do not get any advantage/improvement from using it
b) It helps to detect in advance food safety problems and hygiene gaps, thus providing a remarkable support in managing the working activities
c) It is organised in a complicated way, thus making difficult to use
d) I used it regularly
e) It allows to understand the problem and find a solution with the official vet
f) It is organized in an easy and user-friendly way
g) It is an effective tool in order to support the management of the production process
h) Other

**23) Food labelling** (provide minimum 1 - maximum 3 answers)**:**
a) It provides several information useful for the consumer in order to handle the product correctly
b) It identifies the producer
c) It guarantees the traceability
d) It guarantees the safety of the product and the health of the consumer
e) It is an important tool in order to guarantee food safety, however it is not easily fulfilled
f) It allows a synthetic but effective description of the product
g) It is a law requirement
h) Other
24) In your opinion, which further initiatives should be taken by regional authorities or could be useful in order to improve the safety of salami and soppresse?

_____
_____
_____

# CHAPTER 4. Behaviour of *Listeria monocytogenes* in Belgian and Italian artisanal raw milk cheeses under temperature abuse conditions.

**Redrafted from:**

Roccato, A., & Uyttendaele, M. (2017). Behaviour of *Listeria monocytogenes* in Belgian and Italian artisanal raw milk cheeses under temperature abuse conditions. (*In preparation*)

## ABSTRACT

*L. monocytogenes* is considered one of the main hazards associated to soft and semi-soft cheeses, mainly as result of post-processing contamination which occurs during ripening, slicing of cheese and further manipulation. Farmstead cheese producers have to assure the safety of the food products they put on the market and in order to assess the behaviour of *L. monocytogenes* challenge tests or durability studies can be conducted. However, there is the need to provide more technical details on how to conduct these tests on different food matrices. These details can be useful for both FBO and laboratories performing challenge tests.

Therefore, the present study provides methodological details in order to clarify how to conduct challenge tests and durability studies on cheese. Challenge testing were conducted on two Belgian white-moulded soft to semi-soft ripened raw milk cheeses (one from cow milk and one from goat milk), one Belgian red-smear semi-hard cheese and one Italian semi-hard cheese (no red-smear, nor white-moulded). Cheese slices were stored at different time/temperature conditions (9, 7 and 14 days; 7, 12 and 14°C) and analysed for the enumeration of *L. monocytogenes* and of competing flora (*E. coli* and LAB) and measurement of pH, $a_w$ and NaCl. Durability studies on an occasional naturally contaminated batch red-smear semi-hard cheese was conducted according to the n = 30 multi-units sampling approach suggested by FAFSC SciCom 02-2016.

Challenge tests showed no growth of *L. monocytogenes* in the Italian semi-hard cheese and in the Belgian red-smear semi-hard cheese, while the growth of *L. monocytogenes* was observed in the other two Belgian cheeses stored for 9 days at 7 and 12°C. The two Belgian cheeses supporting growth of *L. monocytogenes* were white-moulded soft to semi-soft cheeses thus confirming the literature findings that these type of cheeses support the growth of *L. monocytogenes* and represent an at-risk product for listeriosis. On the other side the absence of growth of *L. monocytogenes* in the red-smear semi-hard cheese, confirmed also by the results of the durability studies, lead to the conclusion that this type of red-smear cheese is not by definition to be defined as an at risk product for supporting *L. monocytogenes* outgrowth to elevated levels.

In conclusion, the present study suggests that factors as the intrinsic physicochemical parameters (in particular $a_w$) that are related to the 'hardness' of the cheese type play a more important role in determining growth potential of *L. monocytogenes* than the type of cheese (e.g. red-smear, white-moulded) and the type of microbiota present in the cheese.

Finally, the multi-units sampling combined with a Most Probable Number Method for detection of the pathogen was shown to be a valid approach in order to identify the variability of *L. monocytogenes* eventually present in low numbers in the cheese.

## 4.1 Introduction

In the last decades, there has been an increasing interest of consumers towards food products from local farms and small producers. In particular, the public's interest in artisanal and farmstead cheese has grown (Colonna, Durham, & Meunier-Goddik, 2011; Q. Wang, Thompson, & Parsons, 2015). Usually, these products are made from raw milk and using traditional techniques, thus resulting in diverse and rich microflora, which affect the quality and the typical features of these cheeses (Marino, Maifreni, & Rondinini, 2003).

The available studies on the food safety related to the farmstead cheese indicate the occasional presence of food-borne pathogens in raw milk cheese (Brooks et al., 2012; D'Amico, Groves, & Donnelly, 2008). *Listeria monocytogenes* is considered one of the main hazards associated with soft and semi-soft raw milk artisanal cheese (Verraes, Vlaemynck, et al., 2015). Cheese can be contaminated with *L. monocytogenes* through several sources. Contaminated raw milk may result in contaminated cheese if the pathogen is able to survive the cheese manufacturing and ripening process (Kousta, Mataragas, Skandamis, & Drosinos, 2010). In the latter case, *L. monocytogenes* is expected to be found in the core of the cheese, with a quite homogeneous contamination throughout the various units of the cheese batch. However, contaminated raw milk may also introduce the pathogen in the cheese processing site where it can be established, forming biofilms which can persist for long period in the environment (Almeida et al., 2013; Kousta et al., 2010). Post-processing contamination may occur during further manipulation of the cheese, ripening, intermediate storage, display or slicing of cheese, when the bacterium colonizes the environment, equipment, utensils and crates (Lianou & Sofos, 2007). In this case, *L. monocytogenes* is expected to be found on the rind of the cheese as a localized contamination.

Cheese producers have the legal responsibility to assure the safety of the food products they put on the market. In particular, concerning *L. monocytogenes*, according to the Regulation EC 2073/2005, food business operators are recommended to conduct studies to obtain knowledge on the growth potential of this pathogen in the products put on the market under reasonably foreseeable conditions of distribution, storage and use. In order to assess the behaviour of *L. monocytogenes* in food, challenge tests (using artificially inoculated samples) or durability studies (using naturally contaminated samples) can be conducted (EC, 2005).

The revised technical guidance document on challenge tests published by the EU Community Reference Laboratory for *L. monocytogenes* provides specifications (e.g. number of batches; choice of strains; preparation of the inoculum; inoculation of test units,

etc.) on how to conduct the tests in order to assess the growth potential or the growth rate of *L. monocytogenes* in ready-to-eat foods (EURL-Lm, 2014). Still, the latter protocol is quite generic and not detailed for particular food products. As such the protocol for this type of challenge or durability testing is ambiguous and prone to variability in interpretation by experts or service labs performing these types of testing, which in turn renders it complex for artisanal small-scale producers or farmer cooperatives to judge whether the tests conducted will indeed satisfy the competent authority. In Belgium, more technical details were requested by the sector of artisanal cheese producers from the competent authority (the Belgian Agency for the Safety of the Food Chain; FASFC) on the experimental set-up of challenge testing or durability testing when seeking to collect sufficient evidence that their particular type of cheese will not exceed the threshold limit of 100 CFU/g throughout the shelf-life in case of occasional contamination with *L. monocytogenes*. However, also in other countries, including Italy, farmstead cheese producers are struggling on how to elaborate challenge testing and fulfil this requirement of, preferably, providing evidence of no growth potential of this pathogen in artisanal cheeses, and comply with the above mentioned EU legislation concerning *L. monocytogenes* in ready-to-eat foods.

Within Belgium, the Scientific Committee of the Belgian Food Safety Agency (FASFC SciCom) provided clarification on the EURL-*Lm* technical guidance document for conducting challenge tests and durability tests for *Listeria monocytogenes* in ready-to-eat foods for the specific case study of estimation of the growth potential in cheese (FASFC SciCom, 2016). It was explicitly mentioned that in accordance with the EURL-*Lm* technical guidance document, challenge or durability testing does not have the intention to assess the behavior of *L. monocytogenes* during cheese making and ripening but aims to assess the safety of the cheese when set to market throughout its shelf-life. Therefore the artificial inoculation of *L. monocytogenes* in/on cheese is preferably conducted *after* the ripening of the cheese on the first day of the shelf-life (day 0), i.e. the moment that the cheese is ready to be placed on the market as a ready-to-eat food. Moreover, several specifications have to be decided by the cheese producers, the latter preferably in agreement with their buyers (deli-shops or restaurants), if the artisanal cheese producer does not sell all his cheese himself at the farm or his own shop. In fact, information such as what is the (reasonably) expected shelf-life of the cheese and the temperature condition of further ripening (*affinage*) or storage of the cheese at home (or at the shop or catering) have to be provided in order to be able to set-up *L. monocytogenes* challenge testing or durability studies to ensure the safety of the product on the market.

*Listeria monocytogenes* can contaminate cheese via several contamination routes as already mentioned. However, contaminated food processing environment and post-processing contamination represent the most common ways of cheese contamination by *L. monocytogenes* (Lakicevic & Nastasijevic, 2017; Lambertz et al., 2012). In challenge tests, the site of inoculation (core *versus* surface) depends on the most probable contamination route. It is recommended to also measure the pH and $a_w$ at the defined location (core *versus* surface) as cheese is a heterogeneous type of food. In accordance with the EURL-*Lm* technical guidance document, the initial contamination level has to be about 100 CFU/g although results can also be expressed as counts per surface area (per $cm^2$) rather than per gram if considering a post-processing surface contamination. Extrapolation of results obtained on growth potential of the pathogen can only be done to the same type of cheese.

It was also stated in the FASFC SciCom advice 02-2016 that indeed durability tests (using *L. monocytogenes* naturally contaminated cheese) can serve as an alternative for challenge tests if sufficient replicated samples (n = 30) of the contaminated batch are analysed for *L. monocytogenes* using both enumeration according to ISO 11290-Part 2 (with reduced detection limit) (ISO, 1998a) and the principle of the Most Probable Number Method (Blodgett & Garthright, 1998) to estimate low numbers of the pathogen (range of between 1 per 10 g up to 10 CFU/g) being present in the cheese. To assess growth potential, analyses of 30 replicated samples of the same (naturally contaminated) batch is recommended to take place both at (or shortly after) the time the *L. monocytogenes* contamination is established and at the end of the (expected) shelf-life of this contaminated cheese batch (with storage of the cheese under reasonably foreseen conditions of temperature).

The aim of the present study was multiple. The first objective was to investigate to which extent the growth potential established by challenge testing is variable among different types of raw milk artisanal cheeses. Therefore different types of raw milk artisanal cheeses were included: two Belgian white-moulded soft to semi-soft ripened raw milk cheeses (one from cow milk and one from goat milk); one Belgian red-smear semi-hard cheese; and one Italian semi-hard cheese (no red-smear, nor white-moulded). The latter two cheeses were made from raw cow milk. Furthermore, an opportunity occurred to validate the FASFC SciCom advice for conducting durability studies. This is because we were notified of several batches of a natural contaminated red-smear semi-hard raw goat milk cheese residing at a cheese production site (the cheese not being put to market). These batches were contaminated through the use of *L. monocytogenes* raw goat milk delivered to the cheese manufacturing site by one farmer. Thus, as a second objective it was

investigated whether the detailed guidance in the FASFC SciCom 02-2016 advice for conducting durability studies was fit for purpose. Overall, the present study also enabled to compare the results obtained of *L. monocytogenes* growth potential in case of (higher inoculum and artificial) challenge testing *versus* (low level and natural contaminated) durability testing for the same type of red-smear semi-hard cheese.

## 4.2 Materials and Methods

### *4.2.1 Collection of the cheeses for the challenge testing*

Three small cheese producers located in the region of Flanders (Belgium) and two small cheese producers located in Friuli-Venezia-Giulia (FVG) region (Italy) were contacted to provide raw milk cheese. Each producer provided on separate occasions two batches of his respective characteristic artisanal cheese. For the three Belgian cheese producers this concerned respectively either a red-smear semi-hard cheese type made from raw cow milk (cheese 1); a white-moulded soft cheese from raw goat milk (cheese 2); or a white-moulded soft cheese from raw cow milk (cheese 3). The two Italian cheese makers provided, each separately, two batches of a semi-hard raw cow milk cheese (no red-smear nor white-moulded) (cheese 4 thus being identical as cheese 5) (Figure 9).


**Figure 9**. Raw milk cheeses involved in challenge testing: 1) red-smear semi-hard cow milk cheese; 2) white-moulded soft goat milk cheese; 3) white-moulded soft cow milk cheese; 4 & 5) semi-hard cow milk cheese.

In case of the Italian producers, for each batch, one unit of intact big flat round cheese (diameter: 30 to 40 cm; weight: 6 to 7.5 kg) was supplied and subsequently sliced in the lab, while for the Belgian cheeses, as the cheeses were smaller, for each challenge test, 2 or 3 units of intact flat round cheeses (diameter: 11 to 22 cm; weight: 350 grams to 2.5 Kg) were collected from a cheese batch. The cheeses were purchased from the cheese producers shortly after the ripening period when cheeses were judged of sufficient quality by the cheese makers and ready to be set to the market (ripening period varied between 4 to 6 weeks after production; storage during ripening at ca. 12°C). Cheeses were transported to the lab at ambient temperature within one hour for Belgian cheeses and within 24 h for Italian cheeses. Upon arrival in the lab, samples were stored at 4°C and within 48 h cut up in consumer portions and artificially inoculated with *L. monocytogenes* and thus subjected to challenge testing and analysed for microbiological and physicochemical parameters as described below.

### *4.2.2 Set-up of Challenge testing and microbiological and physicochemical analysis of cheese*

For the challenge testing, a *L. monocytogenes* post-processing contamination was simulated in the lab, which could have occurred during ripening of cheese, or during storage of the cheese or cheese consumer portions during refrigerated shop display or at consumer's home refrigerator. As such, inoculation with *L. monocytogenes* was performed either on the cheese-slicing side (core) surface area or on the cheese upper (rind) surface area of sliced consumer portions that were cut in the lab from the intact flat round cheese units purchased.

The challenge testing experimental set-up and storage conditions in the present study were performed based upon the protocol described in the EURL-*Lm* technical guidance document, the detailed guidance of the FASFC SciCom 02-2016 advice, and experience built in prior experiments with challenge testing in cheese (Lahou & Uyttendaele, 2017). In short, for the Belgian cheeses a cocktail of three *L. monocytogenes* food strains used for challenge testing in the Lab of Food Microbiology and Food Preservation at Ghent University (i.e. strains LMG 23194 isolated from Wijnendaele cheese, strain LMG 23192 isolated from liver paté, and strain LMG 26484 isolated from a tuna sandwich spread), all available at the Belgian BCCM® collection at the Lab of Microbiology Ghent University (LMG), were used. For the Italian cheeses, the challenge testing were performed at a later period. The option was taken to make a cocktail of three *L. monocytogenes* strains isolated from cheese i.e.

also strain LMG 23194 isolated from Wijnendaele cheese, but complemented with strain LMG 13305 isolated from a soft cheese associated with a case of meningitis after eating cheese, and strain LMG 23356 isolated from Jalisco cheese. All these *L. monocytogenes* were first subcultured for 24 h at 37°C in Brain Heart Infusion (BHI; Oxoid). Next a subculture was grown at 7°C for 4 days in order to adapt the early stationary phase cells to the refrigerated storage condition of the cheese. It was noted that the strain LMG 23356 failed to grow to high numbers (turbidity) and was thus not used to prepare cocktails for inoculation. Consequently, a 2-strain cocktail was used for the Italian cheese challenge testing whereas a 3-strain cocktail was used for the Belgian cheeses' challenge testing.

Knowing the diameter of the flat-round cheese purchased, it is possible to calculate the cm² of the upper surface area and prepare a template to divide the cheese in equal consumer portions (Figure 10) with each similar known upper triangular surface area (cm² rind) and a resulting similar weight per unit. Knowing also the height of the cheese, subsequently also the slicing surface area showing the inner core of the cheese could be calculated (one rectangular side is the height multiplied by the radius of the cheese). For example, in case of the Italian cheese, the consumer portions had a (upper triangular) rind surface of ca. 30 $cm^2$ and the (rectangular) slicing (core) surface area was ca. 60 $cm^2$; the weight of a portion was ca 200 g (Figure 10).

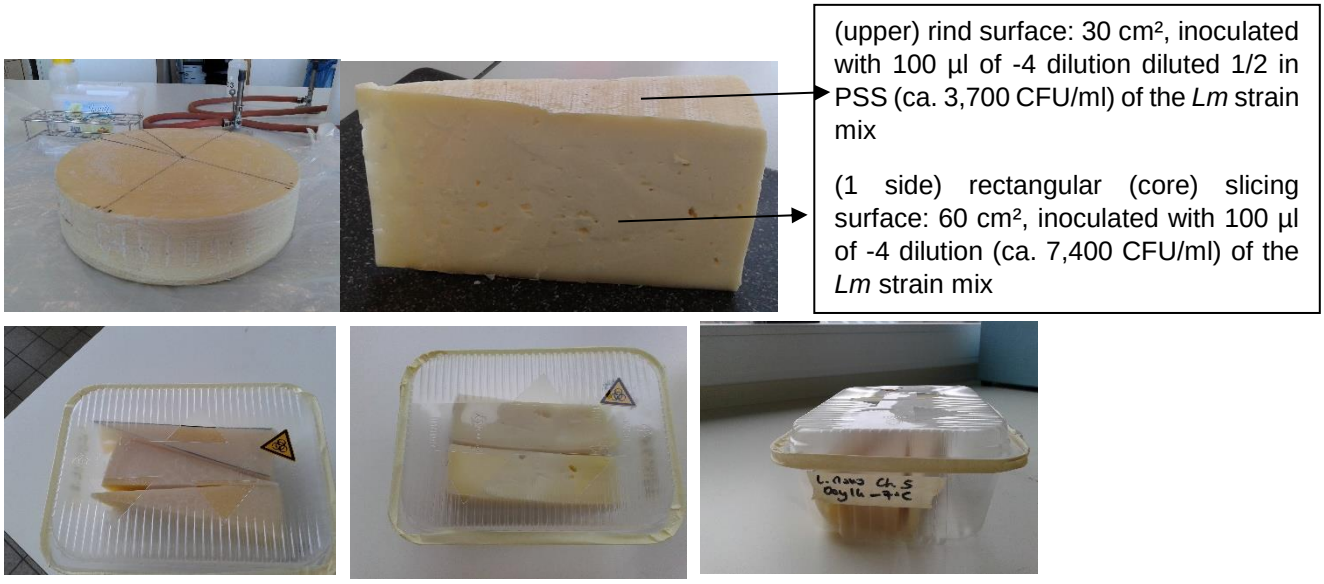



**Figure 10.** Inoculation approach undertaken during challenge testing of raw milk cheese.

Knowing also the density of the mixed three or two-strains inoculum (prior determined by plating on selective *L. monocytogenes* agar medium as being ca. 5.0 to 7.0*$10^8$ CFU/ml), one can calculate the appropriate dilution of the mixed inoculum to be made to have a density so that the inoculation of ca. 100 µl of this proper dilution on the known surface area

of the cheese rind or on the known cheese-slicing core surface will provide a contamination level of ca. 100 *L. monocytogenes* CFU per $cm^2$ surface area. The inoculum volume was homogeneously distributed on the surface area by scattering the inoculum volume in drops on the surface and spreading it with a sterile loop. As rind and core surface areas will differ, a defined inoculum volume of an appropriate dilution of the strain mix was used to inoculate the surface area of the cheese rind whereas a defined inoculum volume of another appropriate dilution of the same mix was used to inoculate the cheese-slicing core surface area (illustrated for the Italian cheese in Figure 10). Furthermore, some cut consumer portions were used to inoculate the (upper) rind surface area whereas other consumer portions cut from the same flat round cheese were used to inoculate the (core) slicing surface area. Thus, (upper) rind & (core) slicing surface were not inoculated on the same single consumer portion to avoid cross-contamination in subsequent sampling for analysis. Consequently, for each sampling day and storage temperature, to have three replicates per sampling day, six consumer portions were inoculated (three serving to inoculate the (core) slicing surface area and three serving to inoculate the (upper) rind surface area). In addition, six non-inoculated consumer portions (inoculated with the same defined volume of sterile physiological saline solution; PSS) were prepared serving as blank samples to measure physicochemical parameters and competing flora either after inoculation. Inoculated consumer portions were put in plastic boxes, which were covered with partially open plastic lids, in order to reduce the possibility of moulds to grow (Figure 10).

Storage was performed at various time/temperature conditions. In particular, the Belgian cheese was analysed after usually 9 days of storage in a ventilated consumer refrigerator at either 7 or 12°C (±1°C). The 9 days were judged by cheese producers already a bit longer that the usual recommended one week use-by date of consumer portions being sold, knowing at least weekly shopping taking place by Belgian consumers. If cheese portions were noted to be deteriorated by moulds growing on the cut consumer portions, sampling occurred a few days earlier (e.g. 6 days for cheese 2, batch 2). The Italian cheese was stored at 7°C for 14 days and at 14°C for only 7 days noting at this higher temperature quicker spoilage by surface overgrowth of moulds on this particular cheese.

The storage temperature of 7°C simulated the maximum (Belgian legal) retail temperature for storage of cheese and maximum recommended consumer refrigeration temperature. Temperatures of 12 to 14°C were chosen in order to simulate the temperatures that can occur during occasional further ripening taking place at cheese producers or cheese deli-

shops and some restaurants, as well it represents temperature abuse during refrigerated domestic storage or storage of cheese in a cellar.

Enumeration of *L. monocytogenes* in the artificially inoculated samples was performed at the beginning of the challenge test immediately after inoculation (day 0) and at the end of the storage period of the cheese. Enumeration of *L. monocytogenes* was performed according to ISO 11290-2 using a reduced detection limit of 1 CFU/$cm^2$ by spreading 1 ml of the primary cheese (rind or core surface) suspension (consisting of x ml corresponding to x $cm^2$ of surface area as mentioned below) on three Agar Listeria according to Ottaviani & Agosti (ALOA; Biolife, Milan, Italy) plates (24 to 48 h incubation at 37°C) (ISO, 1998a). In order to prepare the primary suspension, the surface of the entire rind or core was collected and put in a stomacher bag. Then, knowing the $cm^2$ of the collected surface area, we added the corresponding volume (e.g. in case of the Italian cheese, 30 or 60 ml) of physiological saline solution in order for thus 1 ml to correspond to 1 $cm^2$.

The growth potential within a batch was calculated as the mean of the growth of *L. monocytogenes* as noted from the three individual replicates per batch; whereas for each replicate the growth of *L. monocytogenes* was calculated as the difference between the concentration of *L. monocytogenes* on the cheese rind or core surface area at the end of the challenge test and the concentration of *L. monocytogenes* on the cheese rind or core surface area at the beginning of the test (day 0). This calculation is a deviation from what is mentioned in the EURL-*Lm* technical guidance document on challenge testing. This because due to constraints in time and resources it was not possible to conduct challenge testing for three individual batches, subsequently only two batches were included. Furthermore, considerable intra-batch variability in *L. monocytogenes* growth potential was noted before during challenge testing of cheese (Lahou & Uyttendaele, 2017) and thus it was recommended to include all the data obtained in assessing the growth potential. Therefore, in the present study it was decided to report growth per batch (and this for the two batches included) taking into account the difference during storage of all *L. monocytogenes* counts as noted between intra-batch replicates instead of merely a single value (difference without standard deviation), derived as the median concentration of *L. monocytogenes* of the three replicates at the beginning of the challenge test and the median concentration of *L. monocytogenes* of the three replicates at the end of the challenge test, as stipulated in the EURL-*Lm* technical guidance document and reported by Lahou & Uyttendaele, (2017).

Furthermore, data on physicochemical parameters and competing flora were obtained from the blank samples. At Day 0, before inoculation, presence/absence testing of *L. monocytogenes* was performed on 25-gram samples, according to ISO11290-1 (ISO, 1996). In addition, on each day of analysis (day 0 or 1 and at the end of the challenge test), generic *E. coli*, lactic acid bacteria (LAB), were enumerated for three blank samples (sample units of 25 g, representative sample including both core and rind of cheese and plating of the primary 10-fold suspension or appropriate dilutions in physiological saline solution). Enumeration of generic *E. coli* was performed according ISO 16649-2:2001 (24 h incubation of RAPID' *E. coli* 2 medium at 37°C) (ISO, 2001b). Lactic acid bacteria count was determined according to ISO 15214:1998 (72 h incubation of De Man, Rogosa, Sharpe agar plates (MRS; Oxoid) at 30°C) (ISO, 1998b).

The NaCl concentration, pH and $a_w$ were determined, separately for the rind and for the core of the cheese consumer portions and this for three replicate blank samples and for each time/temperature conditions at the beginning of the challenge test.

The NaCl concentration was determined based upon titrimetric determination of $Cl^-$. A representative test unit of 1 to 2 g 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%; w/v) was added. This solution was then titrated with $AgNO_3$ (0.1 N) to determine the concentration of $Cl^-$ in the solution. Concerning pH and $a_w$, a representative sample of upper surface rind or inner core of the cheese was taken (ca. 5 to 10 g) and pH was measured using a stab electrode (SevenEasy™ pH-meter, Mettler Toledo, Zaventem, Belgium).The water activity ($a_w$) was determined with an $a_w$-kryometer Typ AWK-20 (NAGY Messysteme GmbH, Gaufelden, Germany).

### *4.2.3 Experimental set-up for durability testing of naturally contaminated L. monocytogenes cheese*

Being notified of several batches of a natural contaminated red-smear semi-hard raw goat milk cheese residing at a cheese production site (the cheese not being put to market), there was an opportunity to validate the FASFC SciCom advice for conducting durability studies for naturally contaminated batches. These batches were contaminated through the use of *L. monocytogenes* raw goat milk delivered to the cheese manufacturing site by one farmer. The cheese producer had noted the *L. monocytogenes* contamination in the cheese batch by sending out a (single) sample for analysis to an external service lab at the time the cheese was ready to be set to the market. The sample was analysed both for detection of

*L. monocytogenes* per 25 g according to ISO 11290-1 and enumeration of *L. monocytogenes* according to ISO 11290-2 using a reduced detection limit of 10 CFU/g.

Upon being notified on the positive *L. monocytogenes* result (presence per 25 g but less than 10 CFU/g), the cheese batch was kept for further intermediate storage at 12°C at the cheese producer and subsequent analysis of single test units were further performed on a regular time interval being commissioned by the cheese producer to the external lab. This time apart from *L. monocytogenes* detection per 25 g, also detection per 10 g and per 1 gram was requested as well as again *L. monocytogenes* enumeration. Also pH and $a_w$ were measured on a representative subsample of the cheese (including both rind but mainly the core of the semi-hard cheese).

As it became clear to the cheese producer that this batch of cheese was consistently contaminated with (low levels) *L. monocytogenes* it was decided by the producer to initiate durability studies to assess the growth potential of *L. monocytogenes* in this semi-hard cheese. Thus, the durability study was set-up in the authors' research lab. Thirty replicates of this cheese batch under consideration were analysed for the presence and (estimated) numbers of *L. monocytogenes* at the current time in the shelf-life (at that time of starting the durability studies already 7 weeks far in the shelf-life period) and at the end of the remaining shelf-life two weeks later. The total shelf-life period for this semi-hard cheese was thus set at 9 weeks after the cheese had been judged ready to be set to the market and the first analysis on this cheese batch had been conducted. Throughout this period, the cheese had been stored at 12°C.

The cheese batch comprised ca. 100 individual flat-round cheese units. It was decided to take two representative samples, each ca. 200-gram weight, of at random 15 flat-round cheese units of the batch under consideration. Estimated numbers of *L. monocytogenes* were determined using an MPN approach. The applied MPN approach used triplicate tests of 3 subsequent 10-fold dilutions per sample for which each dilution was subjected to a primary enrichment in demi-Fraser broth (Oxoid, Basingstoke, UK) with subsequent streaking onto *L. monocytogenes* selective agar medium. Thus, three times 11 g of cheese was 10-fold diluted in 99 ml demi-Fraser broth. After homogenization of this primary 10-fold diluted cheese suspension in demi-Fraser, 10 ml was taken to be transferred to an empty sterile test tube and 1ml of this primary suspension was added to a test tube holding 9 ml of sterile Demi-Fraser broth. The corresponding Demi-Fraser suspensions (9 in total, 3 replicates of each the remaining 100 ml primary Demi-Fraser suspension corresponding to 10 g of cheese, the 10 ml Demi-Fraser taking out of the primary suspension corresponding

to 1 g of cheese and the 10 ml Demi-Fraser suspension obtained by 10-fold dilution of 1ml of the primary suspension thus corresponding to 0.1 gram of cheese) were incubated for 24 h at 30°C. The triplicate enrichments of each 10-fold dilution of the cheese were streaked each on 1/3 of an ALOA plate (Agar Listeria according to Ottaviani & Agosti; Biolife, Milan, Italy). These ALOA plates were incubated at 37°C and both after 24 and 48 h investigated for the presence of typical *L. monocytogenes* colonies, which grow with a green- blue colour surrounded by an opaque halo. A selection of typical colonies were confirmed to be *L. monocytogenes* by using a commercial miniaturized biochemical kit (Oxoid Microbact™ Listeria 12L System). In summary, for this durability study, thus for each of the 30 cheese samples taken on a defined day in the shelf-life, per sample 3 ALOA plates were obtained corresponding to the three 10-fold dilutions (Figure 11).

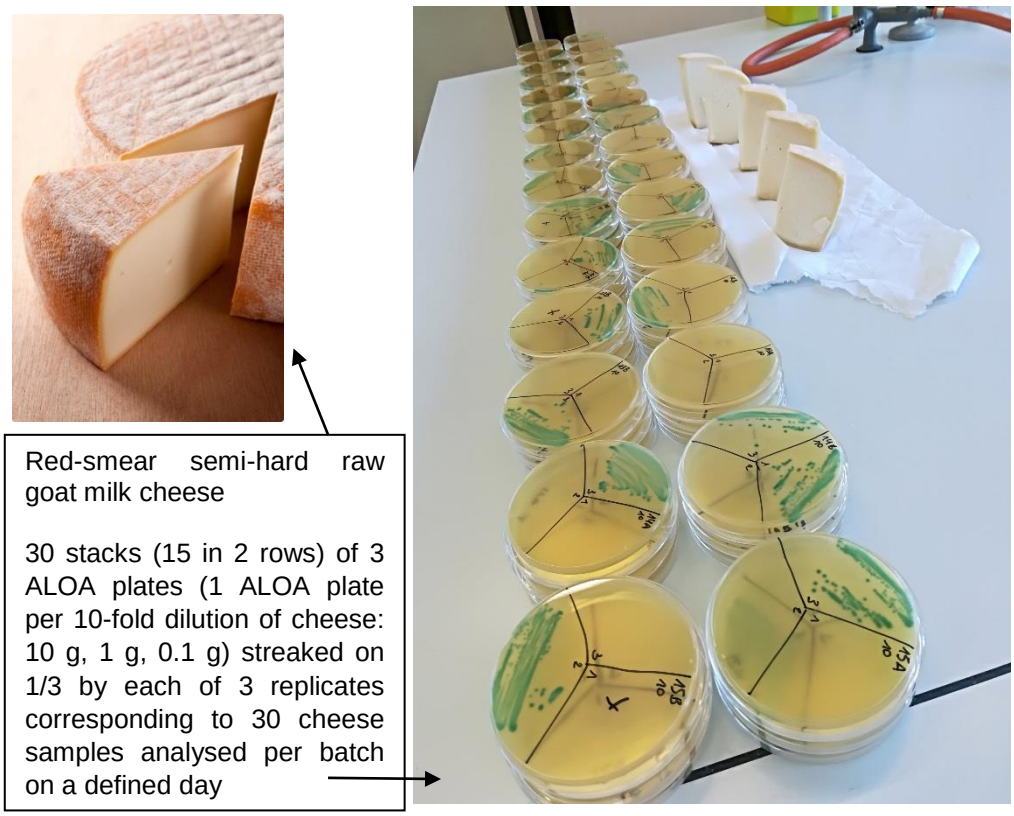



**Figure 11**. Illustration of the MPN approach being executed in the lab for 30 subsamples of naturally contaminated cheese for estimation of *L. monocytogenes* numbers in the framework of the durability study.

Examining these ALOA plates it can be noted how many of the three replicates per dilution showed presence of *L. monocytogenes.* Thus, if *2* replicates for the primary suspension corresponding to enrichment of 10 g show typical colonies, *1* replicate of the next dilution corresponding to 1 g and none (*0*) of further 10-fold dilution corresponding to 0.1 gram show typical colonies a pattern of *2-1-0* is denoted. This pattern of growth is then

read from the MPN table (Dijk et al., 2015) to provide the most probable number, or estimated number of *L. monocytogenes* as well as its 95% confidence interval for this cheese sample. For example, the MPN result of *2-1-0* in this study approach corresponds to an estimated number of 1.5 CFU/10 gram (95% confidence interval 0.4-3.8). This estimated number was in the present study multiplied by 2.5 to have the estimated number expressed 'per 25 g'. The same was done for the maximum estimate of the confidence interval i.e. 3.8 multiplied by 2.5 being thus 10 per 25 gram. Thus, using this maximum estimate as the worst-case scenario, the estimated number of *L.* monocytogenes in this cheese sample will thus be reported in the category of 10 to 25 CFU per 25 gram. A similar calculation is exemplified in Table 13 for other MPN patterns often obtained in the present study.

**Table 13**. Illustration of conversion of MPN number to maximum estimated number and category of *L. monocytogenes* contamination in naturally contaminated cheese using a 3-replicates MPN approach (detection per 10 g, per g, per 0.1 g).

| MPN number | Estimated number/10g | Estimated number/25g | 95% confidence range (min-max) | Max. estimated number per 25g | Category |
|---|---|---|---|---|---|
| 0-0-0 | < 0.30 | <1.0 | 0.00-0.94 | < 2.5 | < 4 CFU/25g |
| 1-0-0 | 0.36 | 0.90 | 0.02-1.70 | 4 | |
| 1-1-0 | 0.74 | 1.85 | 0.13-2.00 | 5 | 4-9 CFU/25g |
| 2-0-0 | 0.92 | 2.30 | 0.15-3.50 | 9 | |
| 2-1-0 | 1.50 | 3.75 | 0.4-3.8 | 10 | 10-25 |
| 3-0-0 | 2.30 | 5.75 | 0.5-9.4 | 24 | CFU/25g |
| 3-1-0 | 4.30 | 10.75 | 0.9-18.1 | 45 | |
| 3-2-0 | 9.30 | 23.75 | 1.8-36.0 | 90 | >1-9 CFU/g |
| 3-2-1 | 15 | 37.5 | 3-38 | 95 | |
| 3-3-0 | 24 | 60 | 4-99 | 248 | ≥10-40 |
| 3-3-1 | 46 | 115 | 9-198 | 495 | CFU/g |
| 3-3-2 | 110 | 275 | 20-400 | 1000 | |
| 3-3-3 | >110 | >275 | 20-400 | >1000 | >40 CFU/g |

The worst-case estimates were thus used to allocate the *L. monocytogenes* MPN patterns for the 30 samples being analysed on one day for this naturally contaminated cheese batch in categories (various orders of magnitude of *L. monocytogenes* contamination) which are presented in the results of this study. In addition, all results for the *L. monocytogenes* enumeration for each of the 30 samples according to ISO 11290-2 using a reduced detection limit of 10 CFU/g by spreading 1 ml of the primary suspension on three Agar *Listeria*

according to Ottaviani & Agosti (ALOA; Biolife, Milan, Italy) plates (24 to 48 h incubation at 37 °C) were reported as results of this study, but in three categories: < 10 CFU/g; ≥ 10-100 CFU/g and ≥ 100 CFU/g.

Apart from the first batch of cheese, produced and identified of being naturally contaminated with *L. monocytogenes,* two more batches of the same cheese type were subsequently produced with contaminated milk from the same goat farm in the weeks following the production of the first batch. When being notified that the first batch of cheese was positive for *L. monocytogenes*, the production of this type of cheese was set on hold, but still these two subsequent batches had already been produced. Again the cheese producer had outsourced single sample units to an external service lab to test for presence of *L. monocytogenes* and measurement of pH and $a_w$ also in these two subsequent batches. For these two additional batches, it was decided to also confirm the no growth of *L. monocytogenes* and restriction of presence of the pathogen to low levels during the shelf-life by subsampling from each batch six units and determine the (estimated) numbers of *L. monocytogenes* at one time point during the shelf-life by the MPN method and enumeration as described above.

## 4.3 Results and discussion

### *4.3.1 Challenge testing*

Challenge testing in this study was performed in order to evaluate the behaviour of *L. monocytogenes* during the shelf-life of different types of cheese, after simulating post-processing contamination. In total, 30 non-contaminated samples and 180 contaminated samples were analysed. All the non-inoculated test units showed absence of *L. monocytogenes* in 25 g.

The intrinsic characteristics (pH, $a_w$, NaCl) of the cheeses are reported in Table 14, showing differences between the tested cheeses. In relation to the Belgian cheeses, heterogeneous values have been observed for all the measured intrinsic characteristics. The different features of the three Belgian cheeses on one hand and the similar production process of the Italian cheeses on the other side, thus explain the results in Table 14.

**Table 14.** Intrinsic characteristics (pH, aw, NaCl) and *L. monocytogenes* growth potential of the cheeses involved in challenge testing. The reported values are expressed as mean values (± Standard Deviation) calculated on three samples for each batch. (T: temperature; NA: not available).

| Cheese* | Batch | Ph | | $a_w$ | | NaCl (%) | | Storage conditions | | *L. monocytogenes* Growth potential** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Core* | *Rind* | *Core* | *Rind* | *Core* | *Rind* | *T (°C)* | *Time (days)* | *Core* | *Rind* |
| ***1*** | ***1*** | 5.37 ± 0.14 | 6.75 ± 0.15 | 0.95 ± 0.00 | 0.93 ± 0.00 | 2.49 ± 0.13 | 1.46 ± 0.08 | 7<br>12 | 9 | -0.34± 0.11<br>-0.14±0.32 | -0.71±0.84<br>-0.01±0.22 |
| | ***2*** | 5.24 ± 0.10 | 6.40 ± 0.12 | 0.96 ± 0.00 | 0.93 ± 0.01 | 2.57 ± 0.14 | 1.32 ± 0.02 | 7<br>12 | 9 | -0.43±0.15<br>-0.64±0.31 | -0.30±0.39<br>-0.10±0.49 |
| ***2*** | ***1*** | 4.87 ± 0.20 | 6.63 ± 0.09 | 0.98 ± 0.00 | 0.97 ± 0.00 | 3.31 ± 0.13 | 2.32 ± 0.10 | 7<br>12 | 9 | 1.97±0.72<br>4.05±0.22 | 2.55±0.72<br>4.10±0.09 |
| | ***2*** | 4.88 ± 0.17 | 7.24 ± 0.13 | 0.98 ± 0.00 | 0.97 ± 0.01 | 2.48 ± 0.27 | 2.44 ± 0.09 | 7<br>12 | 6 | 2.11±0.14<br>4.46±0.35 | 3.15±0.54<br>4.28±0.94 |
| ***3*** | ***1*** | 5.07 ± 0.07 | 6.05 ± 0.10 | 0.99 ± 0.00 | 0.98 ± 0.00 | 1.18 ± 0.21 | 1.19 ± 0.09 | 7<br>12 | 9 | 2.38±0.09<br>3.39±0.82 | 2.33±0.35<br>3.30±0.48 |
| | ***2*** | 5.31 ± 0.19 | 6.81 ± 0.17 | 0.97 ± 0.02 | 0.98 ± 0.00 | 1.72 ± 0.07 | 1.24 ± 0.13 | 7<br>12 | 9 | 2.58±0.16<br>4.67±0.40 | 2.59±0.16<br>2.98±0.19 |
| ***4*** | ***1*** | 5.29 ± 0.05 | 5.45 ± 0.12 | 0.94 ± 0.01 | 0.89 ±0.02 | 1.94 ± 0.21 | 1.77 ± 0.47 | 7<br>14 | 14<br>7 | -1.37±0.71<br>-0.47±0.24 | -0.62±0.13<br>NA |
| | ***2*** | 5.30 ± 0.00 | 5.62 ± 0.01 | 0.95 ± 0.01 | 0.88 ± 0.04 | 1.83 ± 0.48 | 1.50 ± 0.05 | 7<br>14 | 14<br>7 | -1.88±0.48<br>-1.45±0.31 | -0.05±0.16<br>NA |
| ***5*** | ***1*** | 5.23 ± 0.06 | 5.61 ± 0.12 | 0.94 ± 0.02 | 0.90 ± 0.03 | 2.83 ± 0.75 | 2.63 ± 0.67 | 7<br>14 | 14<br>7 | -0.42±0.18<br>0.16±0.08 | NA<br>NA |
| | ***2*** | 5.18 ± 0.05 | 5.64 ± NA | 0.95 ± 0.01 | 0.95 ± NA | 2.55 ± 0.67 | 1.90 ± NA | 7<br>14 | 14<br>7 | -1.31±0.91<br>-0.15±0.62 | -1.06±0.34<br>NA |

*** Cheese 1)** Belgian semi-hard red smear raw cow's milk cheese **2)** Belgian soft white moulded raw goat's milk cheese **3)** Belgian soft white moulded raw cow's milk cheese **4 and 5)** Italian semi-hard, raw cow's milk cheese.
****** the growth potential *within* a batch was calculated as the mean of the growth of *L. monocytogenes* as established from the three individual replicates per batch, whereas for each replicate the growth of *L. monocytogenes* was calculated as the difference between the concentration (in log CFU/cm²) of *L. monocytogenes* on the cheese rind or core at the end of the test (after x days of storage at y °C) and the concentration of *L. monocytogenes* on the cheese rind or core at the beginning of the test (as established by enumeration within 24 h after artificial inoculation of the *L. monocytogenes* three or two strains cocktail).

In fact, each type of Belgian cheese is indeed only produced in an artisanal manner as such at one specific farm or production site, thus each cheese being a unique farmstead cheese. On the other side, the two Italian cheeses (cheese 4 and cheese 5) are produced in the same artisanal manner in the Friuli Venezia Giulia (FVG) region by several producers and thus they represent a unique cheese for this region but similar across the different small businesses that produce this type of cheese.

The producer delivering cheese 4 was a small-scale producer using an unidentified natural ferment from one batch to another, whereas the producer delivering cheese 5 was also an artisanal cheese producer manufacturing other kinds of cheese besides the Latteria cheese included in this study and using a commercial starter culture. Although these two Italian producers are thus somehow different in organisation and management practices they do provide a very similar type of artisanal cheese which belongs to the same cheese type. Physicochemical characteristics for the latter two Italian cheeses are quite similar, except for the sodium chloride content, which was present in higher percentage in cheese 5 than in cheese 4.

In addition, differences in physicochemical characteristics are noted depending upon the sampling location (cheese core *versus* cheese rind surface area). In fact, for all the tested cheeses the pH values measured on the side core surface of the cheeses were lower than the pH values of the upper rind surface. Moreover, the percentage of sodium chloride content was usually higher in the cheese core compared to the rind surface. This result is not unusual and it is the outcome of the diffusion process of salt from the rind to the core, that usually takes place during the ripening period (Montel et al., 2014; Rosshaug, Detmer, Ingmer, & Larsen, 2012).

Conversely, the $a_w$ values of the cheese core surface were higher that the values of the rind surface. Concerning the competing flora, lactic acid bacteria were, as expected, present in high abundance in all cheeses and tested batches (all above $10^7$-$10^8$ CFU/g), while the results of the enumeration of *E. coli* are shown in Figure 12. High numbers of *E. coli* (from $10^4$ to $10^6$ CFU/g) were observed in almost all cheeses except for the Belgian cheese number 2. In addition, heterogeneity of *E. coli* counts between batches was observed in cheeses 1 and 3 (Belgian cheeses) and in cheese 4 (Italian cheese) indicating indeed the artisanal character and non-standardized method of cheese production.

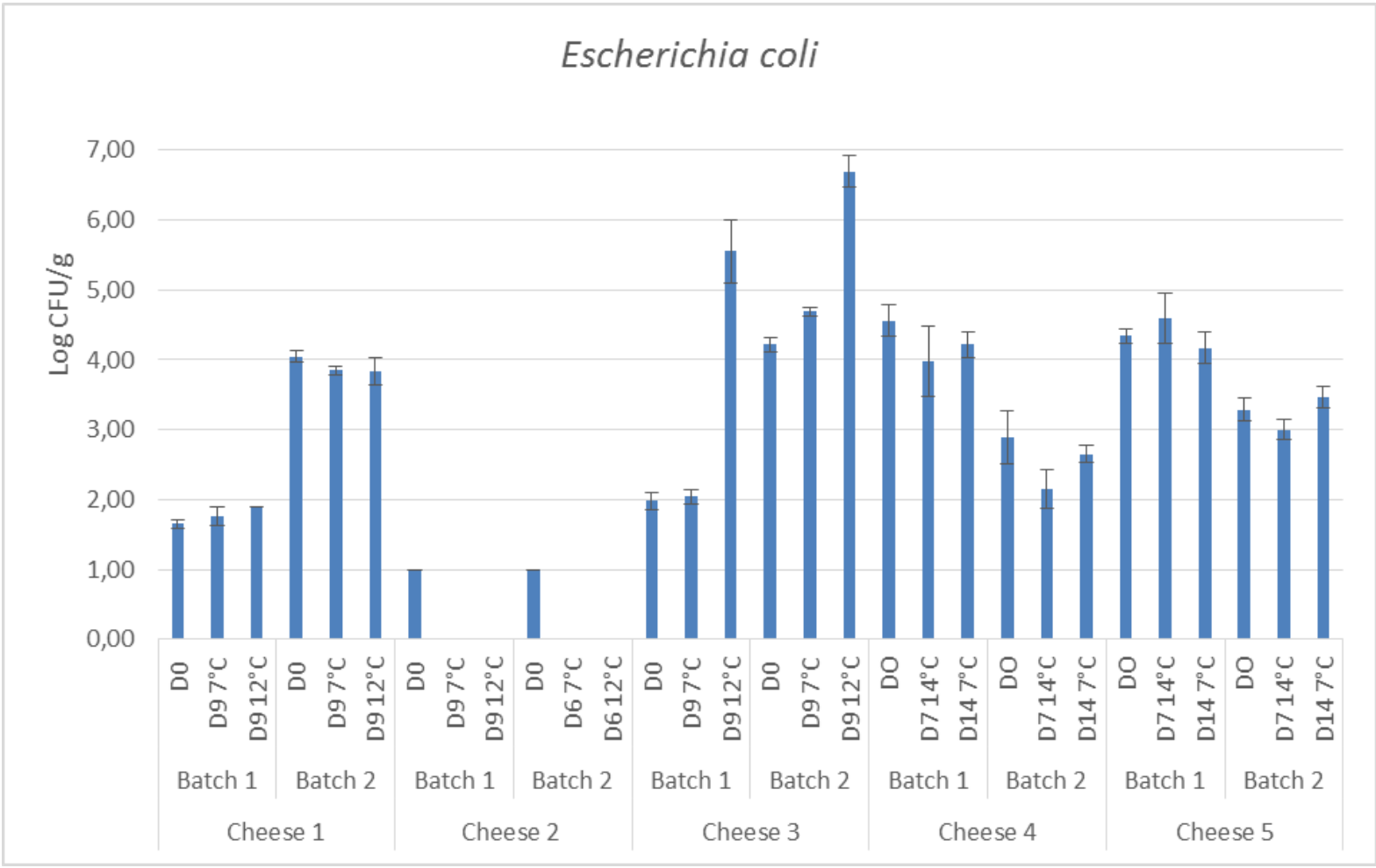



**Figure 12.** *E. co*li enumeration (log CFU/g) in the different tested cheeses (core + rind).

(D0: at day zero, after artificial inoculation; D6: at day 6; D7: at day 7; D9: at day 9; D14: at day 14)

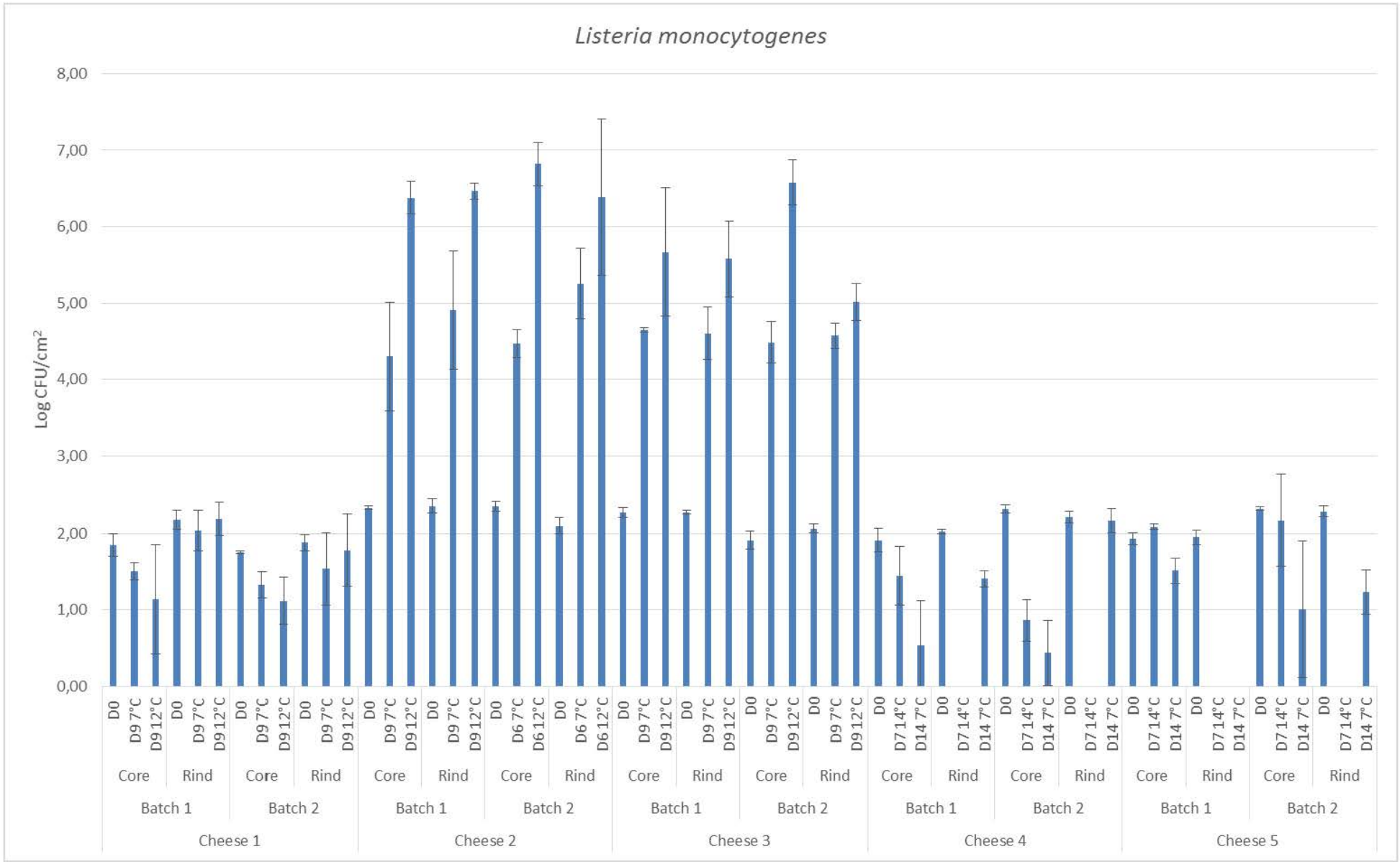



**Figure 13.** *Listeria monocytogenes* enumeration (log CFU/cm$^2$) on the core and the rind of cheeses for each batch during challenge testing. (D0: at day zero, after artificial inoculation; D6: at day 6; D7: at day 7; D9: at day 9; D14: at day 14)

The mean growth potential of *L. monocytogenes* in the various cheeses per batch is reported in Table 14 and the individual results of *L. monocytogenes* counts (mean ± standard deviation) at the time of inoculation (day 0) and after storage of cheese samples under various conditions of time and temperature are shown in Figure 13.

At day 0 all cheeses, batches and fractions have been homogeneously inoculated with similar numbers of *L. monocytogenes* per $cm^2$ of cheese, namely between 1.76 and 2.36 CFU/$cm^2$. The initial inoculum is thus within the limit of 0.5 log of the 2 log (100 CFU) per $cm^2$ target for the challenge test to be acceptable according to the EURL-*Lm* technical guidance document, although in the present case study, because of surface inoculation, the *L. monocytogenes* contamination is indeed expressed per $cm^2$ surface area instead of per gram weight.

After the different storage times and temperatures, there was no substantial growth (≤ 0.5 $log_{10}$ CFU/$cm^2$) in the Italian cheeses and neither in the Belgian cheese number 1. *L. monocytogenes* was able to grow in the Belgian cheeses number 2 and 3. In particular, in cheese 2 and cheese 3, stored for 9 days at 7 and 12°C the growth of *L. monocytogenes* was higher at 12°C (final concentration: from ca. 2 to ca. 5 to 7 log CFU/$cm^2$) than at 7°C (final concentration: from 4 to 5 log CFU/$cm^2$). However, differences in the growth of *L. monocytogenes* between the cheese sliced core surfaces and the upper rind surface area were not observed during the storage time. Moreover, looking at the intra-batch variability no substantial differences were reported between the two batches of the cheeses in which growth of *L. monocytogenes* was observed.

The study of Lahou & Uyttendaele (2017), investigating the behaviour of *L. monocytogenes* in different types of cheeses (soft to semi-hard) as a result of post-processing contamination, showed that storage temperature and cheese type (soft vs. semi-hard) have an influence on the growth potential of *L. monocytogenes*. They reported the highest growth potentials in soft cheeses and at the higher storage temperature (14°C).

The two Belgian cheeses supporting growth of *L. monocytogenes* (both on the core and the upper rind surface area) were white-moulded soft to semi-soft cheeses, for which the recorded water activity values of both the core ($a_w$ in the range of 0.97-0.99) and the rind ($a_w$ in the range of 0.97-0.98) were higher compared to the other cheeses ($a_w$ of the core ranging from 0.94 to 0.96; $a_w$ of the rind ranging from 0.88 to 0.95) and were expected to facilitate growth of *L. monocytogenes*. Whether this cheese type was made of cow milk (cheese 3) or goat milk (cheese 2) did not affect the behaviour of *L. monocytogenes*. Also, white-moulded soft to semi-soft cheeses have been shown before to support the growth of

*L. monocytogenes* (Lahou & Uyttendaele, 2017) or have been included in listeriosis outbreaks (Lunden, Tolvanen, & Korkeala, 2004; Mcintyre, Wilcott, & Naus, 2015). It has been described that traditional raw milk cheeses harbour a rich and diverse microbiota (Montel et al., 2014). A rich and complex microbial community on the cheese rind might help in the control of *L. monocytogenes* (Izquierdo, Marchioni, Aoude-Werner, Hasselmann, & Ennahar, 2009; Mellefont, McMeekin, & Ross, 2008). However, as demonstrated in this study, (reduced) growth may still be possible on these white-moulded soft raw milk cheeses. 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 & Busse, 1991).

As for the other Belgian cheese, a red-smear semi-hard cheese made from raw cow milk, it is generally assumed that red-smear cheese presents an at risk product for listeriosis (Lunden et al., 2004; Rudolf & Scherer, 2001) and these type of cheeses have been associated with outbreaks (Allerberger & Wagner, 2010; Schoder, Rossmanith, Glaser, & Wagner, 2012). However, those red-smear cheeses implicated in outbreaks or recalls (e.g. Hervé red-smear raw milk cheese in Belgium in 2015, Lahou & Uyttendaele, 2017) were soft cheeses and not semi-hard cheeses such as the one involved in the present study, which showed no support of growth of *L. monocytogenes*.

The two Italian cheeses involved in this challenge testing study are also to be classified as a type of semi-hard cheese (although no red-smear or white-moulded) for which the combination of the physicochemical characteristics of pH and water activity for both the core (pH in the range of 5.2 to 5.4 and 5.2 to 5.3; $a_w$ in the range of 0.950 to 0.964 and 0.954 to 0.963) and the rind (pH in the range of 5.3 to 5.7 and 5.5 to 5.8; $a_w$ in the range of 0.866 to 0.931 and 0.903 to 0.960) were close to the values that are described in Annex 3 of EU Regulation 2073/2005 and their combination (pH ≤ 5.0 and $a_w$ ≤ 0.94) suggests the possibility that *L. monocytogenes* will not grow, if taking into account also most likely presence of lactic acid (not measured in this study) being produced during fermentation and ripening.

Nevertheless, this type of challenge test being executed and providing actual experimental results is more convincing and valid for the cheese producers and the local competent authorities than a mere theoretical estimation of no growth by the measurement of pH and $a_w$ of the cheese. It is however, important to note that, although this type of Italian cheese is an artisanal raw milk cheese, with the two producers delivering the cheese operating in a different manner, still a standardized product is being obtained with little variation of pH and

$a_w$ being measured. This consistent achievement of pH and $a_w$ is important to achieve on a continuous basis a safe product.

As for the Belgian red-smear semi-hard cheese, because also being a semi-hard cheese this latter cheese had an overall lower (mean) water activity (0.94) than the two Belgian soft cheeses (0.97 and 0.98 respectively). Still the combination of the (mean) pH and (mean) water activity of this red-smear cheese (respectively 5.3 and 0.95 for the core and 6.6 and 0.93 for the rind) was quite close to the established 'no growth' values in the EU Regulation stipulated above but did not comply. In addition being a 'red-smear' cheese and the association of these type of red-smear cheeses as high-risk foods for *Listeria monocytogenes* growth and causing listeriosis, it was deemed necessary to asses growth potential in a challenge test. Nevertheless, apart from pH and $a_w$, also the cheese will contain lactic acid (not measured in the current study), an organic acid also well known to contribute to *L. monocytogenes* growth inhibition (Vermeulen, Devlieghere, Bernaerts, Van Impe, & Debevere, 2007). The combination of (assumed) lactic acid presence with pH and $a_w$ values close to the 'no growth limit' may have contributed to the no growth of the pathogen during 9 days storage of this cheese (cheese 1) at 7 to 12°C in the challenge test performed. As such it can be concluded that this red-smear type of cheese is not by definition to be defined as an at risk product for supporting *L. monocytogenes* outgrowth to elevated levels.

### *4.3.2 Durability studies*

As mentioned, *L. monocytogenes* contamination (presence per 25 g) of a red-smear semi-hard raw goat milk cheese (batch 1) was established by single unit analysis at an artisanal cheese producer, the time the cheese was ready to set to the market. The contamination was confirmed in two subsequent analysis being conducted respectively 2 and 4 weeks later. During the latter analysis it was shown that the pathogen was present in levels of 'presence per gram' although still < 10 CFU/g (Table 15). However, these analyses were only performed on single test units. When performing a durability study, establishing the distribution of *L. monocytogenes* contamination throughout the batch by taking 30 sample units (already now after 7 weeks shelf-life), it was confirmed that the pathogen was quite homogenously spread (*L. monocytogenes* was detected in all 30 sample units) and was present in low levels (< 10/g in all samples, and (maximum) estimated numbers for 27 out of 30 samples being situated at < 4 to 9 CFU/25g) (Table 16).

**Table 15**. Detection and enumeration of *L. monocytogenes* in three naturally contaminated batches of red-smear raw goat milk cheese during shelf-life (storage at 12°C) by testing single units.
* tests executed by an external service lab in the frame of *L. monocytogenes* monitoring program of the cheese producer

| | **BATCH 1** | | | **BATCH 2** | | **BATCH 3** |
|---|---|---|---|---|---|---|
| **Presence/ absence testing*** | **Start of shelf-life (0 weeks)** | **During shelf-life (2 weeks)** | **During shelf-life (4 weeks)** | **Start of shelf-life (0 weeks)** | **During shelf-life (2 weeks)** | **Start of shelf-life (0 weeks)** |
| | Presence | Presence | Presence | Presence | Presence | Presence |
| per 25 g | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 |
| per 10 g | ND | 0/1 | 0/1 | ND | 1/1 | ND |
| per g | ND | 0/1 | 0/1 | ND | 1/1 | ND |
| **Enumeration*** | | | | | | |
| < 10/g | 1/1 | 1/1 | 1/1 | 1/1 | 1/1 | _ |
| ≥ 10-100/g | _ | _ | _ | _ | _ | 1/1 (10/g) |
| pH | 5.35 | 5.85 | / | 5.40 | 5.95 | 5.55 |
| $a_w$ | 0.97 | 0.95 | 0.94 | 0.97 | 0.94 | 0.97 |

**Table 16**. Maximum estimated numbers of *L. monocytogenes* in a naturally contaminated batch of red-smear raw goat milk cheese during shelf-life (storage at 12°C), by replicate testing units being performed as part of a durability study to assess growth potential of *L. monocytogenes*. [1] mean value of 3 test units

| **Batch 1** | **Multiple test units (n=30)** | |
|---|---|---|
| **MPN Max. estimated numbers** | **During shelf-life (7 weeks)** | **During shelf-life (9 weeks)** |
| < 2.5/25g | 4/30 | 10/30 |
| 4-9 /25g | 23/30 | 19/30 |
| 10-25/25g | 3/30 | 1/30 |
| > 1-9/g | 0/30 | 0/30 |
| ≥ 10-40/g | 0/30 | 0/30 |
| > 40/g | 0/30 | 0/30 |
| **Enumeration** | | |
| < 10/g | 30/30 | 30/30 |
| ≥ 10/g | 0/30 | 0/30 |
| pH[1] | 5.31 | 5.34 |
| $a_w$[1] | 0.94 | 0.93 |

This indicated that up to the 7 weeks of storage at 12°C, the initial low levels of L. monocytogenes present did not grow out. During the durability study, repeating the multi (n = 30) sampling approach at the end of shelf-life (9 weeks) it was again noted that almost all the samples (29 out of 30) showed (maximum) estimated numbers being situated at < 1 to 9 CFU/25g. Thus, it could be concluded that there was no growth of the pathogen in this cheese batch. This could as such not be explained by the combination of (mean) pH and aw (5.31 and 0.938), although, lactic acid also assumed to be present, no growth would be expected. Moreover, as the cheese was kept for a prolonged time at 12°C (at the cheese producers facilities) it was noted that the water activity also gradually decreased from originally 0.967 to 0.941 after 4 weeks, 0.938 after 7 weeks and 0.928 after 9 weeks, thus rendering the cheese more inhibitory to growth of the pathogen, and the cheese as such becoming older and somewhat harder.

Two other batches (batch 2 and batch 3) of the same cheese type, subsequently produced by the cheese producer with contaminated raw goat milk too, were also shown to be positive for *L. monocytogenes* (respectively 'presence per gram' or 'enumerated at the detection limit of 10/g') (Table 15). Again positive test results were obtained during the shelf-life with a multi-unit (n = 6) sampling approach and MPN method now indicating (maximum) estimated numbers being situated at ≥ 1 to 40 CFU/25 g in 6 of 6 samples for batch 3 and 5 of 6 samples for batch 2 with a single sample noted to be above the (maximum) estimated numbers' MPN limit of 40 CFU/g (Table 17).

In batch 2, by enumeration it was indeed noted that the same sample showed *L. monocytogenes* counts of 260 CFU/g (all the others 5 samples showed counts of 10 CFU/g or < 10 CFU/g) (Table 17). Thus, that one sample being above the EU stipulated threshold limit of 100 CFU/g for the pathogen for ready-to-eat foods at the market or during their shelf-life was rendering the batch 2 unacceptable and presenting thus an increased risk for listeriosis. As it was the same type of cheese as batch 1 for which no growth potential was observed in the durability study, and also the pH and $a_w$ (5.35 and 0.93) of this batch 2 was similar as for batch 1 (see Table 16), it is not likely that this elevated number of 260 CFU/g would have occurred due to prior growth of the pathogen in the cheese during shelf-life. It is more likely to have been the result of an overall established higher initial level of the *L. monocytogenes* contamination immediately after production, but not being picked up before because of the single unit sampling being commissioned by the cheese producer to the external lab. This shows the added value and higher probability of finding of thus unacceptable samples if the legally established n = 5 multi-unit sampling approach is being

performed. Still, in this batch 2, also 5 of 6 cheese units showed levels of ≤ 100 CFU/g at 8 weeks of shelf-life and only the sixth sample was noted to have an unacceptable count (> 100 CFU/g).

The batch 3, sampled after 3 weeks of shelf-life and with pH and $a_w$ values (5.21; 0.94) similar to batch 1, showed 5 of 6 cheese units < 10 CFU/g and a sixth sample with a 10 CFU/g count. However, using the MPN approach on the same six samples, 2 out of 6 samples were having (maximum) estimated numbers of ≥ 10 CFU/g (but ≤ 40 CFU/g) whereas thus only one sample showed by enumeration a 10 CFU/g count (Table 17). It is known that an MPN approach, using a prior enrichment and thus growth in 'liquid' suspension before streaking on selective medium might enable better recovery of (low numbers) of cells than direct plating on selective agar media, thus explaining this difference.

**Table 17**. Enumeration versus maximum estimated numbers (by MPN) of *L. monocytogenes* in two naturally contaminated batches of red-smear raw goat milk cheeses during shelf-life (storage at 12°C) (multi-units (n=6) sampling)

| | **Category** | **BATCH 2 During shelf-life (8 weeks)** | **BATCH 3 During shelf-life (3 weeks)** |
|---|---|---|---|
| **MPN Max. estimated numbers** | < 2.5/25g | 0/6 | 0/6 |
| | 4-9/25g | 0/6 | 0/6 |
| | 10-25/25g | 0/6 | 0/6 |
| | > 1-9/g | 0/6 | 4/6 |
| | ≥ 10-40/g | 5/6 | 2/6 |
| | > 40/g | 1/6 | 0/6 |
| **Enumeration** | < 10/g | 3/6 | 5/6 |
| | ≥ 10-100/g | 2/6 | 1/6 |
| | > 100/g | 1/6 (260/g) | 0/6 |

Nevertheless, in batch 3 low numbers of *L. monocytogenes* were confirmed to be present and thus the maintenance of the presence of *Listeria monocytogenes*, as initially established by the single test unit monitoring result, but no growth to elevated levels was established.

Overall, taking into account i) the result of the durability study of the first batch indicating no growth potential and ii) the more restricted confirmation (n = 6) of maintenance of (although somewhat higher) numbers of *L. monocytogenes* initially detected for the two other batches, along with iii) the measurement of pH and $a_w$ of the cheese batches and its (decreasing) evolution to more adverse conditions for growth, it could be concluded that this

type of red-smear semi-hard raw goat milk cheese did not support the growth of *L. monocytogenes*. However, it could also be concluded that no substantial decrease and thus die-off of the pathogen occurred in this cheese. As such these cheese batches would still present an at risk product for pregnant women and other specific vulnerable groups in contracting listeriosis.

### 4.4 Overall conclusion

It seems from the present study that the intrinsic physiochemical parameters such as $a_w$ which is related to the 'hardness' of the cheese type play a more important role in determining growth potential of *L. monocytogenes* than the type or diversity of microbiota being present or added as (natural unidentified or selected identified) starter culture for cheese fermentation. From the challenge tests conducted, it overall supports the classification of these white-moulded *soft* raw milk cheese as at risk products for listeriosis, whereas the *semi-hard* cheeses (whether red-smear or not) were noted to pose less risk for supporting high level *L. monocytogenes* outgrowth.

In fact, looking at the physicochemical parameters of the white-moulded soft cheeses, and in particular at the $a_w$ values, being in the range of 0.97 to 0.99, support to the growth of *L. monocytogenes* could be expected. Besides, water activity is related to the water content of food that in case of soft cheese is above 45% (Monserrat & Mietton, 2014). However, in the present study, the exact water content of the cheese was not measured and the classification of cheese was based on the producer's definition. Besides, the hardness of cheese is not easy to be judge by sensory trials. It is to be noted that for the not-standardized artisanal production of cheese, although a minimum number of weeks of ripening is respected (4 weeks for the Italian cheese and 6 weeks for the Belgian ones), still the number of weeks to bring the cheese to the market might be variable and this may have an impact on the hardness of the cheese, the latter thus being a characteristic susceptible to change between batches. Looking at the measurable intrinsic parameters such as water activity is judged to be a more reliable tool in order to assess if the cheese will support or not the growth of *L. monocytogenes* and this approach is also present in the EU Regulation 2073/2005 and subsequent amendments. Nevertheless, it has to be kept in mind that growth potential of *L. monocytogenes* is the result of the hurdle principle, i.e. the combination of several intrinsic, extrinsic and implicit factors such as $a_w$, pH, presence of lactic acid and other organic acids, atmosphere, composition and activity of competing microbiota, structure

of the cheese. Although some factors might have higher impact and be more determining for growth of *L. monocytogenes* than others, still the estimation of growth potential cannot be reduced to a single parameter.

Furthermore, in the present study, artificial *L. monocytogenes* contamination (inoculum ca. 100 CFU/g) was used to study the growth potential of the pathogen in a red-smear semi-hard cow milk cheese whereas durability studies were used to assess the growth potential in a similar naturally contaminated (levels of ca. 1 to 40 CFU per 25 g) red-smear semi-hard raw goat milk cheese. It was noted that in both cases, no growth of the pathogen was observed, and thus the results of the challenge testing coincided with the results of the durability testing.

Overall, this approach of durability study, using two multi-unit samplings (n = 30) being executed on the same cheese batch with the maximum time difference possible (preferably as close to the time of production and establishment of natural pathogen contamination and the end of shelf-life) and using an MPN approach in parallel to enumeration of *L. monocytogenes* is a valid approach to provide insight in the heterogeneity and levels *L. monocytogenes* contamination and thus capture the variability that might occur in the behaviour of single cells of *L. monocytogenes* if low numbers of the pathogen (< 100 CFU/g) are present such as in these naturally contaminated cheeses.

However, in both cases, challenge testing and durability study, survival of the pathogen throughout the shelf-life was noted in these red-smear semi-hard cheeses made of raw (goat or cow) milk. Thus, it should be kept in mind that in particular for consumption by pregnant women and other specific vulnerable groups being susceptible to listeriosis (Goulet et al., 2012), also low numbers of *L. monocytogenes* are not tolerated in these ready-to-eat foods, and thus absence per 25 g (preferably confirmed by using a multiple sample subunit approach (n = 5) for *L. monocytogenes* testing) needs to be the preferred food safety objective to aim for.

Challenge testing and durability testing are useful tools that FBO could use in order to assess the safety of food products. However, these tests are conducted at present up to the end of the shelf-life, and the temperature abuse is included using the $75^{th}$ percentile of the temperature distribution of the country or 12°C (if no data are available). However, this approach, being conservative, could lead to an overestimation of the exposure of consumers to elevated numbers of *L. monocytogenes*. Therefore, further investigation on simplified distributions of storage time and temperature should be performed in order to improve the effectiveness of challenge tests and durability studies in assessing food safety. This was

undertaken in Chapter 5. Moreover, this type of simplified ‘distributions’ approach to get realistic estimations of ‘reasonably foreseen abuse’ of temperature and time of storage could not only be used in set-up of challenge testing or durability studies but also in relation to risk assessment, aiming at quantifying the risk for listeriosis by consumption of ready-to-eat foods with prolonged shelf-life under refrigeration.

# CHAPTER 5. Analysis of domestic refrigerator temperatures and home storage time distributions for shelf-life studies and food safety risk assessment

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## ABSTRACT

In the framework of food safety, when mimicking the consumer phase, the storage time and temperature used are mainly considered as single point estimates instead of probability distributions. This single-point approach does not take into account the variability within a population and could lead to an overestimation of the parameters. Therefore, the aim of this study was to analyse data on domestic refrigerator temperatures and storage times of chilled food in European countries in order to draw general rules, which could be used either in shelf life testing or risk assessment. In relation to domestic refrigerator temperatures, 15 studies provided pertinent data. Twelve studies presented normal distributions, according to the authors or from the data fitted into distributions. Analysis of temperature distributions revealed that the countries were separated into two groups: northern European countries and southern European countries. The overall variability of European domestic refrigerators is described by a normal distribution: N (7.0, 2.7)°C for southern countries, and, N (6.1, 2.8)°C for the northern countries. Concerning storage times, seven papers were pertinent. Analysis indicated that the storage time was likely to end in the first days or weeks (depending on the product use-by date) after purchase. Data fitting showed the exponential distribution was the most appropriate distribution to describe the time that food spent at consumer's place. The storage time was described by an exponential distribution having as parameter the use-by-date period divided by the common value 4, which is the median value of the 75$^{th}$ percentiles of the retrieved studies. In conclusion, knowing that collecting data is time and money consuming, in the absence of data, and at least for the European market and for refrigerated products, building a domestic refrigerator temperature distribution using a Normal law and a time-to-consumption distribution using an Exponential law would be appropriate.

## 5.1 Introduction

Besides consumer behaviour in the domestic environment, the phenomenon of the changing eating habits of consumers, may have an impact on food safety as well. There is an increased demand for convenience foods such as Refrigerated Processed Foods of Extended Durability (REPFED) and fresh-like, ready-to-eat food products with up to several weeks of shelf-life (Daelman, Jacxsens, Membré, et al., 2013). These are often mildly processed foods with no or few preservatives present, and which mainly rely on modified atmosphere packaging and respect of the cold chain (storage at 4 to max. 7°C) to ensure safety and quality of product until the end of shelf-life (Ragaert, Devlieghere, & Debevere, 2007).

Pathogenic microorganisms may occasionally be present, usually infrequently and at low numbers, in these types of foods, but if products are stored at the appropriate temperature and shelf-life is respected, the risk for consumers of food-borne disease is usually low (Daelman, Jacxsens, Devlieghere, & Uyttendaele, 2013). It is well recognized that temperature is one of the major controlling factors of food quality and food safety because of its influence on microbial growth rates. In fact, bacterial growth (of both pathogenic and spoilage organisms), and subsequently food's shelf-life, mainly depends on the temperature and time of storage.

Therefore, the performance of the cold chain is very important in assuring product quality and safety. Temperature abuse occurs along the food chain and thus, temperature control is especially critical in the last three steps of the cold chain (display cabinets, transport after shopping and domestic refrigerators) (Derens-Bertheau, Osswald, Laguerre, & Alvarez, 2015). In particular, one of the most sensitive parts of the cold chain is domestic storage, where it is already proved that temperature abuse occur frequently in domestic refrigerators which mean temperatures can be in the range of 8 to 10°C (James et al., 2008). According to EFSA opinions on *Listeria monocytogenes* in RTE food, the temperature of domestic refrigerators is highly variable and domestic refrigerator temperatures can therefore have a significant effect on the risk of listeriosis (EFSA Panel on Biological Hazards (BIOHAZ), n.d.; Scientific Opinion of the Panel on Biological Hazards, 2007).

Besides, several studies have pointed out that consumers do not always respect instructions on time and temperature of storage or preparation of refrigerated foods, as indicated on the shelf-life label (Ceuppens, Van Boxstael, Westyn, Devlieghere, & Uyttendaele, 2016; Daelman, Jacxsens, Membre, et al., 2013; Marklinder & Erikkson, 2015; Van Boxstael et al., 2014).

Therefore, in order to meet an appropriate level of protection, it is of paramount importance that both FBOs and risk assessors take into account consumer behaviour – in particular domestic storage temperature and storage time – when assessing food safety. In addition, under Article 3 of Regulation (EC) No 2073/2005, FBOs are obliged to ensure that the food safety criteria applicable throughout the shelf-life of products can be met under reasonably foreseeable conditions of distribution, storage and use. Concerning temperature abuse at the consumer phase, the EU reference laboratory for *Listeria monocytogenes* suggested using either the relevant recorded temperature for the country (75$^{th}$ percentile of the observed home refrigeration temperatures), or if such data is not available, 12°C (EURL-Lm, 2014). On the other hand, for the storage time, the shelf-life test is carried out up to the end of the shelf-life. However, it is to be expected that not all items of a batch of food product are stored up to and consumed on the last day of its shelf-life. Literature data (Daelman, Jacxsens, Membré, et al., 2013) reported that chilled food is usually consumed before the end of the shelf-life and that only a small proportion of consumers do not respect the use-by date. Therefore, if assuming that all packages of chilled food are kept to the end of shelf-life and stored under temperature abuse conditions (e.g. 10°C), this is 'worst case' and overestimating the risk the food product presents because in reality the food product is not all the time 'abused' by all consumers. Thus, taking a deterministic fail-safe approach will lead to unreasonable stringent requirements e.g. shortening shelf life and may lead to more food waste. On the other side, if using distributions instead of using 'worst case' deterministic approaches, the 'high risk' of exposure to *L. monocytogenes* as the case of people that eat food at the end of the shelf-life (or after the use by date) is still always present in the tails of the distributions.

In fact, when establishing the use-by date of chilled, pre-packed, ready-to-eat food products or performing risk assessments, the use of single-point estimates is far from reality. Instead of using single-point estimates of the variables, it is preferable to use distributions, which characterize the full range of potential values and their likelihood of occurrence (Membré & Guillou, 2016; Membré & Valdramidis, 2016). In fact, distributions reflect the variability of the parameters within a population, allowing for more informed decisions. Taking this variability in consideration, recently, Gogou et al. (2015) developed a cold chain database, which contains a large collection of time-temperature profiles from different stakeholders, associated with a probabilistic tool in order to run simulation scenarios of time-temperature evolution of food along the cold chain.

In addition, according to risk assessors, when performing QMRA it is suggested to use

preferably the own country data. However, in absence of own data it could be useful to use at least data from a region close to its own. Nauta et al. (2003), looking at domestic refrigerator temperatures (when performing a retail and consumer phase model for exposure assessment of *Bacillus cereus*), collected the domestic refrigerator temperature of seven different EU countries and built distributions of domestic refrigerator temperatures. He concluded that some geographical trends seem to be present and that in northern countries refrigerator temperatures are usually lower than in southern countries. Therefore, this conclusion suggests that this hypothesis of a difference between northern and southern countries should be better explored and ad hoc data should be provided and eventually used to implement exposure assessment model to obtain more reliable outputs at national level.

Therefore, the main objectives of this study were first, collect data on domestic refrigerator temperatures and time to consumption of chilled food and fit distributions to the available data; second, explore the hypothesis suggested by Nauta et al. (2003) of a difference between northern and southern EU countries; third, define a general rule able to describe, in terms of probability distribution, the domestic refrigerator temperature and the storage time of chilled food in European countries. Data on consumer surveys were collected and organized in order to fit a parametric distribution to the observed data. The key advantage is that the distribution is defined by a limited number of parameters, which can easily be shared and used within different situations, thus providing a useful tool for both FBOs and risk assessors.

## 5.2 Materials and methods

### *5.2.1 Literature search*

A literature search on the storage time of chilled food products at consumer level and on domestic refrigerator temperatures was performed. Studies published from the year 2000 onward and limited to European countries were considered. The search process was performed on two electronic databases, PubMed and Web of Science and on the internet search engine Google Scholar. The keywords used to retrieve pertinent information on domestic refrigerators temperature were “domestic”, “storage” and “temperature” while for the storage time the words “time to consumption” or “home”, “storage” and “time” were used. The information provided by the collected papers was organized in tables reporting the reference, the country, the sample size, the kind of provided data (e.g. raw data, mean and standard deviation), the recording time and position, the device used to measure the

temperature, the shelf-life period and the refrigerated food product considered. Tables 18 and 19 show the above-mentioned information on storage temperature and storage time, respectively.

**Table 18.** Studies on *domestic refrigerator temperatures* (NR: not reported). Studies kept in the temperature analysis are marked in grey.

| Reference | Country | Sample size | Format of data provided | Position | Recording time | Device |
|---|---|---|---|---|---|---|
| Laguerre, Derens, & Palagos, 2002 | FR | n=120 | Mean, SD, percentile | Top, middle and bottom shelves | Every 2-8 mins for 7 days | Data logger |
| WIV-ISP, 2006 | BE | n=3001 | Mean, percentile | NR | NR | NR |
| Azevedo et al., 2005 | P | n=86 | Table of frequency | NR | NR | Digital thermometer |
| Kennedy et al., 2005 | IR | n=100 | Table of frequency | Middle shelf | Every 10 mins for 72 h | Data logger |
| Taoukis, Giannakourou, Koutsoumanis & Bakalis, 2005 | GR | n=250 | Mean, SD | NR | 7 days | Data logger |
| Terpstra, Steenbekkers, de Maertelaere, & Nijhuis, 2005 | NL | n=31 | Min, max, percentile | Door | 24 h | Glass thermometer |
| Breen et al., 2006 | UK | n=24 | Min, max, mode, percentile | NR | NR | Glass thermometer |
| Derens, Palagos, & Guilpart, 2006 | FR | n=251 | Mean, SD | NR | NR | NR |
| Carrasco, Perez-Rodriguez, Valero, Garcia-Gimeno, & Zurera, 2007 | SP | n=30 | Mean, SD, percentile | NR | Every 30 s over 24 h | Data logger |
| Garrido, García-jalón, & Vitas, 2010 | SP | n=33 | Min, max, mean, SD | Top, middle and bottom shelves | NR | Calibrated probe |
| Koutsoumanis, Pavlis, Nychas, & Xanthiakos, 2010 | GR | n=100 | Table of frequency | Top, middle, bottom shelves and door | Every 5 min for 24 h | Data logger |
| WRAP (Waste and Resources Action Programme), 2010 | UK | n=50 | Table of frequency | Top, middle and bottom shelves | 4 days, every 1 min | Data logger |
| Roccato, 2013 | IT | n=106 | Temperature values | Top, bottom and door | Every 15 min for 7 days | Data logger |
| Vegara et al., 2014 | IT | n=84 | Mean, percentile | Middle shelf | 24 h | Digital thermometer |
| (Marklinder & Erikkson, 2015) | SW | n=1770 | Mean, SD | Back and front of top, middle and bottom shelves | 24 h | Refrigerator thermometer |

**Table 19.** Studies on *storage time.* All of them were included in the analysis.

| Reference | Country | Format of data provided | Product | Use-by date (days) |
|---|---|---|---|---|
| Marklinder, Lindblad, Eriksson, Finnson, & Lindqvist, 2004 | SW | Table of frequency | Minced meat | 7 |
| | | | Fresh fish | 7 |
| | | | RTE salad | 7 |
| | | | Salmon | 28 |
| | | | Ham | 21 |
| | | | Cheese | 45 |
| Morelli & Derens, 2009 | FR | Percentile | Smoked salmon | 28 |
| Garrido, García-Jalón, Vitas, & Sanaa, 2010 | SP | Table of frequency | Smoked fish, sliced cooked ham | 30 |
| Koutsoumanis et al., 2010 | GR | Table of frequency | Pasteurised milk | 5 |
| Mataragas, Zwietering, Skandamis, & Drosinos, 2010 | GR | Table of frequency | Sliced cooked cured ham | 60 |
| Daelman, Jacxsens, Membré, et al., 2013 | BE | Table of frequency | Cooked chilled food | 35 |
| Derens-Bertheau et al., 2015 | FR | Mean and percentile | Sliced ham | 30 |

### *5.2.2 Fitting data into distributions*

In relation to the ***storage temperature***, information was reported in various formats: (i) only mean and SD provided, (ii) a set of temperature values, (iii) mean, SD and notable percentiles (e.g. the 95$^{th}$ percentile), or (iv) a 2-dimension array with temperature range and cumulative frequencies (Table 20). In this latter case, each temperature range was summarised to its mean to obtain a temperature cumulative frequency dataset. Data were then fitted into distributions (except when only mean and SD were provided (i)) using the software @Risk (Palisade corporation, version 6.3.1). This software was chosen as it provides the flexibility to analyse data in various formats. For each study, the different distributions provided were ranked according to the root mean squared error (RMSE) as a measure of goodness of fit. Because the devices used to measure the temperature of the refrigerators were located in different positions, in order to have enough number of studies to analyse, the data used in the present study were those of the middle position, the most commonly investigated one. Therefore, only the temperature data of the refrigerator middle shelves were elaborated in the present study, while the temperature data of other positions (door, top or bottom shelves) were not included. Data of studies measuring the domestic refrigerator temperature other than the middle shelf are reported in Table 24 of Annex 2.

**Table 20.** Studies on domestic refrigerator temperatures: statistical parameters provided by the authors or obtained by distribution fitting (NA: not available; NR: not reported).

| Reference | Country | Format in which data were reported | Distribution according to the author | Fitting with @Risk | Goodness of fitting criteria (RMSE) | Mean (°C) | SD (°C) |
|---|---|---|---|---|---|---|---|
| Laguerre et al., 2002 | FR | Mean, SD and percentiles (6.6; 2.3; 25$^{th}$: 5.2; 75$^{th}$: 8.2) | Normal | NA | _ | | |
| WIV-ISP, 2006 | BE | Mean and percentiles (7.0; 25$^{th}$: 5.0; 75$^{th}$: 9.0) | NR | Normal | _ | 7.00 | 2.96 |
| Azevedo et al., 2005 | P | Table of frequency | NR | Weibull | 0.0111 | 6.20 | 2.64 |
| | | | | Triangular | 0.0149 | 6.28 | 2.48 |
| | | | | Logistic | 0.0177 | 6.34 | 2.78 |
| | | | | Normal | 0.0187 | 6.34 | 2.58 |
| Kennedy et al., 2005 | IR | Table of frequency | NR | Normal | 0.0224 | 5.85 | 2.49 |
| | | | | Logistic | 0.0195 | 5.86 | 2.65 |
| | | | | Weibull | 0.0221 | 5.78 | 2.46 |
| Taoukis et al., 2005 | GR | Mean and SD (6.3; 2.7) | Normal | NA | _ | | |
| Terpstra et al., 2005 | NL | Min, max and percentile (3.8; 11.5; 68$^{th}$: 7.0) | NR | Pert | _ | 6.40 | 1.38 |
| Breen et al., 2006 | UK | Min, max and mode (1.0; 12.0; 5.0) | NR | Pert | _ | 6.06 | 2.07 |
| Derens et al., 2006 | FR | Mean, SD (5.9; 2.9) | Normal | NA | _ | | |
| Carrasco et al., 2007 | SP | Mean, SD and percentile (6.6; 2.6; 90$^{th}$: 10.0) | Normal | NA | _ | | |
| Garrido et al., 2010 | SP | Mean, SD, min., max. (7.9; 2.6; 0.6; 14.5) | Normal | NA | _ | | |
| Koutsoumanis et al., 2010 | GR | Table of frequency | NR | Logistic | 0.0083 | 6.35 | 2.62 |
| | | | | Normal | 0.0134 | 6.35 | 2.45 |
| | | | | Weibull | 0.0156 | 6.34 | 2.40 |
| | | | | Triangular | 0.0187 | 6.32 | 2.32 |
| WRAP (Waste and Resources Action Programme), 2010 | UK | Table of frequency | NR | Normal | 0.0177 | 6.54 | 2.93 |
| | | | | Triangular | 0.0149 | 6.48 | 2.76 |
| | | | | Beta General | 0.0154 | 6.44 | 2.86 |
| | | | | Weibull | 0.0153 | 6.46 | 2.87 |
| Roccato, 2013 | IT | Temperature values (n=106) | NR | Logistic | 0.0374 | 7.18 | 1.12 |
| | | | | Normal | 0.0489 | 7.17 | 1.97 |
| | | | | Weibull | 0.051 | 7.17 | 1.96 |
| | | | | Beta General | 0.0589 | 7.17 | 1.98 |
| Vegara et al., 2014 | IT | Mean and percentiles (8.1; 43$^{th}$: 4.0; 51$^{th}$: 10.0) | NR | Lognormal | _ | 8.10 | |
| Marklinder & Erikkson, 2015 | SW | Mean and SD (5.9; 3.1) | NR | Normal | _ | 5.90 | 3.10 |

In Figure 14, the different steps described above are shown, while Table 20 reports the statistical parameters of the retrieved studies, as stated by the authors or according to the fitted distributions.

In addition, in order to assess whether there was, or was not, a difference of mean and SD values as function of the region (southern and northern European countries), the mean and the SD of each country derived from the normal distribution (fitted with @Risk or stated by the authors) were organised in two groups – the southern (code1) and the northern (code 2) – and compared (Table 21).

**Table 21.** Domestic refrigerator studies once temperatures were fitted with the normal distribution (fitted with @Risk or stated by the authors): comparison between northern and southern European countries.

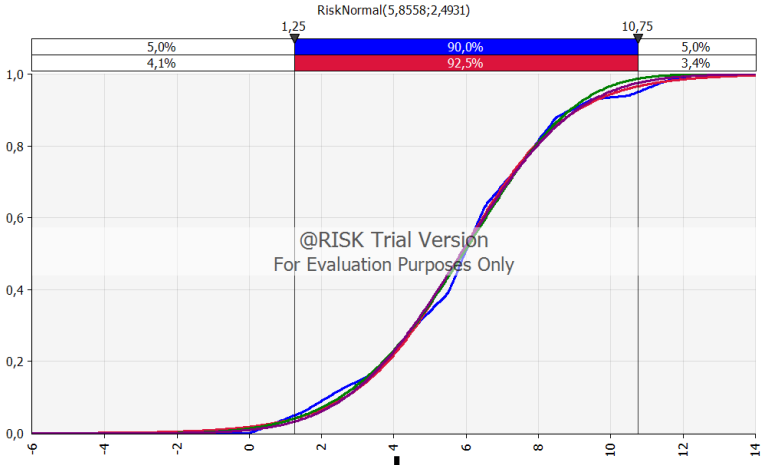
| | Country | Mean (°C) | SD (°C) |
|---|---|---|---|
| Southern countries | GR | 6.4 | 2.5 |
| | GR | 6.3 | 2.7 |
| | P | 6.3 | 2.6 |
| | IT | 7.2 | 2.0 |
| | SP | 6.6 | 2.6 |
| | SP | 7.9 | 2.6 |
| Northern countries | IR | 5.9 | 2.5 |
| | UK | 6.5 | 2.9 |
| | FR | 6.6 | 2.3 |
| | FR | 5.9 | 2.9 |
| | SW | 5.9 | 3.1 |
| | BE | 7.0 | 3.0 |

Because of the low amount of available data, the non-parametric Mann Whitney test ($p < 0.05$) was carried out to see if a statistically significant difference existed between the two groups, using XLSTAT (Addinsoft, version 2015.1.03). Once the difference was established, for both northern and southern groups, an overall distribution was determined as follows: Normal (overall_mean, overall_sd), overall_mean and overall_sd being the mean and the standard deviation of either the northern or the southern group. The overall mean and the overall standard deviation were calculated using the means for each country belonging to the northern or the southern group. Box-plots were used to synthesize the data, providing the principal measures of central tendency and dispersion. In a box-plot representation, the bottom and top of the box are the $25^{th}$ and $75^{th}$ percentiles (Q1 and Q3 respectively), the band in the box is the median and the "+" symbol the mean. The ends of the whiskers represent the lowest figure still within 1.5 IQR (Inter Quartile Range, IQR = Q3 − Q1) of the lower quartile, and the highest figure still within 1.5 IQR of the upper quartile. The dots correspond to the min. and max. of all data..

**a)**

| Reference | Country | Format of data provided | Mean temperature (°C) | Frequency (n) | Mean class (°C) | Cumulative probability |
|---|---|---|---|---|---|---|
| Kennedy et al., 2005 | IR | Table of frequency | >0 | 2 | 0.5 | 0.02 |
| | | | 1-2 | 4 | 1.5 | 0.06 |
| | | | 2-3 | 6 | 2.5 | 0.12 |
| | | | 3-4 | 5 | 3.5 | 0.17 |
| | | | 4-5 | 12 | 4.5 | 0.29 |
| | | | 5-6 | 10 | 5.5 | 0.39 |
| | | | 6-7 | 24 | 6.5 | 0.63 |
| | | | 7-8 | 12 | 7.5 | 0.75 |
| | | | 8-9 | 13 | 8.5 | 0.88 |
| | | | 9-10 | 5 | 9.5 | 0.93 |
| | | | 10-11 | 1 | 10.5 | 0.94 |
| | | | 11-12 | 4 | 11,5 | 0.98 |
| | | | 12-13 | 2 | 12.5 | 1.00 |

**b)**




| | Input | Logistic | Weibull | Normal |
|---|---|---|---|---|
| Minimum | -1,110E-016 | −∞ | -4,930 | −∞ |
| Maximum | 12,500 | +∞ | +∞ | +∞ |
| Mean | 5,845 | 5,862 | 5,789 | 5,856 |
| Mode | N/A | 5,862 | 6,233 | 5,856 |
| Median | 5,958 | 5,862 | 5,919 | 5,856 |
| Std Dev | 2,584 | 2,657 | 2,464 | 2,493 |
| Skewness | 0,0296 | 0,0000 | -0,2514 | 0,0000 |
| Kurtosis | 2,9240 | 4,2000 | 2,8774 | 3,0000 |

**c)**

| Mean squared errors | Distributions |
|---|---|
| 0.0224 | RiskNormal(5.8544,2.4937) |
| 0.0221 | RiskWeibull(4.9612,11.635,RiskShift(-4.8889) |
| 0.0195 | RiskLogistic(5.8619,1.464)) |

**Figure 14.** Workflow followed to extract relevant information from each study on domestic refrigerator temperatures, using one study as an example. a) data organized in tables; b) data fitted into distributions; c) ranking distributions according to the root mean squared error.

Finally, for each country belonging to the northern or the southern group, the values of the normal distribution defined by the overall means and standard deviations previously calculated, were compared with the data provided by the study itself. For studies presenting tables of frequency, several percentiles of the distribution were compared to the ones provided by the normal distribution of the northern or the southern group, while measures of central tendency and dispersion given by the other studies (for which the raw data were not available) were compared with the ones of the northern or southern group.

In relation to the ***storage time***, the same approach as for domestic storage temperature was taken. Only studies providing data collected through questionnaires and surveys have been taken into account. Data collected from the retrieved papers were fitted into distributions using the software @Risk in order to evaluate if a general pattern of distribution could be defined. The fitted distributions were ranked according to the root mean squared error criteria. According to the use-by-date, studies were divided into two groups: long use-by date (> 10 days) *versus* short use-by date (≤ 10 days). In order to find a common rule that could describe the storage time of chilled food as a function of their use-by date, for each study, the value of the use-by date was divided by the 75$^{th}$ percentile of the storage time. For example if the use-by date was 30 days and the 75$^{th}$ percentile 8.5 days, this resulted in 3.5.

The obtained values were used in order to calculate the median for each group of studies and then establish a general rule. Finally, for each study we compare the percentiles (or measures of central tendency and dispersion) of the distribution defined by the rule to the percentiles (or measures of central tendency and dispersion) of the study itself.

## 5.3 Results

### *5.3.1 Temperature*

Among the retrieved papers, 15 studies measured the air temperature of domestic refrigerators while 4 studies recorded the temperature of several types of food stored in the domestic refrigerator. The studies dealing with the temperature measured inside the food were discarded because: a) the number of studies was not enough to be statistically compared; b) the reported data consisted in single point estimates, thus, not appropriate to be used for the aims of this study.

In Table 18, information on the 15 studies used in this paper are reported in chronological and alphabetical order. In order to group the different EU countries of the

available studies, different ways can be used and the criteria taken into account can be political, economic, cultural and geographical. Therefore, a single approach does not exist. France for example can be considered half norther and half southern according to the above mentioned criteria. In our case, taking into account also the opinion of one of the authors (a French speaking person which perceived France as being a northern country due to the cultural similarities of the Region she came from) we decide to consider France as a northern country. This decision was discussed and shared by the authors of the manuscript. Therefore, among the 15 studies, seven studies (two from Greece, two from Italy, two from Spain and one from Portugal) belonged to the southern countries and eight studies (two from France, two from UK, one from Ireland, one from Sweden, one from Belgium and one from Netherlands) were from the northern countries.

Six of the 15 studies did not specify the measured position; the remaining nine studies measured the domestic refrigerator temperatures at the following positions: door (n = 1); middle shelf (n = 2); top, middle and bottom shelves (n = 4); top, middle, bottom shelves and door (n = 1); top, bottom shelves and door (n = 1). Therefore, 7 of 9 studies reported data related to the middle position of refrigerators.

Concerning the kind of data provided, five studies provided tables of frequency or temperature values, six provided parameters such as mean and SD, while four presented a set of temperature values (e.g. mean, percentiles, min., max., etc.); therefore, more than half of the papers did not provide enough data for fitting.

According to the authors, five of fifteen studies presented data showing a normal distribution, seven of the ten remaining studies presented data, which were used in the present study to be fitted into distributions. For all of these seven studies, the normal distribution was one of the appropriate fits according to the ranking criteria adopted (RMSE). The remaining three studies were best described by lognormal (one study) and Pert distributions (two studies).

Concerning the studies presenting a normal fitted distribution, the values of the mean square error indicated that this type of distribution was not always the first choice nor the worst choice. Therefore, for all these reasons, and also for consistency, we decided to test whether it was possible to predict the domestic refrigerator temperature using a normal distribution (Table 20).

Apart from the kind of data provided, twelve studies presented a normal distribution; in particular, six studies were from the northern countries and the other six studies from the southern countries.

The mean and SD values provided by the normal distributions were used in order to calculate, for each group, an overall mean and an overall standard deviation. Therefore, for southern European countries, the distribution of domestic refrigerator temperatures (n = 6) was described by a normal distribution with a mean of 7.0°C and a standard deviation of 2.7°C, while for the northern European countries (n = 6) this resulted in *N* (6.1, 2.8)°C. Figures 15a and 15b show the overall means and standard deviations respectively, obtained for the northern and the southern countries. The Mann-Whitney test showed that there was not a statistically significant difference ($p < 0.05$) between the mean values and the standard deviations of the northern (code 2) and southern (code 1) countries. However, the descriptive box-plots clearly highlighted the existence of a difference (even if not significant) between the means of the two groups. Therefore, it was preferable to keep the two groups of countries separated in further analysis.

The range of the refrigerator temperature means of southern European countries was above 1°C (1.6°C) while in case of northern European countries, this difference was equal to 1°C. The same range calculated for the standard deviation was equal to 0.6°C for southern European countries and 0.8°C for northern European countries. This meant that in case of northern European countries, there was a larger dispersion of measured temperatures than for southern countries, which at the same time had a higher mean temperature than the northern ones.

The comparison between the temperatures provided by the retrieved studies and the temperatures of the fitted normal distribution is reported in Figure 16a for the northern European countries and in Figure 16b for the southern European countries. The results indicated that for the northern countries, the difference between the observed temperatures and the temperatures of the normal distribution was less than ± 0.5°C for 15 of 21 comparable temperatures, while for the remaining temperatures (6 of 21), the difference was above ± 0.5°C (see Table 23 in Annex 2). In relation to the southern countries, the difference between the provided normal distribution and the temperatures obtained by the studies were below ± 0.5°C for 14 of 23 comparable temperatures, above ± 0.5°C for 6 of 23 comparable temperatures and > ±1°C for three temperatures (See Table 23 in Annex 2).

In particular, concerning temperatures > ±1°C, two of three of these belonged to one study (Vegara et al., 2014). The study was conducted on domestic refrigerators in different Italian provinces located in northern and central Italy. The provided data were better fitted to a lognormal distribution instead of a normal one. In addition, > 50% of the surveyed refrigerators had an average temperature higher than 10°C.

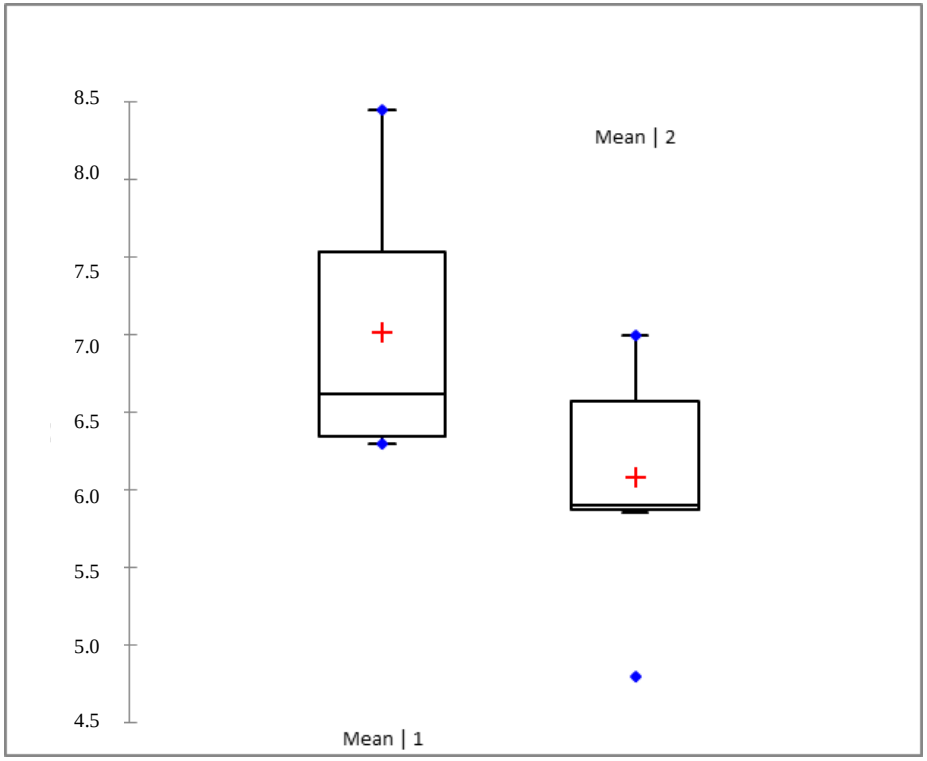



**Figure 15a**. Box and whiskers plot of domestic refrigerator temperatures*: overall means* calculated for the northern (code 2) and southern (code 1) European countries.

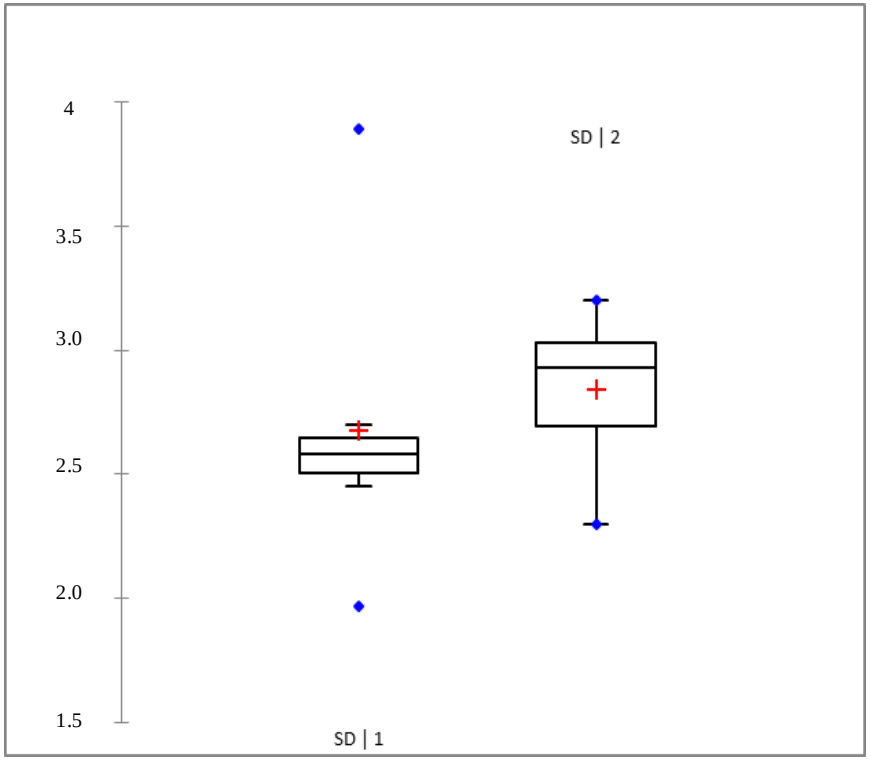



**Figure 15b**. Box and whiskers plot of domestic refrigerator temperatures: *standard deviations* calculated for the northern (code 2) and southern (code 1) European countries.

In a box and whiskers plot representation, the bottom and top of the box are the 25$^{th}$ and 75$^{th}$ percentiles (Q1 and Q3 respectively), the band in the box is the median and the "+"symbol the mean. The ends of the whiskers represent the lowest figure still within 1.5 IQR (Inter Quartile Range, IQR = $Q_3 - Q_1$) of the lower quartile, and the highest figure still within 1.5 IQR of the upper quartile. The dots correspond to the min. and max. of all data.

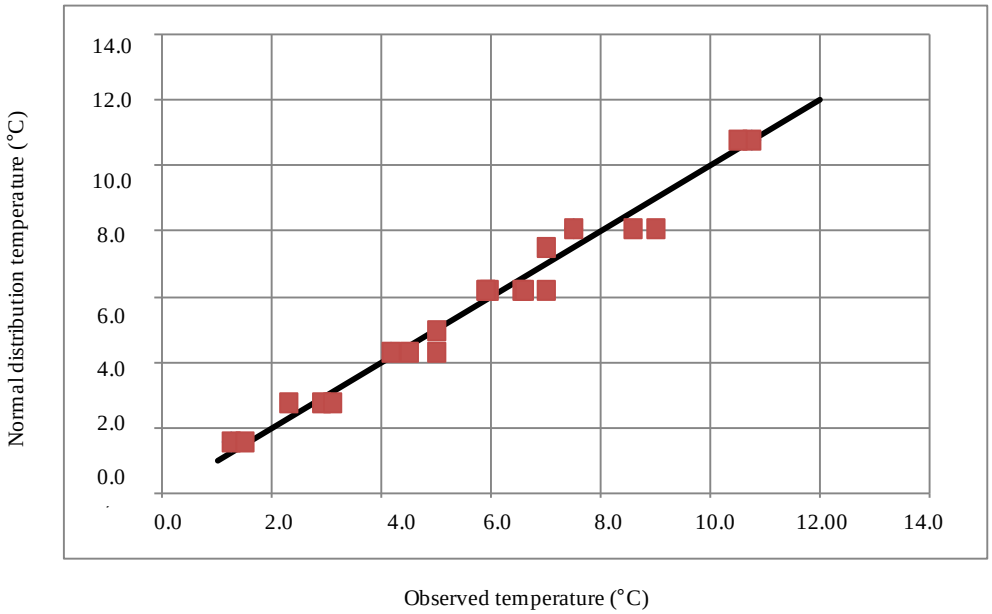



**Figure 16a.** *Northern European countries*. Mean temperature values provided by the retrieved studies *versus* values of the fitted normal distribution. The solid black line depicts the y=x line.

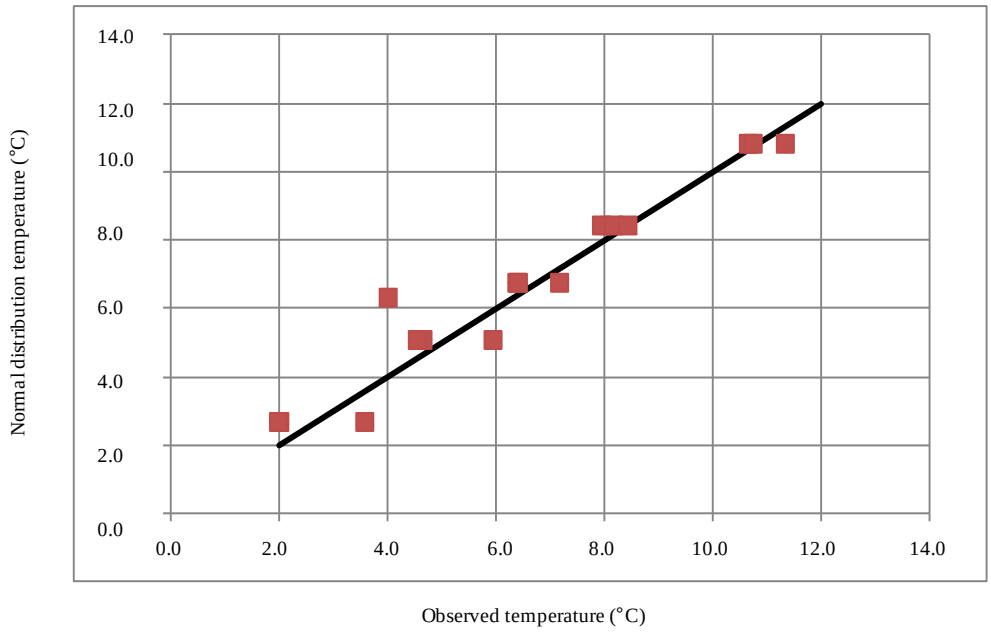



**Figure 16b.** *Southern European countries*. Mean temperature values provided by the retrieved studies *versus* values of the fitted normal distribution. The solid black line depicts the y=x line.

This could explain the difference of > 1°C between the observed values and the normal distribution of southern European countries.

Finally, the derived general rule was: the overall variability of European domestic refrigerator temperatures was described by a normal distribution for the southern countries *N* (7.0, 2.7)°C and another normal distribution for the northern countries *N* (6.1, 2.8)°C.

### *5.3.2 Storage time*

Among the 8 retrieved papers, 7 papers based on surveys were considered eligible while the one based on expert opinion was discarded. Seven articles on storage time matched the defined criteria, providing information through twelve studies in total. Eight studies investigated the storage time of long use-by date (> 10 days) chilled products while four studies dealt with storage time of short use-by date chilled products (≤ 10 days). The results indicated that the storage time was likely to end in the first days or weeks (depending on the product use-by date) after purchase. Data fitting showed an exponential distribution was appropriate to describe the time that food spend at consumers' homes, in most of the cases, and this kind of distribution was also chosen by Chardon & Swart (2016), to predict the time of food in the fridge. Table 25 (see Annex 2) shows the ranking scores of several fitted distributions.

The identified common rule highlighted that the storage time was described by an exponential distribution having as parameter the use-by date period divided by a common value equal to 4, which is the median value of the 75th percentiles of the retrieved studies. Therefore, in order to test if the rule was applicable and taking into account the kind of data provided by the studies, we compared percentiles or measures of central tendency and dispersion of the studies with the same kind of values provided by the exponential distribution obtained applying the common rule.

The comparison between percentiles and measures of central tendency and dispersion of the studies and the exponential distribution for long use-by date and short use-by date chilled products is reported in Table 22.

In the case of the long use-by date products, in correspondence with the $75^{th}$ percentile, the difference between the observed values and the exponential distribution is less than 2 days in 6 of 8 studies, meaning that the products are consumed within a few days after being bought.

**Table 22.** Storage time: comparison between percentiles and measures of central tendency observed in the studies *versus* the exponential distribution values for long use-by date studies and short use-by date studies (NA: not available).

| | Reference | Country | Format of data provided | Product | Use-by date (days) | Values | Days (observed) | Days (exponential distribution) | Differences between "observed" and "exponential distribution" use-by date (days) |
|---|---|---|---|---|---|---|---|---|---|
| **Long Use-by date studies** | Marklinder et al., 2004 | SW | Table of frequency | Salmon | 28 | 25% | 1.5 | 1.5 | 0 |
| | | | | | | 50% | 2.6 | 3.5 | -1 |
| | | | | | | 75% | 5.5 | 7.0 | -2 |
| | | | | | | 95% | 30.3 | 15.1 | 15 |
| | | | | Ham | 21 | 25% | 2.7 | 1.1 | 2 |
| | | | | | | 50% | 4.1 | 2.6 | 1 |
| | | | | | | 75% | 6.3 | 5.3 | 1 |
| | | | | | | 95% | 40.3 | 11.3 | 29 |
| | | | | Cheese | 45 | 25% | 3.4 | 2.3 | 1 |
| | | | | | | 50% | 6.4 | 5.6 | 1 |
| | | | | | | 75% | 11.1 | 11.3 | 0 |
| | | | | | | 95% | 26.9 | 24.3 | 3 |
| | Morelli & Derens, 2009 | FR | Percentile | Smoked salmon | 28 | 45% | 2.0 | 3.0 | -1 |
| | | | | | | 75% | 7.0 | 7.0 | 0 |
| | Garrido, García-Jalón, Vitas, & Sanaa, 2010 | SP | Table of frequency | Smoked fish; sliced cooked ham | 30 | 25% | 2.6 | 1.6 | 1 |
| | | | | | | 50% | 5.1 | 3.8 | 1 |
| | | | | | | 75% | 6.7 | 7.5 | -1 |
| | | | | | | 95% | 9.4 | 16.2 | -7 |
| | Mataragas et al., 2010 | GR | Table of frequency | Sliced cooked cured ham | 60 | 25% | 2.8 | 3.1 | 0 |
| | | | | | | 50% | 5.5 | 7.5 | -2 |
| | | | | | | 75% | 6.9 | 15.0 | -8 |
| | | | | | | 95% | 32.8 | 32.4 | 0 |
| | Daelman, Jacxsens, Membré, et al., 2013 | BE | Table of frequency | Cooked chilled food | 35 | 25% | 1.2 | 1.8 | -1 |
| | | | | | | 50% | 1.9 | 4.4 | -2 |
| | | | | | | 75% | 4.0 | 8.8 | -5 |
| | | | | | | 95% | 11.2 | 18.9 | -8 |
| | Derens-Bertheau et al., 2015 | FR | Mean and percentile | Sliced ham | 30 | mean | 6 days 6 h | 5.4 | NA |
| | | | | | | 40% | < 3 days | 2.8 | NA |
| **Short Use-by date studies** | Marklinder et al., 2004 | SW | Table of frequency | Minced meat | 7 | 25% | 0.0 | 0.4 | 0 |
| | | | | | | 50% | 0.5 | 0.9 | 0 |
| | | | | | | 75% | 1.4 | 1.8 | 0 |
| | | | | | | 95% | 13.3 | 3.8 | 9 |
| | | | | Fresh fish | 7 | 25% | 0.1 | 0.4 | 0 |
| | | | | | | 50% | 0.8 | 0.9 | 0 |
| | | | | | | 75% | 1.8 | 1.8 | 0 |
| | | | | | | 95% | 13.0 | 3.8 | 9 |
| | | | | RTE salad | 7 | 25% | 0.2 | 0.4 | 0 |
| | | | | | | 50% | 0.9 | 0.9 | 0 |
| | | | | | | 75% | 1.9 | 1.8 | 0 |
| | | | | | | 95% | 19.0 | 3.8 | 15 |
| | Koutsoumanis et al., 2010 | GR | Table of frequency | Pasteurized milk | 5 | 25% | 1.0 | 0.3 | 1 |
| | | | | | | 50% | 1.5 | 0.6 | 1 |
| | | | | | | 75% | 2.0 | 1.3 | 1 |
| | | | | | | 95% | 3.0 | 2.7 | 0 |

In addition, as shown in Table 22, negative values of the 75$^{th}$ percentile were recorded, indicating that, in fact, the derived exponential distribution overestimates the storage time, and that in reality, the chilled food is consumed at the beginning of its use-by date.

In relation to short use-by date products (4 studies), the difference between the observed days and the exponential distribution was 0 days in three studies and 1 day in one study, suggesting that the defined rule better describes chilled food with short use-by dates than with long use-by dates (Table 22). It is interesting to note that for short use-by date products, there is a higher likelihood of being consumed after their use-by date, thus increasing the food safety risk posed by these products. In fact, in three of four studies, the observed values of the 95$^{th}$ percentiles were beyond the use-by date period (Table 22).

## 5.4 Discussion

The complexity of the cold chain and the numerous sources of variability require the development of simplified rules/models in order to predict the time-temperature history of chilled products along the cold chain. This study presents an overview of domestic refrigerator temperatures and home storage time of chilled food in Europe, coupled with a proposed general rule that could be used in order to incorporate these factors in shelf-life studies and food safety risk assessment.

In relation to domestic refrigerator temperatures, European studies during the last 16 years have been taken into account. The collected data showed the temperature of domestic refrigerators is still highly variable. In fact, even if there have been considerable developments in the energy efficiency and the refrigeration systems used in domestic refrigerators, these developments have often been divorced from the actual temperatures within the storage compartment (James et al., 2008). In addition, it seems that the operating conditions (e.g. thermostat setting, ambient temperature) have a higher impact on the load temperatures than the equipment design (e.g. dimension, air flow rate, insulation) (Laguerre, Duret, Hoang, & Flick, 2014).

Furthermore, the retrieved studies were grouped into northern and southern countries in order to look for ‘regional’ best fit based upon temperature data set from that region identified. Therefore, the available data were not clustered, as the question/objective was not which countries have more ‘similar’ temperature profiles but to provide a proof of the concept that if needed one can design domestic refrigerator temperature with a ‘northern’ & ‘southern’ distribution. Nevertheless, alternatively one can use an overall ‘EU wide’

temperature design.

The tested hypothesis showed that even if not significant a difference between the two groups exists and thus it will preferable to keep northern and southern EU countries divided. Moreover, the overall 1°C difference will affect the outgrowth of pathogens as *L. monocytogenes* as already stated in the EFSA opinions on *L. monocytogenes* in RTE food in which the importance of temperature control especially at consumer level was highlighted (EFSA Panel on Biological Hazards (BIOHAZ), n.d.; Scientific Opinion of the Panel on Biological Hazards, 2007).

Temperature along with storage time are the two main factors, which determine the date labelling of food. Date labelling is a means to provide consumers a point of reference regarding guarantees on the overall acceptability of food products (DEFeRA, 2011; Van Boxstael et al., 2014); it is not a definitive statement on product safety. In fact, considering that the control of the temperatures to which food products are exposed throughout the food chain is lacking, the use-by date or any similar date cannot be an assurance in terms of food safety (Newsome et al., 2014).

The definition of a shelf-life label includes sequential consideration of product quality issues (changes of texture, colour, flavour, etc.), determination of safety concerns (hazard identification, growth potential of pathogens), in-product assessment (using predictive modelling or lab tests including challenge testing or durability studies) and management making decisions on shelf-life duration, conditions for storage and the shelf-life label – use-by or best-before date – to be mentioned on the product package (FSAI, 2014; NZFSA, 2005).

In order to define the use-by date of a chilled food, the FBO might take into account different time-temperature scenarios. Regarding the temperature, normal use, temperature abuse or reasonable temperature abuse could be tested. In *normal use*, the temperature mean value is used. However, the range of this parameter both at retail and at consumer level is wide. Therefore, this parameter does not reasonably reflect a condition, which should be integrated in shelf-life testing.

When mimicking *temperature abuse*, the 95$^{th}$ percentile of the temperature distribution could be used. Even if realistic, this value is usually quite high (always > 10°C) and might perhaps be better defined as *unreasonable foreseen temperature abuse*. It is questionable if this is feasible or if it makes sense to design a chilled food product and allocate a shelf-life label to ensure wholesomeness of the food product throughout its use-by date based on (a minority of) consumers having refrigerator temperatures as high as that, and which clearly

do not respect the recommended storage conditions mentioned on the packed product. In fact, usually, the recommendation is to store chilled pre-packed product at a temperature of 4 to maximum 7°C (Ceuppens et al., 2016). Finally, *reasonable temperature* abuse could be integrated into shelf-life testing. The EURL document for *L. monocytogenes* challenge testing (EURL-Lm, 2014) recommends taking into account reasonable abuse as a function of the country under consideration, using the 75$^{th}$ percentile of the temperature distribution. However, due to the fact that considerable variability in consumers' refrigerator temperatures among European countries can occur, separate temperature storage conditions depending upon the country where the product is intended to be sold should, thus, be applied. The implementation of this approach could be laborious and time-consuming, particularly because many food products are traded across borders and supermarket chains can have subsidiaries in many countries. Therefore, a compromise could be to use the 75$^{th}$ percentile of the suggested fit distributions for 'northern' and 'southern' EU countries, being respectively 8.0 and 8.5°C. Restricting and agreeing on the '*reasonably foreseen temperature*' in consumers' homes to these two temperatures, representative of the northern and southern parts of Europe, would simplify and meet the need for clearer guidelines on setting use-by dates as highlighted by a number of meat product manufacturers and retailers in a recent survey (Ceuppens et al., 2016). A similar approach could be used in shelf-life modelling or Quantitative Microbial Risk Assessment (QMRA) in order to describe the temperature distribution of other links of the cold chain. In particular, the retail level is another weak point of the cold chain. In fact, the study of Morelli, Noel, Rosset, & Poumeyrol (2012), showed that 70% of the time-temperature profiles of foodstuffs stored at retail level exceeded 7°C, which is considered a risky temperature for the growth of *L. monocytogenes.*

Besides temperature, another important variable to take into account in shelf-life testing is the time spent in the consumer's refrigerator before consumption. In durability studies and also in *L. monocytogenes* challenge testing, recommendations to assess the growth potential of pathogens throughout the set use-by date usually take into account the full period of the set use-by date as the *normal storage time* (EURL-Lm, 2014; Vermeulen, Devlieghere, & Uyttendaele, 2011). It is expected that, in assessing the use-by date, the full-allocated storage time is included in the experimental set-up or in predictive modelling used to bring decisions on the shelf-life. The surveys analysed in the present study show that the consumption of food after the use-by date (abuse) is an unlikely (less than 1%), but still possible event. Thus, the inclusion of a few days extra in these types of shelf-life assessment

studies is recommended to simulate *abuse storage time*. On the contrary, according to literature reports on storage time in the consumer homes that were analysed in the present study, the *normal storage time* as being the end of the use-by date might be better considered as 'unreasonably foreseen' storage time, as it was noted that chilled food is likely to be consumed within a few days of being bought. However, inclusion of this *unreasonably foreseen storage time* (until the end of use-by date given on the package) is still preferred in shelf-life assessment, because use-by dates should indeed provide guarantees to consumers on the quality and safety of food throughout the shelf-life, including up to the last day of the use-by date (or even a few days past; an *abuse storage time*).

On the other hand, microbiological risk assessments have public health goals. They aim to estimate the levels of risk for food-borne disease upon consumption, provide comparisons against benchmarks and produce inputs for determining the acceptable level of risk in food safety management. Quantitative microbiological risk assessment (QMRA) uses mathematical modeling, and when quantifying the risk for the consumer, scenarios can be constructed to predict the range of possible exposures (CAC, 1999; Haas, Rose, & Gerba, 2014). The use of a set of single point 'worst-case' end of use-by date as a storage time in QMRA is a conservative approach and overestimates the likelihood of exposure to unacceptable numbers of pathogenic microorganisms. In contrast to shelf-life testing studies using deterministic values, with risk assessment calculations, it is important that food safety scientists perform probabilistic risk assessment using data derived from surveys. The variability of storage time and refrigerator temperature in the consumer population is then taken into account (Membré & Guillou, 2016). Probability processes help us to better understand and characterize random processes, which is essential when trying to make predictions about future events or trying to make decisions to reduce/increase the probability of events (Cummins, 2016). In the case of chilled foods, probabilistic QMRA enables the inclusion of realistic time-temperature foreseen abuse in the analysis.

Consequently, in order to take into account the variability of the cold chain and the product's characteristics, combining the deterministic and stochastic approaches allows the prediction as well as the description of the time-temperature profiles along the cold chain.
However, in the framework of quantitative risk assessment, it could be useful to consider the possibility to truncate the proposed normal distributions for domestic refrigerator temperature. In fact, when performing Monte Carlo simulations it might be needed to truncate the tails of the distribution in order to avoid unrealistic high values.
The proposed distributions were used in a case study applied to paté and *Listeria*

*monocytogenes* (Membré, 2013). In this case of shelf-life determination, when using single point estimates, *L. monocytogenes* grew above the limit of 100 CFU/g after 12 days of shelf-life while in the case of using the 75$^{th}$ percentile of the domestic refrigerator temperature distribution, the product reached the end of the expected shelf-life (19 days) without exceeding the limit of 100 CFU/g (EC, 2005). The same approach used to assess the risk of non-compliant samples of paté for *L. monocytogenes* showed that when performing risk assessment using a deterministic method, 9.05% of products contained 2 log CFU/g or more when consumed. However, when using stochastic distributions, 0.50% of products contained 2 log CFU/g or more when consumed.

This study presented an approach of dealing with 'temperature' & 'time' available data at consumer level, providing general rules as often this type of data are still used in quite a 'deterministic' way (i.e. looking at the 'worst case' in many challenge test and also in QMRA studies). Providing general rules is not a simple task and they likely are a compromise in order to have a tool to use as a starting point. Therefore, such rules cannot be used systematically but can answer some questions and support the development of more effective predictive methods.

Bibliographic research showed that there is often scarcity of data on consumer behaviour concerning food transport, storage and preparation (Nauta et al., 2003). However, collecting data requires allocation of resources. This study has provided probability density distributions of domestic refrigerator temperatures for northern and southern European countries and of storage times of chilled food with long or short use-by date. Consequently, in the absence of data, at least for the European market and for this group of chilled pre-packed products, these simple rules, useful for both FBOs conducting challenge testing (as described in Chapter 4) and risk assessors performing risk assessment, as illustrated in the present chapter, could be suggested:

- Normal (7.0, 2.7°C) for southern countries and Normal (6.1, 2.8°C) for northern countries for temperature in a consumer's refrigerator;
- Exponential (use-by-date divided by 4) for the time spent at a consumer's home.

## ANNEX 2

**Table 23**. Domestic refrigerator temperatures: comparison between percentiles and measures of central tendency observed in the studies *versus* the normal distribution values for northern and southern countries.

| | Reference | Country | Format of data provided | Values | °C (observed) | °C (normal distribution) | Differences between "observed" and "normal distribution" temperature (°C) |
|---|---|---|---|---|---|---|---|
| Southern group | Azevedo et al., 2005 | P | Table of frequency | 5% | 1.98 | 2.71 | 0.73 |
| | | | | 25% | 4.54 | 5.10 | 0.56 |
| | | | | 50% | 6.41 | 6.78 | 0.37 |
| | | | | 75% | 8.16 | 8.45 | 0.29 |
| | | | | 95% | 10.65 | 10.84 | 0.19 |
| | Koutsoumanis et al., 2010 | GR | Table of frequency | 5% | 2.00 | 2.71 | 0.71 |
| | | | | 25% | 4.64 | 5.10 | 0.46 |
| | | | | 50% | 6.38 | 6.78 | 0.40 |
| | | | | 75% | 7.94 | 8.45 | 0.51 |
| | | | | 95% | 11.33 | 10.84 | -0.49 |
| | Taoukis et al., 2005 | GR | Mean, SD | Mean | 6.30 | 6.78 | 0.48 |
| | | | | SD | 2.70 | 2.47 | -0.23 |
| | Vegara et al., 2014 | IT | Mean, percentile | Mean | 8.10 | 6.78 | -1.32 |
| | | | | 43% | 4.00 | 6.34 | 2.34 |
| | Roccato, 2013 | IT | Temperature values | 5% | 3.57 | 2.71 | -0.86 |
| | | | | 25% | 5.94 | 5.10 | -0.83 |
| | | | | 50% | 7.16 | 6.78 | -0.39 |
| | | | | 75% | 8.43 | 8.45 | 0.02 |
| | | | | 95% | 10.74 | 10.84 | 0.10 |
| | Carrasco et al., 2007 | SP | Mean, SD | Mean | 6.62 | 6.78 | 0.16 |
| | | | | SD | 2.56 | 2.47 | -0.09 |
| | Garrido, Garcia-Jalon, & Vitas, 2010 | SP | Mean, SD | Mean | 7.90 | 6.78 | -1.12 |
| | | | | SD | 2.60 | 2.47 | -0.13 |
| Northern group | Kennedy et al., 2005 | IR | Table of frequency | 5% | 1.25 | 1.60 | 0.35 |
| | | | | 25% | 4.17 | 4.33 | 0.16 |
| | | | | 50% | 5.96 | 6.21 | 0.25 |
| | | | | 75% | 7.50 | 8.08 | 0.58 |
| | | | | 95% | 10.75 | 10.77 | 0.02 |
| | Breen et al., 2006 | UK | Min, max, percentile | Min | 1.00 | NA | _ |
| | | | | Max | 12.00 | NA | _ |
| | | | | 33% | 5.00 | 4.98 | -0.02 |
| | WRAP, 2010 | UK | Table of frequency | 5% | 1.50 | 1.60 | 0.10 |
| | | | | 25% | 4.50 | 4.33 | -0.18 |
| | | | | 50% | 6.56 | 6.21 | -0.35 |
| | | | | 75% | 8.58 | 8.08 | -0.50 |
| | | | | 95% | 10.50 | 10.77 | 0.27 |

**Table 23 (continued).** Domestic refrigerator temperatures: comparison between percentiles and measures of central tendency observed in the studies *versus* the normal distribution values for northern and southern countries.

| | Reference | Country | Format of data provided | Values | °C (observed) | °C (normal distribution) | Differences between "observed" and "normal distribution" temperature (°C) |
|---|---|---|---|---|---|---|---|
| Northern group | Laguerre et al., 2002 | FR | Mean, SD | Mean<br>SD | 6.60<br>2.30 | 6.21<br>2.79 | -0.39<br>0.49 |
| | Derens et al., 2006 | FR | Mean, SD | Mean<br>SD | 5.90<br>2.90 | 6.21<br>2.79 | 0.31<br>-0.11 |
| | WIV-ISP, 2004 | BE | Mean, percentile | Mean<br>25%<br>75% | 7.00<br>5.00<br>9.00 | 6.21<br>4.33<br>8.08 | -0.79<br>-0.68<br>-0.92 |
| | Terpstra et al., 2005 | NL | Min, max, percentile | Min<br>Max<br>68% | 3.80<br>11.50<br>7.00 | NA<br>NA<br>7.51 | –<br>–<br>0.51 |
| | Marklinder et al., 2015 | SW | Mean, SD | Mean<br>SD | 5.90<br>3.10 | 6.21<br>2.79 | 0.31<br>-0.31 |

**Table 24.** Domestic refrigerator temperatures: data from studies measuring the temperature in locations other than the middle shelf.

| Reference | Position | Format of data provided | Values (°C) |
|---|---|---|---|
| Laguerre et al., 2002 (FR) | Top shelf | Mean, SD, min, max, median, percentile | 6.7; 2.5; -1.6; 12.0; 7.0; $25^{th}$: 4.9; $75^{th}$: 8.2 |
| | Middle shelf | Mean, SD, min, max, median, percentile | 6.4; 2.4; -0.2; 10.7; 6.9; $25^{th}$: 4.8; $75^{th}$: 7.8 |
| | Bottom shelf | Mean, SD, min, max, median, percentile | 6.5; 2.7; -1.0; 11.6; 6.7; $25^{th}$: 5.2; $75^{th}$: 8.5 |
| Koutsoumanis et al., 2010 (GR) | Upper | Mean, SD, min, max, median | 7.57; 2.95; -1.77; 14.47; 7.60 |
| | Middle | Mean, SD, min, max, median | 6.31; 2.66; -0,70; 13.03; 6.29 |
| | Lower | Mean, SD, min, max, median | 6.69; 3.29; -2.69; 18.08; 6.40 |
| | Door | Mean, SD, min, max, median | 8.40; 3.00; 1.18; 14.94; 8.32 |
| Garrido, Garcia-Jalon, & Vitas, 2010 (SP) | Top, middle and bottom shelves | The data are not reported. The authors stated that "no correlation were observed between temperatures and zones of the fridge" | |
| WRAP, 2010 (UK) | Top | Mean | 5.9 |
| | Middle | Mean | 4.7 |
| | Bottom | Mean | 9.6 |
| Marklinder et al., 2015 (SW) | Top shelf (front) | Mean and SD | 7.5; 4.1 |
| | Top shelf (back) | Mean and SD | 6.2; 3.7 |
| | Middle shelf (front) | Mean and SD | 5.9; 3.1 |
| | Middle shelf (back) | Mean and SD | 4.8; 3.2 |
| | Bottom shelf (front) | Mean and SD | 6.8; 3.6 |
| | Bottom shelf (back) | Mean and SD | 6.1; 3.8 |

**Table 25.** Storage time: ranking score of the fitted distributions (NA: not available).

*AIC: Akaike Information Criterion

| Reference | Country | Format of data provided | Product | Fitting @risk | Goodness of fitting criteria (RMSE) |
|---|---|---|---|---|---|
| Marklinder et al., 2004 | SW | Table of frequency | Minced meat | Gamma<br>Exponential | 0.140<br>0.145 |
| | | | Fresh fish | Gamma<br>Exponential | 0.127<br>0.131 |
| | | | RTE salad | Weibull<br>Gamma<br>Exponential | 0.100<br>0.102<br>0.119 |
| | | | Salmon | Weibull<br>Gamma<br>Exponential | 0.0759<br>0.0742<br>0.0783 |
| | | | Ham | Weibull<br>Lognormal<br>Exponential | 0.0614<br>0.0591<br>0.0629 |
| | | | Cheese | Weibull<br>Gamma<br>Lognormal<br>Exponential | 0.0724<br>0.0680<br>0.0587<br>0.0917 |
| Morelli and Derens, 2009 | FR | Percentile | Smoked salmon | NA | NA |
| Garrido, Garcia-Jalon, Vitas, Sanaa, 2010 | SP | Table of frequency | Smoked fish, sliced cooked ham | Beta General<br>Exponential | 0.022<br>0.114 |
| Kotsoumanis et al., 2010 | GR | Table of frequency | Pasteurised milk | Gamma<br>Weibull<br>Exponential | 0.008<br>0.009<br>0.122 |
| Mataragas et al., 2010 | GR | Table of frequency | Sliced cooked cured ham | Pearson5<br>Exponential | 6125.2438 (AIC)*<br>6466.1131 (AIC)* |
| Daelman, Jacxsens, Membré et al., 2013 | BE | Table of frequency | Cooked chilled food | Beta General<br>Gamma<br>Exponential | 0.035<br>0.032<br>0.046 |
| Derens et al., 2015 | FR | Mean and percentile | Sliced ham | NA | NA |

# CHAPTER 6. Survival of *Salmonella* Typhimurium in poultry-based meat preparations during grilling, frying and baking

**Redrafted from:**

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## ABSTRACT

The burden of food-borne diseases still represents a threat to public health and the domestic setting accounts for the great majority of strong-evidence food-borne *Salmonella* outbreaks in Europe. Next to cross-contamination, inadequate cooking procedure is considered as one of the most important factors contributing to food-borne illness.

The few studies which have assessed the effect of domestic cooking on the presence and numbers of pathogens in different types of meat have shown that consumer-style cooking methods can allow bacteria to survive and that the probability of eating home-cooked poultry meat that still contains surviving bacteria after heating is higher than previously assumed. Thus, the main purpose of this study was to reproduce and assess the effect of several types of cooking treatments (according to label instructions and not following label instructions) on the presence and numbers of *Salmonella* Typhimurium DT 104 artificially inoculated in five types of poultry-based meat preparations (burgers, sausages, ready-to-cook-kebabs, quail roulades and extruded roulades) that are likely to be contaminated by *Salmonella*. Three contamination levels (10 CFU/g; 100 CFU/g and 1,000 CFU/g) and three cooking techniques (grilling, frying and baking) were applied.

Cooking treatments performed according to label instructions eliminated *Salmonella* Typhimurium (absence per 25 g) for contamination levels of 10 and 100 CFU/g but not for contamination levels of 1,000 CFU/g. After improper cooking, 26 out of 78 samples were *Salmonella*-positive, and 23 out of these 26 samples were artificially contaminated with bacterial loads between 100 and 1,000 CFU/g. Nine out of 26 samples provided quantifiable results with a minimum level of 1.4 MPN/g in kebabs (initial inoculum level: 100 CFU/g) after grilling and a maximum level of 170 MPN/g recorded in sausages (initial inoculum level: 1,000 CFU/g) after grilling. Kebabs were the most common *Salmonella*-positive meat product after cooking, followed by sausages, burgers and extruded roulades. In relation to the type of cooking treatment applied, *Salmonella* Typhimurium was detected mostly after frying.

Thus, following label instructions mostly, but not always, produced safe cooked poultry-based meat preparations, while the application of inadequate cooking treatments was not able to assure complete elimination of *Salmonella* from the products even with a low contamination level (10 CFU/g). Consequently, there is a need to develop guidelines for producers and consumers and promote multidisciplinary educational campaigns in order to provide information on safe cooking practices.

## 6.1 Introduction

*Salmonella* spp. is a common and widely distributed zoonotic food-borne pathogen in the European Union (EU). Epidemiological data indicate that *Salmonella* is still the main causative agent of strong-evidence food-borne outbreaks in Europe and households were identified as the setting for the majority of these outbreaks (EFSA & ECDC, 2016).

Since 2000, *Salmonella* Typhimurium has been the most commonly detected serovar in Italy, contributing 40% of human isolates reported in 2009 (ISS, 2011). Epidemiological studies show that in Italy, poultry represented after pork, the second most common source of salmonellosis (Mughini-Gras et al., 2014).

At the European level, the laying hen reservoir was estimated to be the most important source, contributing 43.8% of cases, followed by pigs (26.9%). Turkeys and broilers were estimated to be less important sources of *Salmonella*, contributing 4.0% and 3.4% of cases, respectively (Pires, de Knegt, & Hald, 2011).

Poultry meat is usually consumed after cooking, still the only widely used and most effective method to eliminate vegetative pathogenic microorganisms causing food-borne disease from contaminated meat.

Among different food handling practices (e.g. cooling, separate raw and cooked food, cleaning and cooking), cooking is an important factor in controlling food-borne disease (Kennedy et al., 2011; Luber, 2009; Medeiros et al., 2001; Taché & Carpentier, 2014), but at least 30% of consumers undercook meat (Angelillo, Foresta, Scozzafava, & Pavia, 2001; Phang & Bruhn, 2011). Moreover, 50% to 88% of consumers think that subjective evaluation (visual inspection) is acceptable to determine the end of the cooking process (Lazou, Georgiadis, Pentieva, McKevitt, & Iossifidou, 2012; Redmond & Griffith, 2003). Observational studies reported up to 93% of consumers rely on visual indicators to determine the doneness of meat products (Redmond & Griffith, 2003; Sampers et al., 2012).

The recommended time-temperature combination of 70°C for 2 minutes (ACMSF, 2007) to produce > 6 logarithm reduction of the most heat resistant bacterium, *Listeria monocytogenes*, is effective in terms of minimising the risks posed by food-borne pathogens and is a critical control point for the food industry. Nevertheless, the required time-temperature combination to eliminate or decrease any reasonably expected level of *Salmonella* to a level that guarantees food safety depends upon several factors, including the heat resistance of the microorganism, the heat transfer rate that is affected by the structure of food and the distribution of the *Salmonella* in the food (superficial or deep). The effectiveness of heat treatment on *Salmonella* is also affected by product composition (fat

content, NaCl, pH and water activity) and geometry (volume and size) (Bermudez-Aguirre & Corradini, 2012; de Jong, Van Asselt, Zwietering, Nauta, & de Jonge, 2012; Juneja, 2007; Juneja & Eblen, 2000; NACMF, 2007; Silva & Gibbs, 2012). Moreover, bacterial pathogens occur exclusively on the surface of whole pieces of meat, but in comminuted products, like sausages and burgers, are also in the interior. Therefore, undercooking comminuted products is more likely to allow pathogen survival.

Few studies have been performed in order to assess the effect of domestic cooking on the presence and numbers of pathogens in different types of meat. Sampers, Habib, De Zutter, Dumoulin, & Uyttendaele (2010) tested the heat resistance of *Campylobacter* spp. in inoculated and naturally contaminated chicken burgers cooked in a frying pan. Even if *Campylobacter* was already below the detection limit (< 10 CFU/g) before the burger was thoroughly cooked (i.e. observing colour change), the frying time differed according to burger thickness, and the evaluation of adequate cooking could be a problem for consumers due to the slight visual difference between semi- and well-cooked burgers. Bergsma, Fischer, Van Asselt, Zwietering, & de Jong (2007) concluded that although fried chicken breast fillets looked cooked, *Campylobacter jejuni* might not be inactivated and the probability of eating home-cooked poultry that contains surviving bacteria is higher than previously assumed. Finally, retail sausages were contaminated with *Salmonella* Typhimurium DT 104, then fried, grilled or barbequed (Mattick, Bailey, Jørgensen, & Humphrey, 2002). *Salmonella* survived in high enough numbers to be quantified depending on the cooking method and the time applied (Mattick et al., 2002).

The purpose of this study was to reproduce and assess the effect of domestic-style cooking on the presence and numbers of *Salmonella* Typhimurium in different types of poultry-based meat preparations available on the market, that are likely to be contaminated by *Salmonella* due to their raw material being of animal origin. Poultry-based meat preparations were cooked either in accordance with label instructions, as provided by the manufacturer, or simulating plausible insufficient cooking conditions, not following label instructions, by applying shorter cooking times.

## 6.2 Materials and methods

### *6.2.1 Selection of poultry-based meat preparations and analysis performed on food at the delivery day*

Five poultry-based meat preparations were studied: burgers, sausages, ready-to-cook kebabs, quail roulades and extruded roulades. Ingredients and physical parameters of the final products are shown (Table 26). The main constituent was poultry meat; however, with the exception of burgers, the products contained also pork or bacon. Quail roulades were strips of quail and chicken breast meat mixed with milk powder and spices then wrapped with bacon. Extruded roulades were minced turkey and pork meat mixed with the other ingredients to produce an extrudable batter.

Products were obtained from local poultry meat industries in northern Italy, which delivered the commercial poultry meat patty formulation (for burgers) and commercial sausage mixture (for sausages), as well as the ready-to-cook products (i.e. kebabs, quail roulades and extruded roulades) to the laboratory, at refrigeration temperature (4°C), within maximum two days from the time of production. Cooking instructions as given on the labels and provided by the food manufacturers are shown (Table 27).

On the delivery day, for rapid detection of *Salmonella*, the food matrices (n=65) were analysed by real-time PCR using a commercial kit validated to ISO 16140 by the manufacturer (AES Chemunex, Biomerieux Company, Combourg, France). After incubation in Buffered Peptone Water (BPW) for 16 to 20 h at 37°C, 10 µl of pre-enriched sample was used to perform DNA extraction and amplification, using the manufacturer's recommended thermal profiles in an Opticon II PCR machine (MJ Research, Bio-Rad Laboratories, Hercules, CA, USA). Sensitivity of 98.1% (CI, 89.9–100.0) and specificity of 95.5% (CI, 93.8–96.8) were calculated for this PCR assay (Lettini et al., 2012). Moreover, detection and quantification of *Salmonella* spp. were also performed according to, respectively, the ISO 6579:2002/Amd1:2007 (ISO, 2007) classical detection method per 25 g of meat, and the ISO/TS 6579-2:2012 (ISO, 2012) miniaturized Most Probable Number (mini-MPN) method (starting from the 1/10 primary suspension of 25 g of meat, prepared for detection). Estimated numbers from the mini-MPN method were expressed as MPN/g; lower limit of quantification = 1.3 MPN/g; upper limit of quantification = 710 MPN/g. Finally, detected *Salmonella* were serotyped (White–Kauffmann-Le Minor scheme) by slide agglutination with O and H antigen specific sera (Staten Serum Institute, Copenhagen, Denmark).

**Table 26**. Ingredients and physical parameters of the poultry-based meat preparations prepared and studied.

| Product | Ingredients as provided by the manufacturer | Physical parameters of the finished products |
|---|---|---|
| Meat patty formulation for burgers | Minced turkey meat (82%); water, wheat flour, ground rice, salt, sodium ascorbate, flavours (18%) | Weight: 104 ± 3 g<br>Diameter: 9.5 ± 0.5 cm<br>Thickness: 1.3 ± 0.2 cm |
| Poultry sausage mixture for sausages | Minced chicken meat (50%); bacon (25%); water, salt, dextrose, saccharose, lactose, vegetable fibers, flavours, antioxidants E300 and E301, acidity corrector E331, spices, food colour E120 (25%) | Weight: 75 g<br>Diameter: 2.3 cm |
| Kebabs | Chicken meat (54%); sausages 34.5% (pork 35%, turkey meat 28%, water, chicken meat 8%, salt, peas, carrots, dextrose, lactose, natural flavours, spices, antioxidants: ascorbic acid, sodium ascorbate, sodium acetate); chuncks of bacon (7%); peppers (4.5%) | Weight: 160 g<br>Diameter (chicken meat): 3.8 ± 0.6 cm<br>Diameter (sausages): 2.5 ± 0.3 cm |
| Quail roulades | Strips of quail breast (45%); strips of chicken breast (34%); bacon (18%); mixture of herbs and spices (1.5%); milk powder (1%); rosemary | Weight: 400 g<br>Diameter: 6 cm |
| Extruded roulades | Minced turkey meat (65%); minced pork (25%); salt, skimmed powder milk, milk proteins, egg white, natural flavours, dehydrated egg, yeast extract, cheese, vegetables, herbs and spices, vegetable oil (10%) | Weight: 700 g<br>Diameter: 7 cm |

**Table 27**. Cooking treatments performed and core post-cook temperatures measured on the poultry-based meat preparations.

| Product | Label instructions[a] | T (°C) | Tμ[b] (°C) | Improper cooking[a] | T (°C) | Tμ[b] (°C) |
|---|---|---|---|---|---|---|
| Burgers | Grill: 10 min | 72.8; 74.3; 73.6 | 73.5 | Grill: 5 min | 65.8; 67.6; 69.2 | 67.5 |
| | Pan: 10 min | 82.5; 83.8; 86.2 | 84.1 | Pan: 5 min | 71.3; 73.8; 76.5 | 73.8 |
| | Oven: 12 min at 180°C | 69.4; 70.2; 71.5 | 70.3 | Oven: 8 min at 180°C | 65.7; 66.8; 68.4 | 66.9 |
| Sausages | Grill: 10 min | 71.3; 72.7; 77.3 | 73.7 | Grill: 5 min | 68.2; 70.5; 60.6 | 66.4 |
| | Pan: 8 min | 80.5; 81.7; 82.3 | 81.5 | Pan: 4 min | 60.2; 51.1; 65.2 | 58.8 |
| Kebabs | Grill: 25 min | 75.2; 74.6; 74.4 | 74.7 | Grill: 15 min | 76.3; 70.2; 76.3 | 74.2 |
| | Pan: 20 min | 68.5; 64.3; 65.6 | 66.1 | Pan: 12 min | 67.5; 67.3; 64.2 | 66.3 |
| | Oven: 25 min at 180°C | 80.2; 81.5; 79.8 | 80.5 | Oven: 20 min at 180°C | 68.7; 71.2; 70.3 | 70.0 |
| Quail roulades | Oven: 40 min at 180°C | 65.3; 61.8; 60.4 | 62.5 | Oven: 35 min at 180°C | 51.3; 48.5; 51.9 | 50.5 |
| Extruded roulades | Oven: 60 min at 180°C | 93.2; 86.7; 91.3 | 90.4 | Oven: 45 min at 180°C | 71.8; 71.4; 68.5 | 70.5 |
| | Pan: 15 min | 76.4; 74.7; 72.5 | 74.5 | Pan: 10 min | 66.1; 65.2; 62.4 | 64.5 |

[a] Grill and pan cooking was performed on a "gas stove using a medium heat flame" whereas oven cooking was a "electric oven on fan-bake setting".
[b] Tμ: mean temperature

Cooking treatments were conducted on naturally contaminated products (positive PCR result) or artificially contaminated products (negative PCR result).

Following the first tests on naturally contaminated products, which in most cases contained numbers of *Salmonella* spp. that were too low (< 1-10 MPN/g) to quantify the inactivation of *Salmonella*, it was decided to proceed with artificially contaminated products. The food batches, which were negative by real-time PCR were then used for the artificial contamination.

### *6.2.2 Artificial contamination procedure*

A broth culture of *Salmonella* Typhimurium DT104 (2324/5 2010Salm strain, isolated from poultry minced meat – culture collection, the Italian *Salmonella* Reference Laboratory), was prepared in order to obtain a suspension with an optical density at 600 nm of 1. Next, this suspension was serially diluted and appropriate dilutions were used to obtain three residual contamination levels in the products: ca. 10 CFU/g, ca. 100 CFU/g and ca. 1,000 CFU/g.

These levels of contamination were selected for two purposes: to enable quantitative monitoring of *Salmonella* inactivation and to be relevant inoculum levels. According to Straver et al. (2007) and to tests performed on naturally contaminated products in the present study, 10 CFU/g is considered a plausible level of contamination of poultry-based meat preparations available on the market, while 100 and 1,000 CFU/g represented the intermediate and worst case scenarios, respectively, in which *Salmonella*, after accidental contamination (at low levels of < 1-10/g), had already multiplied due to prior storage under temperature abuse (Roccato et al., 2012).

For burgers for each inoculum level, 10 ml of appropriate dilution was added to 1,500 g of commercial poultry meat patty formulation, mixed thoroughly and portions pressed into a Petri dish in order to obtain the final burger dimensions (Table 26). For sausages for each inoculum level, 15 ml of appropriate dilution was added to 1,600 g of commercial poultry sausage mixture, mixed thoroughly and stuffed into a bovine natural collagen gut casing using a commercial sausage filler, provided by a local sausage producer (see Table 26 for sausage dimensions). After each use, the sausage filler was sterilized (121°C, 30 minutes). Kebabs, quail roulades and extruded roulades were contaminated with *Salmonella* Typhimurium using a spray, which from previous tests had proven to be the most efficient and effective contamination method; 72 kebabs, 42 quail roulades and 54 extruded roulades were used.

After artificial contamination, for each inoculum level, three samples were analysed to verify the presence/numbers of *Salmonella* Typhimurium. Contaminated food was stored overnight at 4°C prior to cooking.

### *6.2.3 Cooking treatments: grilling, frying and baking*

The cooking treatments were: grilling (burgers, sausages and kebabs), frying (burgers, sausages, kebabs and extruded roulades) and fan-baking in an electric oven (kebabs, quail roulades and extruded roulades). The cooking treatments were according to label instructions provided by the food manufacturers, while less stringent but plausible cooking times, termed "improper cooking", were also used. For each level of inoculum and type of cooking treatment, two (burgers and sausages) or three (kebabs, quail roulades and extruded roulades) meat samples were cooked simultaneously.

Meat products were grilled in a cast-iron pan (diameter: 28 cm), or fried in a stainless steel pan containing 25 ml of olive oil (diameter: 30 cm), in both cases using a medium heat gas-flame, with regular turning during cooking. Meat products were baked in an oven pan in an electric oven on fan-bake setting. All kitchen cooking equipment used was domestic equipment. Time-temperature combinations for cooking treatments are shown (Table 27).

After cooking, the products were transferred to kitchen plates and core temperatures measured using a thermocouple (P200 Profi-Digital thermometer, TFA, Wertheim, Germany) calibrated between 0°C (melting ice) and 100°C (boiling water) prior to use. For kebabs, the thermocouple was inserted into the centre of one poultry meat piece, avoiding the wooden skewer. The thermocouple was kept into the centre of the food until the temperature indicated on the screen was stable.

Products were photographed before and after cooking as evidence of plausible cooking, since consumers often use colour changes of meat as visual indicators of meats' readiness for consumption.

The presence and numbers of *Salmonella* Typhimurium were determined in cooked meats as previously described in paragraph 6.2.1. Detected *Salmonella* were serotyped according to White–Kauffmann-Le Minor scheme.

## 6.3 Results

### *6.3.1 Physical parameters of products and cooking treatments applied*

Twenty-two different types of consumer-style cooking procedures were applied to the different types of products. Table 26 reports the ingredients and the physical parameters of the studied products while Table 27 shows the different types of cooking treatments and the temperatures obtained after cooking for each product type and cooking treatment. The dimensions of the products differed widely (e.g. weight ranged from 75 g for the sausages to 700 g for the extruded roulades) (Table 26).

Concerning the cooking treatments performed according to label instructions, the maximum mean temperature was recorded during fan-baking of the extruded roulades (90°C) while for improper cooking, the maximum temperature was registered during frying of burgers and grilling of kebabs (74°C) (Table 27).

Photographs of the meat samples were taken at the end of each cooking treatment (Figures 17 and 18). Clearly, colour differences were not a reliable tool to distinguish between the cooking treatment performed according to label instruction and the improper cooking. Colour differences were clearer when meats were cut and the colour of the interior became visible (especially in the case of burgers and sausages).

### *6.3.2 Detection and quantification of Salmonella spp.*

#### *6.3.2.1 Naturally-occurring Salmonella spp.*

Meat samples were analysed upon arrival in the laboratory, and thus, before artificial contamination with *Salmonella* Typhimurium: 40 of 65 samples contained *Salmonella* spp. Among the 40 positive samples, 24 provided quantifiable results: 18 samples were between the lower limit of quantification (1.3 MPN/g) and 10 MPN/g and 6 samples were above 10 MPN/g with a maximum number of 34 MPN/g. Isolated *Salmonella* spp. were serotyped: burgers contained *Salmonella* Newport, sausages contained *Salmonella* Montevideo and kebabs contained the monophasic variant of *Salmonella* Typhimurium. The identified serotypes in quail roulades, in order of frequency, were *Salmonella* Indiana, *Salmonella* Typhimurium and *Salmonella* Blockley while in extruded roulades, the identified serotypes were *Salmonella* Schwarzengrund, *Salmonella* Hadar, *Salmonella* Newport and the monophasic variant of *Salmonella* Typhimurium.

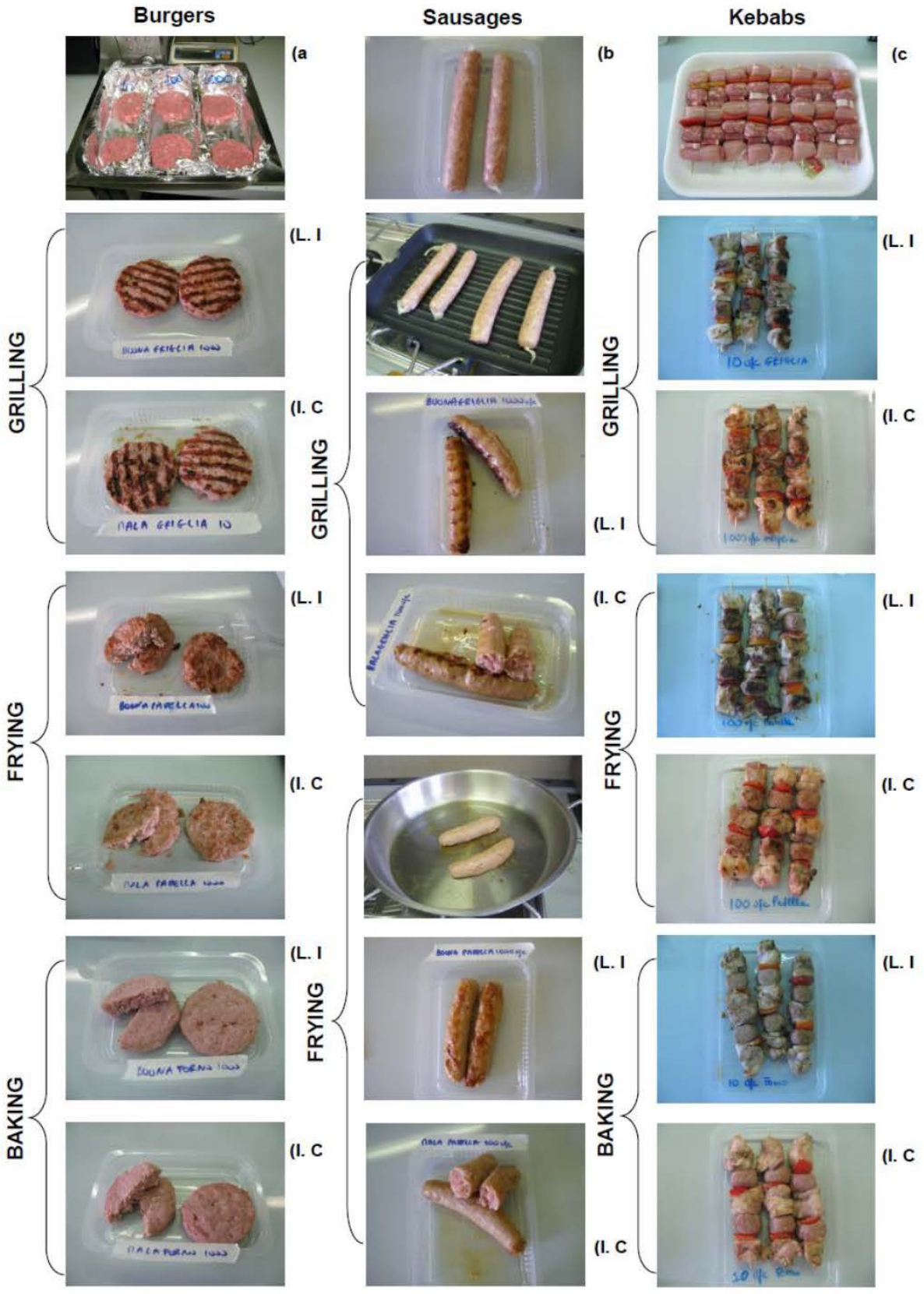


**Figure 17.** External appearance of burgers (a), sausages (b), and kebabs (c), before and after cooking treatments (L.I: Label Instructions; I.C: Improper Cooking).

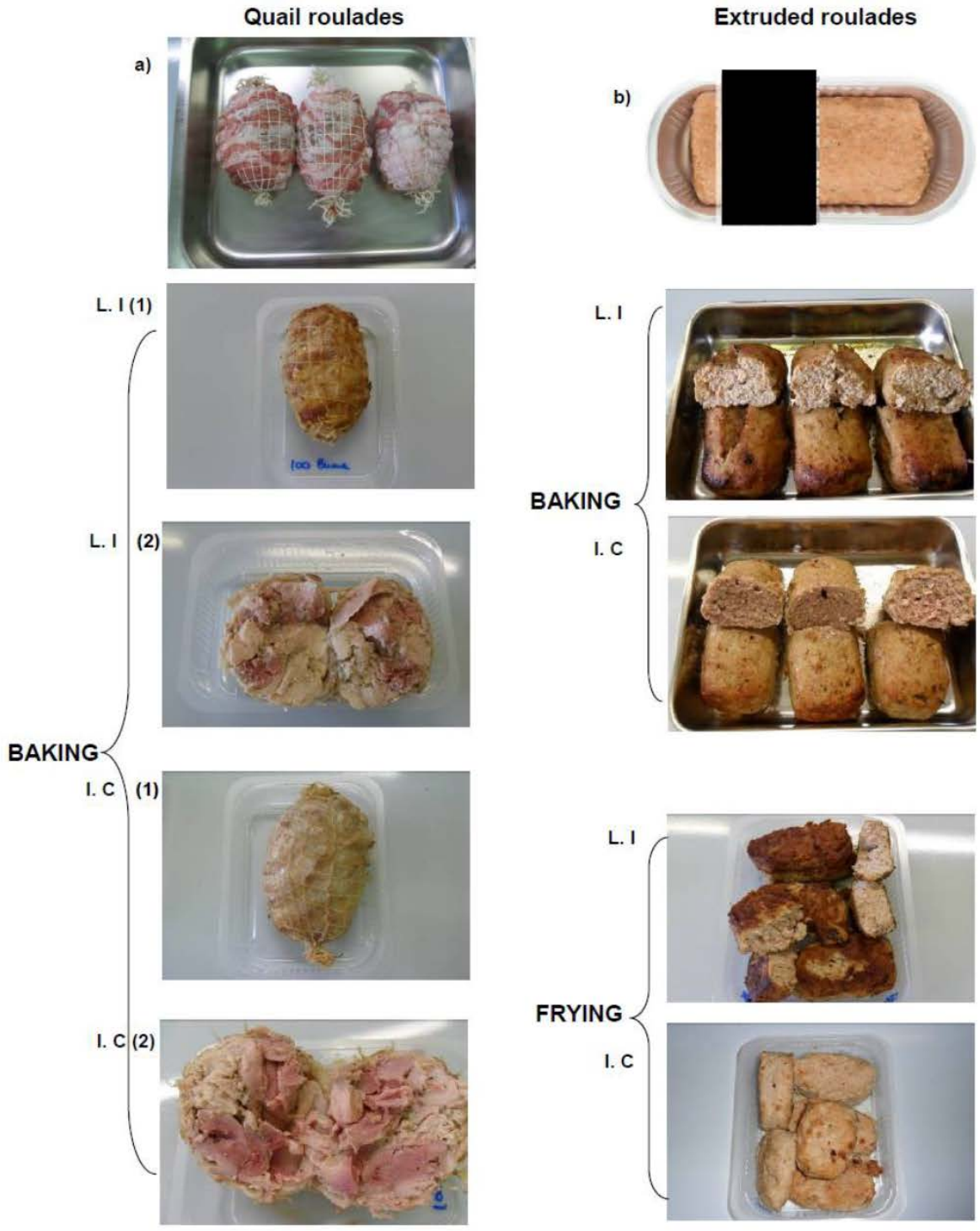


**Figure 18.** External appearance of quail roulades (a) and extruded roulades (b), before and after cooking treatments (L.I: Label Instructions; I.C: Improper Cooking).

### *6.3.2.2 Survival of inoculated Salmonella Typhimurium*

The results of detection and quantification of *Salmonella* Typhimurium from products after artificial contamination and after cooking treatments for burgers, sausages and kebabs are reported in Table 28, while these data obtained for quail roulades and extruded roulades are shown in Table 29.

After cooking treatments performed according to label instructions, *Salmonella* Typhimurium was not recovered from 76 of 78 samples (of 25 g). The two residual *Salmonella*-positive samples, one kebab after frying and one quail roulade after baking, had initial inoculum levels of approximately 1,000 CFU/g.

After improper cooking, *Salmonella* Typhimurium was detected in 26 of 78 samples. Three of these 26 samples had initial inoculum levels of 10 CFU/g. All three samples were sausages: two had been fried and one grilled. The number of *Salmonella* Typhimurium was always below the lower limit of quantification (< 1.3 MPN/g) in these samples. Eight of 26 improperly cooked samples with an initial inoculum level of 100 CFU/g contained residual *Salmonella* Typhimurium after cooking treatments; they were sausages and kebabs, with a maximum level of 19 MPN/g in sausages after frying and 3.2 MPN/g in kebabs after baking. Finally, 15 of 26 improperly cooked samples with an initial 1,000 CFU/g inoculum level, still contained viable *Salmonella* Typhimurium after cooking treatments; 5 of 15 samples provided quantifiable results (Table 28). In four samples, numbers above 10 MPN/g were recorded (one burger after grilling; two sausages, one after grilling and another after frying; one kebab after baking) and a level of 100 MPN/g was recorded in one sausage after grilling.

In sausages, 0.8 to 1.4 logarithm reductions of *Salmonella* were observed after grilling and a 1.3 logarithm reduction after frying, with recorded core mean temperatures of 66°C and 59°C, respectively. For grilled burgers, a 1.9 logarithm reduction was measured (mean core temperature: 67°C) while a 1.6 logarithm reduction was quantified in kebabs after baking (mean core temperature: 70°C). Finally, a 3.0 logarithm reduction occurred on quail roulades and extruded roulades after each of the tested cooking treatments.

Among the residual *Salmonella*-positive products after improper cooking, 23 of 26 had been artificially contaminated with the higher levels of *Salmonella*, between 100 and 1,000 CFU/g. Among the meat types, kebabs were most frequently *Salmonella*-positive (12 of 18 kebabs contained *Salmonella* post-cooking): 5 post-cook kebabs were baked, while 4 and 3 kebabs were grilled or fried, respectively. The other *Salmonella*-positive meats post-cooking were sausages (4 of 6 were fried), 3 burgers after grilling, and 2 extruded roulades after frying.

In relation to the type of cooking treatment, *Salmonella* Typhimurium was detected mostly after frying: in fact 12 of 28 *Salmonella*-positive products (including both cooking according to label instructions and improper cooking) were cooked by frying, for which the recorded mean core temperatures were between 66°C and 84°C after cooking according to label instructions and between 59°C and 74°C after improper cooking. Concerning the other two cooking types, 10 *Salmonella*-positive meats were grilled, while 6 were fan-baked. The core mean temperatures obtained after cooking according to label instructions were between 73°C and 75°C for grilling and between 70°C and 90°C for fan-baking, while in case of the improper cooking, temperatures post-cooking were between 66°C and 74°C and between 50°C and 70°C, for grilling and fan-baking, respectively.

After improper cooking, the temperature in 15 of 26 *Salmonella*-positive samples did not reach 70°C while in nine of these samples, mean temperatures ≥ 70°C were recorded. In particular, only 4 of 11 tested improper cooking treatments produced temperatures of ≥ 70°C, namely during the frying of burgers, the grilling and baking of kebabs and the baking of extruded roulades (Table 27). Even cooking treatments performed according to label instructions did not always assure final temperatures of 70°C: in fact, this temperature was not reached in 2 of 11 proper cooking treatments (frying of kebabs and baking of quail roulades; Table 27). Thus, the survival of *Salmonella* was mostly associated with cooking temperatures <70°C. However, in other cases, such as the cooking of kebabs, in which 70°C was reached in two of three cooking treatments, *Salmonella* was still detected after cooking, highlighting that the "safe" temperature of 70°C is not always able to eliminate *Salmonella*.

**Table 28.** Survival of *Salmonella* Typhimurium after cooking treatment, either according to product label instructions or after improper cooking, of (artificially) contaminated poultry meat burgers, sausages and kebabs.

| | **Burgers** | | | | **Sausages** | | | | **Kebabs** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Level 10 CFU/g** | Detection[a] | | MPN/g[b] | | Detection[a] | | MPN/g[b] | | Detection[a] | | MPN/g[b] | |
| After contamination | 3/3 | | 2/3 (6.1; 11) | | 3/3 | | 2/3 (1.6; 13) | | 3/3 | | 3/3 (3.8; 5.9; 6.1) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After grilling | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 1/2 | 0/2 | 0/3 | 0/3 | 0/3 | 0/3 |
| After frying | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 2/2 | 0/2 | 0/3 | 0/3 | 0/3 | 0/3 |
| After baking | 0/2 | 0/2 | 0/2 | 0/2 | - | - | - | - | 0/3 | 0/3 | 0/3 | 0/3 |
| **Level 100 CFU/g** | Detection | | MPN/g | | Detection | | MPN/g | | Detection | | MPN/g | |
| After contamination | 3/3 | | 3/3 (110; 170; 380) | | 3/3 | | 3/3 (41; 96; 140) | | 3/3 | | 3/3 (13; 110; 140) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After grilling | 0/2 | 0/2 | 1/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/3 | 0/3 | 2/3 | 1/3 (1.4) |
| After frying | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 2/2 | 2/2 (1.6; 19) | 0/3 | 0/3 | 1/3 | 0/3 |
| After baking | 0/2 | 0/2 | 0/2 | 0/2 | - | - | - | - | 0/3 | 0/3 | 2/3 | 1/3 (3.2) |
| **Level 1,000 CFU/g** | Detection | | MPN/g | | Detection | | MPN/g | | Detection | | MPN/g | |
| After contamination | 3/3 | | 3/3 (>710) | | 3/3 | | 3/3 (240; 710; 710) | | 3/3 | | 3/3 (59; >710; >710) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After grilling | 0/2 | 0/2 | 2/2 | 1/2 (13) | 0/2 | 0/2 | 2/2 | 2/2 (41; 170) | 0/3 | 0/3 | 2/3 | 0/3 |
| After frying | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 2/2 | 1/2 (45) | 1/3 | 0/3 | 2/3 | 0/3 |
| After baking | 0/2 | 0/2 | 0/2 | 0/2 | - | - | - | - | 0/3 | 0/3 | 3/3 | 1/3 (26) |

[a] Detection per 25 g according to ISO 6579:2002/Amd1:2007.

[b] MPN/g: estimated number of surviving *Salmonella* according to ISO/TS 6579-2:2012.

**Table 29.** Survival of *Salmonella* Typhimurium after cooking treatment, either according to product label instructions or after improper cooking, of (artificially) contaminated quail roulades and extruded roulades.

| | **Quail roulades** | | | | **Extruded roulades**[c] | | | |
|---|---|---|---|---|---|---|---|---|
| **Level 10 CFU/g** | Detection[a] | | MPN/g[b] | | Detection[a] | | MPN/g[b] | |
| After contamination | 3/3 | | 3/3 (3.8; 8.5; 13) | | 2/2 | | 2/2 (380) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After baking | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| **Level 100 CFU/g** | Detection | | MPN/g | | Detection | | MPN/g | |
| After contamination | 2/2 | | 2/2 (66; 110) | | 2/2 | | 2/2 (>710) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After baking | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| **Level 1,000 CFU/g** | Detection | | MPN/g | | Detection | | MPN/g | |
| After contamination | 3/3 | | 3/3 (710) | | 2/2 | | 2/2 (>710) | |
| Cooking treatment | Label instructions | | Improper cooking | | Label instructions | | Improper cooking | |
| | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g |
| After baking | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |

[a] Detection per 25 g according to ISO 6579:2002/Amd1:2007.

[b] MPN/g: estimated number of surviving *Salmonella* according to ISO/TS 6579-2:2012.

[c] *Salmonella* Typhimurium was detected in 2 of 3 1,000 CFU/g samples after frying.

## 6.4 Discussion

Even though *Salmonella* is a well-known and widely studied bacterium, few studies have investigated its behaviour during food preparation under domestic conditions. Redmond & Griffith (2003) found that 46 to 50% of consumers undercooked meat loaves and hamburgers, and 83% failed to cook a roast chicken for the recommended time. Moreover, an observational study on consumer practices in the kitchen concluded that up to a third of the participants undercooked their chicken (van Asselt et al., 2009). Furthermore, whether a cooking procedure will eliminate *Salmonella* from the heat treated meat will depend from several factors. Doyle & Mazzotta (2000) highlighted that the thermal inactivation rate for *Salmonella* varies based on the strain, the food product, and environmental factors. Total solid content, pH, water activity, fat content, some food additives and redox potential can affect *Salmonella* thermal inactivation. In addition, the structure of food (e.g., minced meat *versus* whole piece of meat) can have an impact on the heat transfer rate. In fact, the paper of Mogollón et al. (2009) studying the effect of muscle structure on *Salmonella* survival in beef showed higher thermal resistance in the whole-muscle compared to the ground meat (no difference among the degree of grinding). In fact, the different structure of food can affect several factors: the physical arrangement of the food components (e.g. fat tissue that leads to thermal protection), the status of water (e.g. lower water activity is associated to higher thermal resistance), and the attachment of bacterial cells to solid or liquid media (bacteria suspended in the liquid component of food makes organisms more susceptible to thermal inactivation).

In addition, the type of method to accomplish heat transfer i.e. the type of cooking method can influence the elimination of *Salmonella* as well. Nowadays, various cooking methods are used in meat preparation: oven cooking, grilling, frying and microwaving, but the effectiveness of the heat treatments achieved in domestic kitchens is unclear. Rhee, Lee, Hillers, McCurdy, & Kang (2003), evaluating the effect of consumer-style cooking methods on *E. coli* O157 levels in ground beef patties, showed that grill-cooking methods (double-sided grill *versus* single-sided grill) produced significantly different pathogen reductions, and concluded that consumers should be advised to cook patties in a grill that cooks on both sides simultaneously, or to turn patties frequently (every 30 s) when cooking on one side only.

Many consumers (up to 80%) know that undercooking represents a risk factor associated with food-borne diseases and that thoroughly cooking meat decreases the risk of food poisoning. Moreover, most consumers know how to check the doneness of cooked

meat; however, the majority do not employ a thermometer to do so (Bergsma et al., 2007; Fischer et al., 2007; Kennedy et al., 2011). Consumer decisions on how to cook meat are based on several factors, including taste, palatability and perceived food safety risk. However, taste was clearly the most important factor affecting how meat is cooked; in fact, consumers are concerned that the temperatures reached during (over) cooking can affect the juiciness and flavour of meat (Ralston, Brent, Starke, Riggins, & Lin, 2001).

Consequently, the main goal of this study was to analyse changes in the presence and level of *Salmonella* Typhimurium in artificially contaminated poultry-based meat preparations as a result of the application of different cooking methods.

When products were cooked following label instructions, *Salmonella* Typhimurium (absence per 25 g) was eliminated from meats with lower initial levels (10 and 100 CFU/g), but not when contamination levels were higher (1,000 CFU/g). In fact, *Salmonella* was still detected in two 25 g samples: one kebab and one quail roulade; therefore, the results of the present study show that the recommended cooking times for these types of products are only marginally safe.

Improper cooking produced inadequate heat treatments, which were not able to eliminate *Salmonella* Typhimurium from the products, and even with low initial contamination levels of 10 CFU/g, *Salmonella* was occasionally recovered after cooking treatments. When initial contamination levels were higher (100 or 1,000 CFU/g), improper cooking produced less than 2 logarithm reductions, and 15 of 26 improperly cooked meat samples, initially contaminated with 1,000 CFU *Salmonella*/g, still contained viable *Salmonella* Typhimurium after cooking treatments. These data show that thoroughly cooking these various types of products is important from a food safety point of view. Furthermore, using visual inspection of the internal colour as a tool to assess the doneness of poultry-based meat preparations is not a reliable method to verify microbiological safety of the meat, as the inside can look adequately cooked while *Salmonella* still survive.

Consequently, this study highlighted the role that the consumer has in shaping the risk associated with microbial hazards, in this specific case-study related to *Salmonella* in poultry-based meat preparations, either properly cooking according to label instructions or cooking inadequately.

On the other side, when defining the thermal treatments in order to inactivate pathogens in food, food industry makes use of the available D-values that is the time at a certain temperature needed in order to obtain 1 log reduction of the pathogen of concern. Literature data show that D-values are most often derived from experiments in liquid

matrices (broth, milk) and to a lesser extent in solid matrices and if the case often in quite artificial circumstances (usually small pieces and thin layers of meat) (Van Asselt & Zwietering, 2006). Generally, cells on agar surface exhibited higher heat resistance than those in broth (Wang, Devlieghere, Geeraerd, & Uyttendaele, 2017). In other studies, simulated home-pan frying of meat was performed and throughout the heat treatment, the temperature at the core or the surface was monitored using temperature probes. Corresponding F70-values were 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 and indeed residual pathogen cells were occasionally isolated (Lahou et al., 2015). However, data collection of temperature profiles are quite cumbersome and quite hard to collect in an appropriate manner, especially when having small pieces of meat (e.g. kebabs) and when doing oven baking. It was not within the scope of the study at the time to collect this type of data. The focus of the present study was to 'validate' the producer's cooking instructions on the label concerning food safety and inactivation of pathogens achieved using reasonable numbers of pathogens expected to be present (even if artificially inoculated) on the meat under consideration.

Moreover, (Juneja et al., 2010) did also do some similar experiments and conclude that D-values and inactivation models developed from such controlled experiments would not provide a reasonable prediction of results likely to be obtained when cooking on commercial grills.

Besides, our data show that the temperature of 70°C, considered appropriate in order to inactivate most pathogenic bacteria, was only sometimes reached at the end of the applied cooking treatments. This temperature was recorded after 4 of 11 improper cooking treatments and 9 of 11 cooking treatments following label instructions. Unexpectedly, 9 of 12 Salmonella-positive kebabs recorded a temperature equal or above 70°C after improper cooking, which may plausibly be due to uneven temperature distribution and/or the protective effect of some ingredients such as fat.

Thus, considering that even when label instructions were adhered to, the temperature reached was not always 70°C, and that 70°C cannot always be considered safe, since *Salmonella* survived cooking to this temperature, the cooking instructions given on labels were not entirely satisfactory. Clearly, safe cooking instructions need to be provided to consumers with an appropriate safety margin to account for the wide range of conditions in which meat products will actually be prepared and heat-treated.

In the United States, the National Advisory Committee on Microbiological Criteria for Foods (NACMF, 2007) recommended that producers should provide clear label indications of suitable time-temperature combinations for cooking and must take into account how consumers might interpret the cooking instructions and how they may actually prepare and cook the product. On the other hand, consumers should follow temperatures recommended on labels and should use thermometers to measure the endpoint temperature reached after cooking. However, only 34% of American consumers use a thermometer to assess the doneness of food (McCurdy, Hillers, & Cann, 2005) and very few European households do so (Bearth et al., 2014).

Nevertheless, the presence of relevant and clear messages on food packaging could be a successful way to improve food-handling practices by consumers in their own homes. However, to be effective, the information on food labels should be reliable, accurate, accessible to, and utilized by consumers. Therefore, efforts should be made both that producers improve label information and that consumers are educated. Educational campaigns should be aimed at raising consumer awareness of their role and responsibility in the domestic kitchens. In this framework, food safety education of children, based on developing awareness of the reasons for good hygienic practices via practical examples, has proved to be effective in improving long-term protective hygiene-related behaviours (Losasso et al., 2014).

Educational efforts should aim to develop consumer consciousness that raw meat can regularly contain microbiological hazards, and that a proper cooking process is the best tool to eliminate these hazards. In this framework, reading and compliance with label instructions coupled with measuring the internal temperature of meat are useful methods to assess readiness for consumption. However, considering that many consumers report that thermometers are inconvenient and difficult to use (Byrd-Bredbenner et al., 2013), more emphasis should be placed on using better visual indicators of adequate cooking, including cooking until juices run clear. Concurrently, scientific information should be provided to the food industry, allowing food business operators to provide clear and science-based cooking instructions on labels, thus giving consumers a proper risk management tool.

In conclusion, this study highlighted the key role played by consumers in the context of food safety: the studied domestic cooking practice reveals that the probability of eating poultry-based meat preparations that still contain *Salmonella* after home cooking is likely an everyday reality.

Consequently, improvements to food safety are only feasible with a multidisciplinary approach that sees the involvement and collaboration of natural and social sciences (Fischer, de Jong, de Jonge, Frewer, & Nauta, 2005), and the participation of food industries and consumer associations in order to develop solutions to food safety problems in the real world.

In order to improve the scientific knowledge on the effect of consumer's practices in the domestic environment on the behaviour of microorganisms in food, the effect of domestic storage practices will be investigated in the following chapter.

# CHAPTER 7. Effect of domestic storage and thawing practices on *Salmonella* in poultry-based meat preparations

**Redrafted from:**

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## ABSTRACT

Among consumer food handling practices, time-temperature abuse has been reported as one of the most common contributory factors in salmonellosis outbreaks were the evidence is strong.

The present study performed tests on burgers, sausages and kebabs in plausible conditions of storage. In particular, the effect of refrigerator temperatures (4°C versus 8 or 12°C, which were the temperatures recorded in 33 and 3%, respectively, of domestic refrigerators in Italy), with or without prior temperature abuse (25°C for 2 h, simulating transport of meats from shop to home) was investigated. In addition, the impact of the thawing method (overnight in refrigerator at 8°C versus on the kitchen countertop at 23°C) on the presence and numbers of *Salmonella* bacteria was assessed. Storage tests were carried out on naturally or artificially (*Salmonella enterica* serovar Typhimurium at ca. 10 CFU/g) contaminated products, while freezing-thawing tests were conducted only on artificially contaminated products (*Salmonella* Typhimurium at ca. 10, 100 and 1,000 CFU/g).

The results from the artificially contaminated products showed a significant ($p < 0.05$) growth of *Salmonella* Typhimurium at 12°C (i.e. from ca. 8 most probable number [MPN]/g to > 710 MPN/g) in kebabs after 7 and 10 days but more moderate growth in sausages (i.e. from ca. 14 MPN/g to a maximum of 96 MPN/g after 9 days storage). During storage at 8°C an increase of *Salmonella* Typhimurium from 1 to 2 log MPN/g was recorded in 1 of 3 sausages analysed at day 7 and in 4 kebabs.

Storage of naturally contaminated burgers or sausages (contamination at or below 1 MPN/g) at 4, 8, or 12°C and short time of temperature abuse (2 h at 25°C) did not facilitate an increase in the presence and numbers of *Salmonella* bacteria.

Thawing overnight in the refrigerator led to either a moderate reduction or no change of *Salmonella* Typhimurium numbers in burgers, sausages and kebabs while after thawing overnight on the kitchen countertop (23°C) a significant ($p < 0.05$) increase of *Salmonella* Typhimurium was recorded in kebabs.

Overall, this study showed that domestic storage and thawing practices can affect food safety, and that time-temperature abuse can cause a substantial increase of *Salmonella* numbers in some types of poultry-based meat preparations, highlighting that efforts for the dissemination of consumer guidelines on the correct storage and handling of meats need to be continued.

## 7.1 Introduction

Reduction of the incidence of food-borne diseases and governing food safety remain important priorities both for food industries and for competent authorities because of the consequences of such diseases on public health, the loss of consumer trust in the food supply chain, and the economic impact on both food producers and society.

As already stated in the introduction of chapter 6, *Salmonella* bacteria remain the most frequently reported cause of food-borne outbreaks in the European Union and more than half of *Salmonella* outbreaks with strong-evidence were traced to foods consumed at home (EFSA & ECDC, 2016).

Nevertheless, surveys on risk perception in European consumers demonstrated that they are very confident about being able to personally take action to avoid bacterial contamination; rather, they expressed higher concerns about chemical risks than for microbial contaminants (EFSA, 2010; Kher et al., 2013). Several factors contribute to outbreaks of food-borne illness in the home. Most food eaten is prepared at home, thereby contributing to the likelihood of food handling mistakes to occur in this setting. In addition, most consumers consider the domestic environment as a safe place (Byrd-Bredbenner et al., 2013; Taché & Carpentier, 2014), thus underestimating the role of personal handling of products in contamination in the domestic environment. Moreover, home kitchens are multipurpose areas and are much more than just food preparation and storage places (Scott, 2003). Studies have shown that surfaces in the domestic environment are contaminated with pathogenic and non-pathogenic microorganisms and that, in some cases, kitchen locations are more contaminated with faecal coliforms than bathrooms (Azevedo, Albano, Silva, & Teixeira, 2014; Catellani, Scapin, Alberghini, Radu, & Giaccone, 2014; Haysom & Sharp, 2005; Redmond & Griffith, 2009). Several consumer-based research studies have pointed out that after purchase of food, improper transport, handling (cross-contamination), storage and/or cooking frequently happens, thus allowing survival, spread and multiplication of microorganisms (Anderson et al., 2004; Beumer & Kusumaningrum, 2003; Kennedy et al., 2011).

Among consumer food handling practices, time-temperature abuse has been reported as one of the most common contributory factors in salmonellosis outbreaks with strong-evidence and in food-borne illness in general (EFSA & ECDC, 2016; WHO, 2006). Several studies have been conducted on the temperature performance of domestic refrigerators, demonstrating that the majority of the refrigerators throughout the world are running at higher than recommended temperatures (0 to 5°C) and that the temperature can vary

significantly according to the internal position measured (James et al., 2008). Moreover, Nauta, Litman, Barker, & Carlin (2003) hypothesized a geographical distribution of refrigerator temperatures with the northern countries usually recording lower mean refrigerator temperatures than the southern countries. Studies conducted in the United Kingdom, Ireland, Greece and New Zealand (Gilbert et al., 2007; James & Evans, 1992a, 1992b; Kennedy et al., 2005; Koutsoumanis et al., 2010) showed that from 55 to 64% of refrigerators operate at a temperature of > 5°C, and this goes up to 80% in the case of domestic refrigerators in France and Italy (Cibin, Roccato, Ruffa, Barrucci, & Ricci, 2012; Laguerre et al., 2002). In addition, concerning storage temperature, from 44 to 75% of European consumers are not aware of the temperature of their domestic refrigerator (Garrido, García-Jalón, & Vitas, 2010; Lagendijk, Asserè, Derens, & Carpentier, 2008; Marklinder et al., 2004).

Most consumers were not familiar with the expression "cold chain" and only 10 to 20% of consumers bought perishable foods (like raw meat) at the end of shopping in order to reduce the potential for temperature abuse during transport home (Jevsnik,Hlebec, & Raspor, 2008a; Ovca & Jevšnik, 2009). In relation to food transport, the majority of consumers used the car trunk to transport food items purchased at stores (Gilbert et al., 2007; Kim et al., 2013) and transport took from 25 min (Jevsnik, Hlebec, & Raspor, 2008) to more than 2 h (Gilbert et al., 2007; Karabudak, Bas, & Kiziltan, 2008; Kennedy et al., 2005). An insulated bag to maintain temperature while carrying meat or perishable food home was used by only 15 to 18% of consumers (Gilbert et al., 2007; Jevsnik, Hlebec, & Raspor, 2008), with the rate dropping to 5% in the case of Turkish consumers (Karabudak et al., 2008).

Freezing is a common type of storage adopted by 50 to 60% of European consumers in order to extend meat storage life (Gilbert et al., 2007; Jevsnik et al., 2008; Migliorati et al., 2015; Sampers et al., 2012). Several of these studies reported that 46 to 60% of consumers preferred to thaw food on the kitchen countertop at room temperature (23 to 25°C) while only 26 to 30% thawed food in the refrigerator (Angelillo et al., 2001; Langiano et al., 2012; *personal communication*).

The purpose of the present study was to reproduce consumers' storage and thawing practices in an experimental kitchen in order to record the effects of different refrigeration temperatures and thawing methods on the presence and numbers of *Salmonella* bacteria in naturally (by preference) or artificially contaminated poultry-based meat preparations (burgers, sausages and ready-to-cook kebabs) commonly found in retail stores in Italy.

## 7.2 Materials and methods

### *7.2.1 Selection of poultry-based meat preparations and microbiological analyses performed on the delivery day*

Three poultry-based meat preparations were studied: burgers, sausages and ready-to-cook kebabs. The above-mentioned kind of food was also submitted to the cooking trails previously described in chapter 6.

The ingredients, physical parameters and the shelf lives of the final products, as provided by the manufacturer, are shown (Table 30). The main ingredient was poultry meat (chicken or turkey); however, with the exception of burgers, the products also contained pork or bacon. The products were obtained from local poultry meat industries in northern Italy, which delivered the commercial poultry meat patty formulation (for burgers) and commercial sausage mixture (for sausages), and ready-to-cook kebabs to the laboratory, at refrigeration temperature (4°C), within a maximum of 2 days from the time of production.

As already described in chapter 6-paragraph 6.2.1, in order to have a rapid detection of *Salmonella*, on the delivery day, the poultry-based meat preparations were analysed, by real-time PCR using a commercial kit validated by the manufacturer (AES Chemunex, Biomerieux Company, Combourg, France) to comply with ISO 16140. In addition, detection and quantification of *Salmonella* bacteria were also performed according to, respectively, the ISO 6579:2002/Amd1:2007 (ISO, 2007) classical detection method for 25 g amounts of meat and the ISO/TS 6579-2:2012 (ISO, 2012) miniaturized Most Probable Number (MPN) method, (starting from the 1/10 primary suspension of 25 g meat, prepared for detection). Estimated numbers from the miniaturized MPN method were expressed as MPN/g; the lower limit of quantification was 1.3 MPN/g and the upper limit of quantification was 710 MPN/g. Finally, all *Salmonella* bacteria detected were serotyped (White–Kauffmann-Le Minor scheme) by slide agglutination with O and H antigen-specific sera (Staten Serum Institute, Copenhagen, Denmark).

Before potential artificial contamination with *Salmonella enterica* serovar Typhimurium, 16 samples of the commercial poultry meat patty formulation were analysed before preparing burgers, 18 commercial sausage mixture samples were analysed before preparing sausages and 36 kebabs were analysed before experimental trials.

Storage and freezing-thawing tests were conducted on naturally contaminated products (positive PCR result) and artificially contaminated products (negative PCR result).

**Table30**. Ingredients, physical parameters, and shelf-lives of the poultry-based meat preparations purchased in northern Italy, as provided by the manufacturer.

| Product | Ingredients | Physical parameters of finished products | Shelf life (days) |
|---|---|---|---|
| Meat patty formulation for burgers | Minced turkey meat (82%); water, wheat flour, ground rice, salt, sodium ascorbate, flavours (18%) | Weight: 104 ± 3 g<br>Diameter: 9.5 ± 0.5 cm<br>Thickness: 1.3 ± 0.2 cm | 11 |
| Poultry sausage mixture for sausages | Minced chicken meat (50%); bacon (25%); water, salt, dextrose, saccharose, lactose, vegetable fibers, flavours, antioxidants E300 and E301, acidity corrector E331, spices, food colour E120 (25%) | Weight: 75 g<br>Diameter: 2.3 cm | 10 |
| Kebabs | Chicken meat (54%); sausages (pork [35%], turkey meat [28%], water, chicken meat [8%], salt, peas, carrots, dextrose, lactose, natural flavours, spices, antioxidants: ascorbic acid, sodium ascorbate, sodium acetate) (34.5%); bacon (7%); peppers (4.5%) | Weight: 160 g<br>Diameter of chicken meat: 3.8 ± 0.6 cm<br>Diameter of sausages: 2.5 ± 0.3 cm | 9 |

### *7.2.2 Artificial contamination procedure*

A strain of *Salmonella* Typhimurium DT104 (2324/5 2010Salm strain, culture collection at the Italian *Salmonella* Reference Laboratory), isolated from minced poultry meat and kept in cryobank, was spread on nutrient agar and incubated at 37°C for 18 h. Afterwards, the strain was suspended in buffered peptone water in order to obtain a suspension with an optical density at 600 nm of 1. Next, this suspension was serially diluted in buffered peptone water, and appropriate dilutions were used to obtain three residual contamination levels in the food products: ca. 10, ca. 100, and ca. 1,000 CFU/g.

In storage tests, only the 10 CFU/g level of artificial contamination was tested on poultry-based meat preparations, as this is considered a plausible level of contamination of poultry-based meat preparations available on the market, in light of both data in literature (Straver et al., 2007) and the results on food products naturally contaminated with *Salmonella*, described in chapter 6 paragraph 6.3.2.1. For the freezing-thawing tests, in order to be able to quantify any potential reduction of *Salmonella* bacteria due to the freezing-thawing practices, in addition to the level of contamination of 10 CFU/g, an additional two levels of contamination were tested, namely 100 and 1,000 CFU/g, considered the intermediate and worst-case scenario, respectively, in which *Salmonella* bacteria after accidental contamination (at low levels of < 1 to 10 CFU/g), had already multiplied due to prior storage under temperature abuse.

According to the kind of test and, consequently, the number of samples needed, for burgers at each inoculum level, 7 to 12 ml of the appropriate dilution was added to 1,000 to 1,800 g of commercial poultry meat patty formulation and mixed thoroughly for five minutes into a planetary mixer (KitchenAid professional, model KMP05 PRO) with a flat aluminium beater, and portions pressed into a Petri dish in order to obtain final burger weights and dimensions (Table 30). For sausages at each inoculum level, 6 to 12 ml of the appropriate dilution was added to 900 to 1,800 g of commercial sausage mixture and mixed thoroughly as described above, and the mixture stuffed into a bovine natural collagen gut casing using a commercial sausage filler, provided by a local sausage producer. Sausages of 75 g in weight, 2.3 in cm diameter, and 15 cm in length were prepared. After each use, the sausage filler was sterilized (121°C for 30 mins). Ready-to-cook kebabs were contaminated with *Salmonella* Typhimurium using a spray, which from the tests described in chapter 6-paragraph 6.2.2, had proven to be the most efficient and effective contamination method. Each kebab was contaminated with 1.0 ml of appropriate dilution for the level of contamination desired.

After artificial contamination, for each inoculum level, three replicates were analysed to verify the presence and estimated numbers of *Salmonella* Typhimurium. Once artificially inoculated, burgers and sausages were packed in groups of three in stomacher bags, while kebabs were packed in groups of three in modified atmosphere (15% oxygen, 60% carbon dioxide, and 10% nitrogen) using a bell-shaped vacuum packer (Orved VM16).

### *7.2.3 Storage and freezing-thawing tests*

Naturally or artificially contaminated poultry-based meat preparations were stored at three different temperatures (4, 8 or 12°C [± 1°C]) during the shelf-life period. The temperatures were selected in order to study the behaviour of *Salmonella* in the food when it was stored according to label instructions (keep at 0 to 4°C) and at two different refrigeration temperatures, namely, 8 and 12°C, which are the temperatures recorded in 33% and 3% of domestic refrigerators, respectively, in a study performed in Italy (Cibin et al., 2012).

For each type of contaminated product, three replicate samples were taken at two to three time points for detection and enumeration of *Salmonella* and were analysed as described above. Burgers were analysed after 4, 8, and 11 days of storage, while sausages, due to logistic difficulties in sending the commercial sausage mixture to the laboratory on a day as close as possible to the time of production, were analysed after 7 and 10 days of

storage. Kebabs were analysed after 4, 7, and 9 days of storage: for each sample, portions from all the items on a kebab skewer were collected and homogenised in order to start the analyses.

Moreover, in keeping with the goal of simulating storage habits and behaviours that are likely to be adopted by consumers, poultry-based meat preparations were kept, after artificial contamination, at 25°C for 2 h (temperature abuse), in order to simulate a plausible scenario of not storing food in the refrigerator for a short time after purchase, as reported in literature (Kennedy et al., 2005). After temperature abuse, products were stored in domestic refrigerators at one of three different temperatures, as previously described (storage tests). The core temperature of the products before and after temperature abuse was measured using a thermocouple (P200 Profi-Digital thermometer, TFA, Wertheim, Germany) calibrated between 0°C (melting ice) and 100°C (boiling water) prior to use. For kebabs, the thermocouple was inserted into the centre of one poultry meat piece, avoiding the wooden skewer.

Finally, freezing-thawing tests were carried out on artificially contaminated burgers, sausages, and kebabs, which were frozen at -22°C for 15 days and subsequently thawed overnight in disposable plastic containers at room temperature on the kitchen countertop (23°C) or in a domestic refrigerator (8°C). For each type of thawing method and level of contamination, three replicates were analysed to detect and estimate the numbers of *Salmonella* Typhimurium bacteria.

### *7.2.4 Data analysis*

In order to perform statistical tests, for each storage and thawing temperature, the results of the quantitative analyses performed on samples were analysed together, irrespective of the time point and the level of artificial contamination. All analyses were performed with SPSS Statistics version 22 at a significance level of 95% ($p = 0.05$). The Kruskal-Wallis test was used for non-parametric statistical analysis of the *Salmonella* numbers in the different meat products at different temperatures. The Bonferroni correction was applied to control the familywise error rate at 5% for all multiple pairwise comparisons.

## 7.3 Results

### *7.3.1 Storage tests: detection and quantification of Salmonella bacteria*

Analysis of meat samples upon arrival in the laboratory provided the following results: 6 of 16 commercial poultry meat patty formulation samples contained *Salmonella* bacteria. Of the six positive samples, four provided enumerable results ranging from 1.3 to 3.8 MPN/g. *Salmonella* bacteria were detected in 8 of 18 sausage mixture samples at numbers below 1.3 MPN/g (except that one sample had 1.6 MPN/g) and finally, 1 of 36 kebabs was found positive for *Salmonella* bacteria, leading to the decision to carry out storage tests only on artificially contaminated kebabs. The *Salmonella* isolates were serotyped: the poultry meat patty formulation contained mainly *S. enterica* serovar Newport (four of six isolates), as well as *Salmonella* Typhimurium and *S. enterica* serovar Brandenburg, while the sausage mixture and the kebabs contained *S. enterica* serovar Montevideo (all eight isolates) and the monophasic variant of *Salmonella* Typhimurium, respectively.

The results of the detection and estimation of numbers of *Salmonella* bacteria in naturally or artificially contaminated poultry-based meat preparations before (day zero) and after the storage tests are reported in Table 31.

The temperature abuse treatment at 25°C for 2 h did not affect the presence or numbers of *Salmonella* in naturally contaminated burgers and sausages. In fact, *Salmonella* bacteria were detected in 10 of 13 samples and 9 of 12 samples analysed before and after temperature abuse, respectively, with numbers below or at the lower limit of detection of the MPN method. In artificially contaminated kebabs, *Salmonella* Typhimurium was always detected in the samples analysed, with numbers ranging from 5 to 13 MPN/g before temperature abuse and from 3 to 8 MPN/g after temperature abuse. The recorded core temperatures before the 2 h of temperature abuse were, respectively, 3, 5, and 6°C for burgers, sausages, and kebabs, while the core temperatures after temperature abuse were 19, 17, and 18°C, respectively.

**Table 31**. Detection and numbers of *Salmonella* bacteria during storage tests, with or without temperature abuse, performed on naturally or artificially contaminated burgers, sausages, and kebabs.

| Meat preparation | Day 0[a] Det.[b] | Day 0[a] MPN/g[c] | Temp (°C) | Day 4 Det. | Day 4 MPN/g | Day 8 Det. | Day 8 MPN/g | Day 11 Det. | Day 11 MPN/g |
|---|---|---|---|---|---|---|---|---|---|
| **Burgers** | | | | | | | | | |
| **NC**[d] | 7/8 | 1/8 (1.6) | 4 | 3/3 | 1/3 (1.3) | 3/3 | 0/3 | 3/3 | 0/3 |
| | | | 8 | 0/3 | 0/3 | 1/3 | 0/3 | 1/3 | 0/3 |
| | | | 12 | 3/3 | 0/3 | 0/3 | 1/3 (3.8) | 3/3 | 0/3 |
| **NC** | Before temp abuse (2 h, 25°C) 5/8 | 2/8 (3.8, 3.8) | 4 | 1/3 | 0/3 | 2/3 | 0/3 | 2/3 | 0/3 |
| | After temp abuse 6/9 | 0/9 | 8 | 0/3 | 0/3 | 1/3 | 0/3 | 3/3 | 0/3 |
| | | | 12 | 1/3 | 1/3 (3.2) | 1/3 | 1/3 (1.6) | 1/3 | 1/3 (1.6) |
| **AC (10 CFU/g)** [e] | 4/4 | 4/4 (8.1; 8.1; 8.1; 21) | 4 | 3/3 | 3/3 (3.2; 6.1; 12) | 3/3 | 3/3 (8.1; 8.1; 8.9) | 3/3 | 3/3 (1.6; 13; 41) |
| | | | 8 | 3/3 | 3/3 (1.6; 8.1; 19) | 3/3 | 3/3 (8.9; 13; 19) | 3/3 | 3/3 (8.9; 13; 19) |
| | **Day 0** Det. | **Day 0** MPN/g | **Temp (°C)** | **Day 7** Det. | **Day 7** MPN/g | **Day 10** Det. | **Day 10** MPN/g | - | - |
| **Sausages** | | | | | | | | | |
| **NC** | 1/5 | 0/5 | 4 | 1/3 | 0/3 | 0/3 | 0/3 | - | - |
| | | | 8 | 0/3 | 0/3 | 2/3 | 0/3 | - | - |
| | | | 12 | 0/3 | 0/3 | 1/3 | 0/3 | - | - |
| **NC** | Before temp abuse (2 h, 25°C) 5/5 | 1/5 (1.6) | 4 | 3/3 | 1/3 (1.6) | 2/3 | 0/3 | - | - |
| | After temp abuse 3/3 | 1/3 (1.6) | 8 | 3/3 | 1/3 (6.1) | 3/3 | 1/3 (1.6) | | - |
| | | | 12 | 3/3 | 1/3 (1.6) | 2/3 | 0/3 | - | - |
| **AC (10 CFU/g)** | 3/3 | 3/3 (5.9; 18; 19) | 4 | 3/3 | 3/3 (1.6; 4.9; 8.5) | 3/3 | 3/3 (6.1; 12; 14) | - | - |
| | | | 8 | 3/3 | 3/3 (5.9; 66; 380) | 3/3 | 3/3 (8.5; 18; 19) | - | - |
| | | | 12 | 3/3 | 3/3 (12; 31; 43) | 3/3 | 3/3 (12; 26; 96) | - | - |
| | **Day 0** Det. | **Day 0** MPN/g | **Temp (°C)** | **Day 4** Det. | **Day 4** MPN/g | **Day 7** Det. | **Day 7** MPN/g | **Day 9** Det. | **Day 9** MPN/g |
| **Kebabs** | | | | | | | | | |
| **AC (10 CFU/g)** | 3/3 | 3/3 (1.6; 8.1; 13) | 4 | 3/3 | 3/3 (8.1; 31; 41) | 3/3 | 3/3 (1.4; 8,1; 13) | 2/3 | 3/3 (8.9; 12; 12) |
| | | | 8 | 3/3 | 3/3 (18; 21; 41) | 3/3 | 3/3 (11; 110; 240) | 2/3 | 3/3 (61; 170; 220) |
| | | | 12 | 3/3 | 3/3 (21; 61; 710) | 3/3 | 3/3 (>710) | 3/3 | 3/3 (>710) |
| **AC (10 CFU/g)** | Before temp abuse (2 h, 25°C) 4/4 | 4/4 (5.1; 6.1; 8.2; 13) | 4 | 3/3 | 2/3 (3.2; 13) | 3/3 | 3/3 (3.1; 3.8; 13) | 3/3 | 3/3 (1.4, 1.4; 8.5) |
| | After temp abuse | | 8 | 3/3 | 2/3 (6.1; 8.1) | 3/3 | 3/3 (5.9; 6.1; 13) | 3/3 | 3/3 (1.4; 3.8; 6.1) |
| | 4/4 | 4/4 (3.2; 5.9; 8.1; 8.1) | 12 | 3/3 | 3/3 (8.5; 110; 710) | 3/3 | 3/3 (2.8; 110; 710) | 3/3 | 3/3 (4.1; 20; 31) |

[a] Day zero is the day of delivery or immediately after artificial contamination.
[b] Det., detection per 25 g according to ISO 6579:2002/Amd1:2007. Number of positive samples/number of analysed samples.
[c] MPN/g, estimated number of surviving *Salmonella* bacteria according to ISO/TS 6579-2:2012. Number of samples with a quantity above 1.3 MPN/g/number of analysed samples.
[d] NC, naturally contaminated.
[e] AC, artificially contaminated.

As expected, the storage temperature of 4°C did not facilitate an increase in the presence or estimated numbers of *Salmonella* bacteria in either naturally or artificially contaminated poultry-based meat preparations throughout the extent of the shelf-life. In naturally contaminated samples (burgers and sausages), *Salmonella* bacteria were detected in 20 of 30 samples during the shelf-life period established by the manufacturer, with levels of contamination at or below the lower limit of the MPN method. In the case of artificially contaminated samples (burgers, sausages, and kebabs), *Salmonella* Typhimurium was always detected in numbers comparable to those of the initial artificial inoculation at day zero.

The storage of products at 8°C led to different results between naturally and artificially contaminated samples. In fact, with regard to naturally contaminated burgers and sausages with or without temperature abuse before storage, *Salmonella* bacteria were detected in 18 of 26 samples at day zero in numbers at or below the lower detection limit of the MPN method. During the storage period (11 days for burgers and 10 days for sausages), *Salmonella* bacteria were detected in 14 of 30 samples in numbers at or below the MPN detection limit. In artificially contaminated samples, namely, burgers, sausages, and kebabs, *Salmonella* Typhimurium was always detected and estimated MPNs were obtained in 19 of 24 samples analysed during storage with levels comparable to the numbers initially present after artificial contamination. Thus, at 8°C, most of the time, no substantial growth of *Salmonella* Typhimurium was noted, although in one of three sausages analysed on day 7, a count of 380 MPN/g was estimated. In case of kebabs stored at 8°C, four of six samples analysed at the end of the storage contained estimated numbers of *Salmonella* Typhimurium between 110 and 240 MPN/g.

The higher storage temperature of 12°C led to different results according to the type of meat preparation and type of contamination (natural *versus* artificial). With regard to naturally contaminated burgers (with or without temperature abuse before storage), *Salmonella* bacteria were detected in 12 of 16 samples before storage (with estimated numbers always at or below the lower MPN detection limit) and in 9 of 18 samples during the storage period (11 days). Of the nine positive samples, four contained estimated *Salmonella* numbers between 1.6 and 3.8 MPN/g. At this storage temperature, no growth of *Salmonella* occurred in naturally contaminated sausages (with or without temperature abuse before storage): in fact *Salmonella* bacteria were detected in 9 of 13 sausage samples initially, in numbers at or below 1.3 MPN/g (the lower detection limit), and in 6 of 12 samples during the storage period (10 days) with numbers at or below the lower limit of the MPN

method. However, in the case of sausages artificially contaminated with *Salmonella* Typhimurium (ca. 10 CFU/g), one of three samples contained 96 MPN/g at the end of the shelf-life. Finally, in artificially contaminated kebabs (with or without temperature abuse at 25°C) a statistically significant ($p < 0.05$) growth was recorded. In fact, the initial numbers of *Salmonella* Typhimurium (ca.10 MPN/g) reached 710 MPN/g after 4 days of storage in two of six samples and exceeded the upper limit of the MPN method (710 MPN/g) at the end of the shelf-life (days 7 and 9) for all 12 kebab samples.

### *7.3.2 Freezing-thawing tests: detection and quantification of Salmonella bacteria*

In Table 32, the results of freezing-thawing tests on detection and levels of *Salmonella* bacteria in artificially contaminated samples of burgers, sausages, and kebabs are reported. Some differences were noted in *Salmonella* Typhimurium numbers after freezing-thawing according to the kind of poultry-based meat preparation and the thawing temperature.

In particular, in the kebabs, overnight thawing at room temperature (23°C) caused a significant ($p < 0.05$) increase of *Salmonella* Typhimurium, with numbers exceeding the upper limit of the MPN method (> 710 MPN/g) for all three levels of contamination tested. The thawing of the same kind of food in the refrigerator did not change the numbers of *Salmonella* Typhimurium for all three levels of contamination tested.

In the case of sausages, no *Salmonella* growth was observed after thawing at ambient temperature, as *Salmonella* numbers were maintained at the initial artificial inoculation level, while overnight thawing at refrigerator temperature (8°C) led to a significant ($p < 0.05$) decrease of *Salmonella* Typhimurium numbers.

Finally, for the burgers, lower numbers of *Salmonella* Typhimurium were recorded after thawing at both temperatures, but the difference was not statistically significant.

**Table 32**. Detection and numbers of *Salmonella* Typhimurium on artificially contaminated burgers, sausages, and kebabs subjected to freezing-thawing tests.

| Contamination conditions | Burgers | | Sausages | | | | Kebabs | |
|---|---|---|---|---|---|---|---|---|
| | Det.[a] | MPN/g[b] | Det. | MPN/g | Det. | MPN/g | Det. | MPN/g |
| **10 CFU/g** | | | | | | | | |
| After inoculation | 4/4 | 4/4 (3.8; 4.9; 8; 21) | 3/3 | 3/3 (3.8; 6.1; 8.1) | 3/3 | 3/3 (13, 21; 21) | 4/4 | 4/4 (6.1; 13; 45; 110) |
| Thawing at 23°C | 2/3 | 2/3 (1.3; 3.8) | 3/3 | 3/3 (3.8; 13; 14) | - | - | 3/3 | 3/3 (>710) |
| Thawing at 8°C | 3/3 | 2/3 (1.4; 1.6) | - | - | 3/3 | 2/3 (1.6; 6.1) | 3/3 | 3/3 (3.2; 7.4; 17) |
| **100 CFU/g** | | | | | | | | |
| After inoculation | 4/4 | 3/4 (41; 110; >710) | 3/3 | 3/3 (110; 170; 240) | 3/3 | 3/3 (380, 380; 710) | 4/4 | 4/4 (66; 110; 170; 380) |
| Thawing at 23°C | 2/3 | 2/3 (8.8; 8.9) | 3/3 | 3/3 (66; 67; 240) | - | - | 3/3 | 3/3 (>710) |
| Thawing at 8°C | 3/3 | 3/3 (3.8; 8.9; 21) | - | - | 3/3 | 3/3 (12; 19; 21) | 3/3 | 3/3 (66; 96; 380) |
| **1,000 CFU/g** | | | | | | | | |
| After inoculation | 3/4 | 3/4 (21; >710; >710) | 3/3 | 3/3 (3.2; >710; >710) | 3/3 | 3/3 (>710) | 4/4 | 4/4 (380; 710; 710; >710) |
| Thawing at 23°C | 3/3 | 3/3 (6.7; 45; 380) | 3/3 | 3/3 (710, 710; >710) | - | - | 3/3 | 3/3 (>710) |
| Thawing at 8°C | 3/3 | 3/3 (3.2; 6.9; 11) | - | - | 3/3 | 3/3 (15; 45; 56) | 3/3 | 3/3 (710; >710; >710) |

[a] Det., detection per 25 g according to ISO 6579:2002/Amd1:2007. Number of positive samples/number of analysed samples.
[b] MPN/g, estimated number of surviving *Salmonella* bacteria according to ISO/TS 6579-2:2012. Number of samples with a quantity above 1.3 MPN/g/number of analysed samples.

## 7.4 Discussion

Domestic kitchens and time-temperature abuse during domestic storage have been identified as the main setting and contributory factors involved in food-borne outbreaks (Gonzales-Barron, Redmond, & Butler, 2010; Taoukis et al., 2005). Moreover, in the framework of "home food safety", intended to describe the total sum of measures used to prevent contamination with pathogens, temperature abuse practices during transport of food and due to thawing methods can occur, as shown by consumer-based studies (Bearth et al., 2014; Terpstra et al., 2005). However, how such time-temperature fluctuating conditions affect the behaviour of *Salmonella* bacteria, the preeminent causative agent of food-borne outbreaks in the European Union, has been investigated only rarely (Hwang & Sheen, 2011; Lianou & Koutsoumanis, 2009; Scientific Opinion of the Panel on Biological Hazards, 2007), while many studies have focused mainly on *Listeria monocytogenes*, due to its well-known

psychrotrophic nature. The present study, therefore, provides some data on the behaviour of *Salmonella* bacteria in three different types of poultry-meat preparations (burgers, sausages, kebabs) that were naturally or artificially contaminated with *Salmonella* and submitted to time-temperature abuse both during refrigerated storage (and also prior to storage, simulating shop-to-home transport) and during freezing-thawing.

With regard to the temperature abuse prior to refrigerated storage, the study of Kim et al. (2013), highlighted that refrigerated foods (e.g. eggs, milk, and fresh meat) left in the car trunk exposed to sunlight reached 20°C within 40 min and 30°C within 90 to 110 min, thus reaching the temperature danger zone (5 to 60°C) for food-borne pathogens to grow. For this reason, the refrigeration of perishable foods within 2 h is recommended, and when the outdoor temperature reaches 32°C, the time between purchase and refrigerated storage should be reduced to 1 h (USDA, 2011). In the present study, exposure of the poultry-based meat preparations to 25°C for 2 h before refrigerated storage did not have any effect on the presence or estimated numbers of *Salmonella* bacteria in either the naturally or the artificially contaminated poultry-based meat preparations. This was in spite of the average internal temperatures of the products recorded after this temperature abuse ranging from 17 to 20°C, and, thus, being above the minimum growth temperature of *Salmonella* bacteria. It is likely that the 2 h of temperature abuse was still within the microorganism's lag-phase, and therefore, growth of *Salmonella* was not observed. This lends support to the *Salmonella*-related safety of the 2-h rule, which is commonly used as a maximum guideline time for the non-refrigerated holding of perishable products.

Our observations on the stability of the presence and estimated numbers of *Salmonella* bacteria during refrigerated storage at 4°C are supported by previous studies on *Salmonella* survival in a variety of artificially contaminated (> 3 log units) raw chicken products during refrigerated storage (Bailey, Lyon, Lyon, & Windham, 2000; Betts, Everis, & Paish, 2003; Oscar, 2011; Pintar et al., 2007; Pradhan et al., 2012). In these studies, *Salmonella* could survive but was not able to proliferate at 4°C during storage periods ranging from 8 to 21 days. In fact, food safety authorities (e.g. the Food Standard Agency and the U.S. Department of Agriculture) of different countries recommend that refrigerated foods be stored between 4 and 5°C as an intrinsic part of safe food handling, in order to inhibit or prevent growth of spoilage and pathogenic organisms, such as *Salmonella*.

Storage at 8°C, for most of the meat products sampled, did not support an increase in the presence and estimated numbers of *Salmonella* bacteria, although in a few artificially contaminated sausages and kebabs, the estimated numbers of *Salmonella* Typhimurium

were on the order of 2 log units. The latter findings are in agreement with studies conducted on artificially contaminated (> 3 log units) chicken meat samples (chicken, breast, and thighs) stored at 8°C, in which significant ($p < 0.05$) increases of *Salmonella* numbers within 9 days of storage were recorded (Betts et al., 2003; Pradhan et al., 2012; Zaher & Fujikawa, 2011).

Storage at 12°C facilitated a significant ($p < 0.05$) increase of *Salmonella* Typhimurium in artificially contaminated kebabs (10 CFU/g). In fact, *Salmonella* numbers exceeding the upper limit of the MPN method (> 710 MPN/g) were found in seven of nine kebabs analysed during the shelf-life period. Studies investigating the behaviour of *Salmonella* on artificially inoculated chicken meat stored at 12°C observed a growth of *Salmonella* from 10 CFU/g to 2,900 CFU/g within the 9-day shelf-life (Betts et al., 2003; Oscar, 2011, 2014; Zaher & Fujikawa, 2011), thus confirming that improper refrigeration temperatures can have a substantial effect on the growth of this pathogen.

With regard to freezing-thawing, other studies investigating the behaviour of *Salmonella* bacteria during frozen storage concluded that *Salmonella* could survive at frozen storage temperatures (Dominguez & Schaffner, 2009; Pradhan et al., 2012). However, how thawing practices could affect the numbers of *Salmonella* in meat products has rarely been investigated. The study of Lianou & Koutsoumanis (2009) observed no significant changes in *Salmonella* Enteritidis counts on artificially contaminated ground beef samples during abusive thawing (25°C for 12 h), while according to Manios & Skandamis (2015), thawing of beef patty samples on the kitchen counter (20°C for 12 h) resulted in significant increases in *Salmonella* populations. Statistical tests performed on the thawing data collected in this study led in some cases to non-significant results. This is mainly related to the fact that in order to have a sufficient number of samples to be analysed, for each kind of food and thawing method, the estimated numbers of *Salmonella* bacteria from the three different levels of contamination were analysed together. However, the present study highlighted that the type of thawing method and the kind of food affect the numbers of *Salmonella*. In fact, thawing overnight in the refrigerator (8°C) led to either a moderate reduction or no change in *Salmonella* Typhimurium numbers in burgers, sausages and kebabs. Thawing overnight on the kitchen countertop (23°C) caused significant increases in *Salmonella* Typhimurium numbers in kebabs, but occasionally, the numbers remained stable or were even reduced in sausages and burgers, respectively. This phenomenon could be explained by possible different time-temperatures profiles, as described by Manios & Skandamis (2015), of different food matrices. Consequently, the recommendation to defrost poultry meat at a

temperature between 5°C and 7°C, i.e. in a refrigerator, is still pertinent in order to prevent the growth of microorganisms like *Salmonella* (Damen & Steenbekkers, 2007), as was observed (although not consistently) in the present study.

Overall, the results of the present study highlighted that domestic and consumer-related storage and thawing practices could affect poultry meat safety in regard to *Salmonella.* Time-temperature abuse allowed a substantial increase of *Salmonella* numbers in poultry-based meat preparations, although the actual observed behaviour of *Salmonella* (no change in presence or numbers, either reduction or growth) is dependent upon several factors, such as the exact extent of temperature abuse (with 12°C being more supportive for growth than 8°C), the type of strain (with artificially inoculated strain facilitating growth and notably larger increases in *Salmonella* numbers upon temperature abuse than natural strains) and the type of meat preparation (with kebabs being more supportive of growth than sausages and burgers).

The latter result could be due to the fact that, even though kebabs were packed in a modified atmosphere, while burgers and sausages were not, they were composed of pieces of whole meat tissue. Thus, while burgers and sausages contained added salt and spices, likely with an inhibitory effect on bacterial growth (Sampers et al., 2010), the structure and composition of kebabs in the packaging may have allowed the presence of more exudates, which can provide nutrients to bacteria. In addition, we were unable to detect any naturally occurring *Salmonella* bacteria among the kebabs, and so these were studied only with artificial *Salmonella* contamination. In contrast to fully viable bacteria used for artificial contamination, bacterial cells in naturally contaminated foods are frequently impaired by sublethal injury as a result of having been exposed to adverse conditions during food processing (Jasson, Baert, & Uyttendaele, 2011). Therefore, retarded growth due to a longer lag phase might have occurred in the present study.

Although the results of the present study, using poultry-based meat preparations that were both naturally and artificially contaminated with *Salmonella*, show that temperature abuse (prior to storage, during refrigerated storage, or during freezing-thawing) did not consistently, for all samples, lead to a substantial increase in the presence or estimated numbers of *Salmonella* bacteria in the products under consideration, there was occasional substantial growth of *Salmonella* in artificially contaminated samples. Therefore, efforts to disseminate guidelines for consumers on correct storage and handling of food need to be continued. This is of particular importance given the widespread lack of consumer knowledge of safe food handling practices in the kitchen. Studies have reported that large

proportions of consumers (up to 93%) do not know the recommended refrigerator operating temperature range and do not have a thermometer with which to measure it (Evans & Redmond, 2014; Nesbitt et al., 2014). Part of this lack of knowledge may stem from the fact that most consumers do not consider themselves responsible for food safety to the same degree as professional food handlers (Jevsnik, Hlebec, & Raspor, 2008; Ovca & Jevšnik, 2009). According to Rosati & Saba (2004), the majority of Italian consumers identify the food industry and public institutions as bearing the main responsibility for assuring food safety, while considering themselves as having the least responsibility.

The results presented in this chapter and in chapter 6 (i.e. temperature abuse and domestic cooking), highlighted the role that the consumer has in shaping the risks associated with microbial hazards adopting either adequate or improper behaviours. These results represent useful information for food business operators and competent authorities for improving the information reported on the labels, adding clear indications on how to properly cook, store and handle food products. At the same time, as already stated, consumers have to be educated in order to effectively take up the provided information. Thus, on-going efforts taken by food safety authorities and organisations such as the International Scientific Forum on Home Hygiene remain necessary to set up effective educational campaigns addressing specific topics on consumer food handling-related behaviours.

# CHAPTER 8. General discussion

Ensuring food safety along the “from farm to fork chain” is the main goal of food business operators, competent authorities and consumers as well, and a shared responsibility. In 2004, with the so called “hygiene package regulations” (Reg. 852/2004; Reg. 853/2004; Reg. 854/2004), the European food safety policy has substantially changed. The new strategy has been developed based on the admission that end product testing alone was unable to assure safe food production and that the main responsibility of food safety belonged to the FBO. Hence, the HACCP principles and the application of good manufacturing and hygienic practices along the production process were the main tools that FBO have to adopt in order to eliminate or reduce the identified hazards to an acceptable level in the perspective of public health.

Moreover, the continuous evolution of the food market and of consumer’s preferences towards food products may affect food safety as well. In fact, the growing interest of consumers for artisanal/local food, which is mainly produced in micro and small-scale companies, made this kind of production system a valuable way in order to provide a wide choice of food products, the maintenance of diversity in land use and a new income especially for the rural areas. Therefore, the investigation of the production process of artisanal food products and the identification of a minimum number of control points that can be easily monitored and recorded with accessible tools could represent an effective way to implement FSMS in micro/small-scale enterprises and thus assure food safety.

Nevertheless, once food is ready to be set to the market, food producers and consumers have the shared responsibility to guarantee its safety. Therefore, producers have to take into account the effect on microbial behaviour of reasonably foreseeable conditions of distribution, storage and use of the products they put on the market and consumers have to handle properly food products in the domestic environment, once they bought them.

The above-mentioned aspects have been investigated in the present PhD and the obtained results have been combined in order to provide useful suggestions, addressing food business operators, competent authority and consumers.

## 8.1 Food safety control of the artisanal/local food production system

The development and implementation of effective pre-requisite programs (PRPs) and HACCP, as part of the FSMS, is mainly dependent on the availability of enough financial, technical and managerial resources. Large food companies, in most cases, have the needed resources to build up and measure an effective FSMS (Jacxsens et al., 2011). On the other side, micro and small-scale companies are less likely to invest in hygiene and food safety due to several constraints. In primis, the development of an effective FSMS requires people with enough expertise and knowledge. This means that training of people is needed and therefore financial resources have to be allocated for this aim (Ramalho, de Moura, & Cunha, 2015).

The production system of artisanal, traditional food such as salami and soppresse from Veneto region and raw milk cheeses is characterized by very small production facilities/premises, with products sold directly by the farmer to the consumer or at local markets. For micro/small-scale enterprises like these of the artisanal salami and soppresse production but also those producing farmstead cheeses, hygiene training and food safety education of the personnel involved in food handling could represent a heavy financial burden (Verraes, et al., 2015). In addition, the record keeping and documentation, as required by the application of HACCP and PRPs, could be seen just as paperwork by micro/small-scale enterprises, thus representing an obstacle instead of a useful tool in order to keep the production process under control (Luning et al., 2015). The case study of artisanal salami and soppresse produced in Veneto region of Italy, described in Chapters 2 and 3 along with the case study of ensuring safety of artisanal raw milk cheeses, described in Chapter 4 allowed us to make some reflections about the potential approach to adopt in order to ensure food safety of an artisanal/local food production system.

These artisanal products are processed with traditional know-how. They are fermented products and to some extent rely on the natural contamination of the raw materials by microbiota that occur during animal slaughter or from the production environment. The natural microbiological contamination may comprise a wide variety of taxa including both Gram-positive and Gram-negative bacteria and both beneficial bacteria (such as lactic acid bacteria which are supposed to dominate during fermentation processes) and spoilage bacteria, as well as occasionally some pathogenic bacteria such as STEC, *Salmonella* or *L. monocytogenes* being identified as the most important pathogens in fermented foods. These pathogenic bacteria may increase or decrease during the fermentation process depending

on the dynamics of the competing microbiota and evolution of the intrinsic factors (pH, $a_w$) either facilitating or presenting adverse conditions for the growth of pathogens.

Literature data on the presence and persistence of several pathogenic microorganisms in fermented meat products indicated different behaviours according to the pathogen investigated. In fact, *Salmonella enterica* usually die during the fermentation process (Mataragas et al., 2015) while large variations in the reduction of STEC are reported (Holck et al., 2011). In relation to *L. monocytogenes*, the study of Thevenot et al. (2005) showed that the pathogen tend to decrease during the ripening period of fermented meat products. However, several studies indicated that the cells of *L. monocytogenes* resulted more resistant than cells of *E. coli* O157:H7 and *S.* Typhimurium to fermentation and drying of fermented sausages and thus a low decrease of *L. monocytogenes* counts was observed In fermented meat products (Nightingale et al., 2006; Porto-Fett et al., 2010).

The behaviour of *L. monocytogenes* has been studied also in other fermented products. In cheese, for example, some studies indicated a decreasing trend of the pathogen (Angelidis, Boutsiouki, & Papageorgiou, 2010; Bellio et al., 2016; Chatelard-Chauvin, Pelissier, Hulin, & Montel, 2015; Finazzi et al., 2011; Ortenzi, Branciari, Primavilla, Ranucci, & Valiani, 2015) during fermentation and ripening. In contrast, other studies pointed out that *L. monocytogenes* might survive (Bellio et al., 2016; Valero, Hernandez, & González-García, 2014) or grow (Gameiro, Ferreira-Dias, Ferreira, & Brito, 2007) in the cheese.

Therefore, it is clear that the behaviour of *L. monocytogenes* in fermented foods is a complex phenomenon that might be influenced by the antilisterial properties of some starter cultures (e.g. LAB) which could be naturally present in the products or selected and added (Cornu, Billoir, Bergis, Beaufort, & Zuliani, 2011; Izquierdo et al., 2009; Reis, Paula, Casarotti, & Penna, 2012; Sip, Więckowicz, Olejnik-Schmidt, & Grajek, 2012).

In our study, the investigation of microbial hazards associated to artisanal salami and soppresse (Chapter 2) and of *L. monocytogenes* behaviour in several types of raw milk cheeses (Chapter 4) indicated that while *Salmonella* and STEC died during the ripening period of artisanal salami and soppresse, *L. monocytogenes* showed different behaviours according to the food matrix but also variability was recorded even within the same food category (i.e. raw milk cheeses). In fact, in case of salami and soppresse, *L. monocytogenes* was still detected in some samples at the end of the ripening period, thus even if it was not able to grow, the environmental conditions allowed its maintenance. On the other side, concerning raw milk cheeses, *L. monocytogenes* was able to grow in white-moulded soft/semi-soft cheeses while it was not able to grow but still survived in semi-hard cheeses

(independently if red-smear cheese or not). In case of the red-smear cheese (a semi-hard cheese) in chapter 4 we actually dealt with two ways of contamination. In fact when performing challenge tests, it was shown that the semi-hard red-smear cheese (artificially) contaminated with *L. monocytogenes* on the surface as post-contamination during (further) ripening or storage in deli-shops or at consumer home was not able to support the growth of the above-mentioned pathogen. In the durability study (using the same type of red-smear semi-hard cheese) we dealt with a *L. monocytogenes* contamination that was naturally present in the raw milk. Thus, in the latter case also the production process and the ripening that could have affected the survival of *L. monocytogenes* were included. Also in this durability study (the pathogen being introduced from the raw material), just as in the challenge test study (the pathogen inoculated on the surface) the semi-hard red-smear cheese was not able to support the growth of the pathogen.
The study of Schvartzman et al. (2011), exploring the behaviour of *L. monocytogenes* during red-smear cheeses making and ripening reported no growth of *L. monocytogenes* during raw milk cheese making, whereas growth did occur in pasteurised milk. During ripening, growth occurred in raw milk cheese, but inactivation occurred in pasteurised milk cheese.

The microbiota naturally present in artisanal food products, provide the specific taste and organoleptic qualities associated with artisanal foods and appreciated by consumers but also it is stated for artisanal fermented food that these microbiota present in high numbers represents a competition and hurdle for the development of pathogens (Dal Bello et al., 2010; Montel et al., 2014). Still, the fermentation process of salami and soppresse, constituted by a drying step and a ripening step, is characterized by a low level of standardization as it depends greatly on the environmental conditions, such as temperature and humidity. Consequently, deviations of physical-chemical parameters as pH and $a_w$, which are important hurdles for the growth of pathogenic bacteria, eventually present, can occur. Thus, in order to assure the safety of artisanal food products, a delicate balance between the composition of the autochthonous microflora of the food and the control of the environmental conditions, which can affect important technological parameters (e.g. pH and $a_w$) and thus the behaviour of microorganisms, is required.

Controlling the development of the indigenous microbiota under the given environmental conditions encountered along the production process is a challenging task. Understanding this phenomenon and managing this equilibrium is important. Nowadays, metagenomics approaches are used to unravel the composition of microbiota and to

investigate the impact of technological parameters on the microbiota developed during curing and ripening of fermented food products (Cocolin & Ercolini, 2015; Ferrocino et al., 2017). It could be an option in due time also to apply this type of genetic tools for better understanding of the microbiota dynamics during the production process of artisanal salami and soppresse produced in Veneto region of Italy.

Also for the cheese some 'metagenomics' studies exist (Delcenserie et al., 2014; Lusk et al., 2012; Mangia, Fancello, & Deiana, 2015; Van Hoorde, Verstraete, Vandamme, & Huys, 2008) and they might provide better understanding of microbiota composition involved in the manufacturing process. Still, DNA-based metagenomics approaches are descriptive in what type of bacteria are present; they do not provide information on functionality. If defined lactic acid bacteria are identified, it is still recommended to isolate and in-vitro assess in co-cultures or challenge studies the actual antimicrobial properties and their ability to produce bacteriocins and specifically inhibit growth of for example *L. monocytogenes*.

The present case-study of the Italian artisanal salami and soppresse only used plate counts and measurement of pH and $a_w$ as these are easy tools to apply on a routine basis by competent authorities and small-scale producers to manage the safety of their food products. Initiative of the competent authorities, which allocated time and resources in order to become familiar with this type of production system and the support of food science skilled personnel at the IZSVenezie research institute together with the collaboration of the producers, was noted to be in the present study a good approach in identifying the main hazards associated with the food product and setting up monitoring and control measures (Chapter 2). In the present case-study weight loss of the salami or soppresse has been identified as the most effective tool to be used by small-scale FBO to monitor its production process and initiate further analysis (if needed) to help manage food safety of its products. Although this correlation between the weight loss and $a_w$, which occur during the ripening period was not good enough for accurate predictions of $a_w$, still the weight loss was an easy tool to be applied. In addition, FBO can monitor other important parameters in order to control the main hazard associate with salami/soppresse, which is represented by *L. monocytogenes*. In fact, the control and recording of the parameters that affect the ripening process as the temperature of the drying and ripening rooms, the amount of salt and additives added to the minced meat, the humidity of the drying/ripening room and the duration of the drying/ripening period could be easily collected. Collect and keep record of the above-mentioned parameters is important in order to have an indication of the goodness

of the ripening process. In fact a high variability of the parameters could indicate a deviation of the ripening process and thus that the safety and the organoleptic features of the products are not assured.

Similar considerations could be done for the other artisanal food product investigated in this study: raw milk cheese (Chapter 4). In fact, the production process of artisanal or farmstead raw milk cheeses is conducted in a less standardized environment than the industrial one and thus also the fermentation process depends much on the proper development of the lactic acid bacteria starter cultures added or naturally present in the milk. In fact, as already stated, LAB represent a competitive flora that can control the growth of *L. monocytogenes*, one of the main hazards identified also in cheese.

However, the presence of LAB itself do not assure an inhibitory effect on *L. monocytogenes*. In fact, besides LAB, other factors might affect the behaviour of the above-mentioned pathogen. Firstly, the composition of the anti-*Listeria* consortia and its ability to affect the behaviour of *L. monocytogenes* should be investigated (Montel et al., 2014). Moreover, it seems that the measurement of pH and $a_w$ is of importance especially in semi-hard cheeses, where the control of these parameters might properly control and inhibit the growth of *L. monocytogenes*, as shown in chapter 4.

In addition, because during the ripening of the cheese, as during manufacture, the particular dynamism of the intrinsic characteristics of the matrix and its physicochemical properties (e.g. pH, temperature, lactic acid content and water activity) will affect the very unpredictable behaviour of the microorganisms (including pathogens), it is recommended to measure the 'lactic acid' concentration generically produced by all LAB but also other limiting environmental factors not accounted for, such as acetic or other organic acids produced by the background flora (Schvartzman et al., 2011).

However, even if LAB were present in high concentrations (above $10^7$-$10^8$ CFU/g), growth of *L. monocytogenes* was recorded in the Belgian soft cheeses (Chapter 4), which showed measured values of pH (5.00-7.00) and $a_w$ (0.97-0.99) expected to support growth of the pathogen, according to the available information reported in literature (Koutsoumanis, Kendall, & Sofos, 2004; Tienungoon et al., 2000).

Thus, the main strategy in order to control the presence and numbers of *L. monocytogenes* in artisanal food products is still the prevention, which means the application of proper measures of GMP and GHP (e.g. cleaning and disinfection) along the production process. In this context, it is of paramount importance to start with raw materials (i.e. raw meat or raw milk) with no pathogens present (or at least detectable absent per 25 g or 25 ml in a sampling

and testing approach). In this way, testing could be done just to confirm the effectiveness of the preventive approach adopted.

Moreover, when sampling and testing is included in the FSMS, usually a single-unit sampling is taken by small-scale producers as it is costly (usually the analysis are performed by an external lab). Nevertheless, single-unit sampling do have limitations in case of non-homogenous distribution of pathogens as shown in chapter 4. Therefore, the approach could be control the absence of the pathogen per 25 g in a multiple-unit sampling (n = 5) approach, as legally recommended.

Consequently, for these micro/small-scale FBOs, it was possible to apply the concept of flexibility expressed in the European legislation and assure the safety of artisanal/local food products through the development and application of a simplified FSMS (EFSA Panel on Biological Hazards et al., 2017). This system is based on the understanding of the production process and the activities that can contribute to an increased occurrence of the hazards. The control points are founded on the identification of the PRPs to control the hazards that may occur at each stage. These PRPs are based on those described in the Commission Notice 2016/C 278/01, but with the inclusion of an additional PRP on 'product information and customer awareness'. In fact, producers (also the small ones) should provide (e.g. labelling, leaflets) sufficient information to promote proper handling, storage and preparation by consumers.

In addition, it was shown that the development of a collaborative approach, involving dialogue and efforts of both competent authorities and producers and with support of scientific researchers was much appreciated by the producers and adopted also in the long-term in their everyday activities as was expressed when questioning these food producers (Chapter 3).

It was also noted that the combination of overall theoretical knowledge on food microbiology and the behaviour of microorganisms in food, as impacted by the composition of the food and its intrinsic (pH, $a_w$, competing microbiota) & extrinsic factors (storage conditions i.e. time and temperature), are along with experimental lab data (i.e. measurements of pH, $a_w$ and microbiological analysis of both naturally and artificially contaminated foods) bringing a wealth of information for decision making for ensuring food safety. This shows that the development of any FSMS requires knowledge on several subject matters.

Motivation to implement good manufacturing and hygienic practices (GMP/GHP) and the concept of HACCP starts with awareness of the importance of food-borne pathogens and how they could be introduced in the food or food production environment and develop

and persist in there. This should be part of the basic knowledge of each FBO. Therefore, continuous education and training of the FBOs and the personnel operating in food companies (independently of the size) on the above mentioned topics is required in order to firstly build and next apply correctly GMP/GHP during the production process. During the case study on artisanal salami and soppresse it was noted when questioning these food producers (Chapter 3) that in particular also training and education that had been going on was well appreciated by FBOs. Regional legislation in several EU Member States made food hygiene training of small-scale FBOs mandatory, thus recognizing the education of FBOs as a crucial line of defence in the prevention of foodborne illnesses. The need of training and education was also expressed by the respondents of the self-reported questionnaire on food hygiene knowledge and practices administered to small-scale artisanal salami and soppresse producers of Veneto region in Italy. In fact, 15% (5/34) of respondents suggested to develop additional training and education activities and 73 % (25/34) indicated they would prefer to use available financial resources mainly for further "training and education". In addition, most of the respondents recognized indeed the importance of food safety legislation in order to prevent the arising of problems affecting food safety. Therefore, the development of a fit for purpose training program, which answers to the actual needs of small-scale FBOs coupled with a positive attitude of the operator itself towards training and education could represent an effective tool in order to increase the food safety and hygiene knowledge of FBOs and thus improve the safety of artisanal/local food products. However, several studies (McIntyre et al., 2013; Roberts et al., 2008) pointed out that increasing the level of knowledge through training does not necessarily lead to changes in the attitude and thus the behaviour of FBO. Nevertheless, knowledge is the starting point in order to build a sense of awareness and responsibility and therefore produce an effective change of the behaviour.

Overall, it can be concluded that it is evident that the safety of artisanal meat/food products is the result of the interplay of many factors and actors. The support and cooperation with local and national food safety authorities coupled with fit for purpose training and education, can answer to the effective needs of the food operators and provide the needed information and expertise, which are the starting points for the development of the awareness and motivation among small-scale producers to implement food safety management systems. This approach could represent a winning strategy, enabling avoidance of marketing potentially hazardous artisanal food products and contributing to

improve the perception of not only good quality but also good safety attributed to traditional food among consumers.

### 8.2 How to deal with storage abuse practices in food safety assessment

Another aspect that both FBOs and risk managers (CA) have to take into account in the framework of food safety assessment is the consumer behaviour in the domestic environment. In fact, according to epidemiological data, the domestic environment is the major setting for outbreaks of food-borne illness. Therefore, the effect of consumer's behavior on microbial growth has to be considered by FBOs and risk assessors, with these last ones providing useful information to risk managers and thus to the CA in order to manage food safety and public health.

It is widely recognized that storage time and temperature are the two main factors that affect microbial growth. The effect of temperature abuse on microbial growth was studied in Chapter 4, where several types of raw milk cheeses (e.g. from semi-hard to soft cheeses) artificially or naturally contaminated with *L. monocytogenes* were stored at different time-temperature combinations (i.e. 9, 7 and 14 days; 7, 12 and 14°C). Growth of *L. monocytogenes* was recorded in two (artificially contaminated) white-moulded soft to semi-soft Belgian cheeses and the growth was higher at 12°C (final concentration: from ca. 5 to 7 log CFU/cm$^2$) than at 7°C (final concentration: from 4 to 5 log CFU/cm$^2$). The study of Lahou & Uyttendaele (2017), highlighted that the growth potential of *L. monocytogenes* is affected by the cheese type (soft *versus* semi-hard) as well as the storage temperature.
However, other studies investigating the behaviour of *L. monocytogenes* at different temperatures, pointed out that the lower the storage temperature, the higher the bacterial count and the longer the survival of *L. monocytogenes*. The study of Bellio et al. (2016), reported a reduction of *L. monocytogenes* in cheese samples stored at 10°C compared to the storage at 4°C, and this behaviour was explained by the increase of *L. monocytogenes* metabolism with the higher temperature, probably leading to its earlier inactivation due to autolysis. Moreover, the greater competition from (more active) LAB populations in cheese stored at higher temperatures may inhibit *L. monocytogenes* growth and/or survival (Kagkli, Iliopoulos, Stergiou, Lazaridou, & Nychas, 2009). Valero et al. (2014) concluded that storage of cheese at room temperature (i.e. 22°C) produced an accelerated decay rate of *L. monocytogenes* mainly due to the loss of humidity and LAB growth.

However, several tools are available for FBO in order to assure the safety of food products set to the market. Besides challenge tests and durability studies (described in chapter 4), predictive modelling represents another tool (suggested also by Annex 3 of EU Regulation 2073/2005) that FBO can use in order to predict the behavior of microorganisms. The study of Schvartzman et al. (2011) on the behavior of *Listeria monocytogenes* during manufacture and ripening of smeared cheese made with pasteurised or raw milk, clearly highlighted how challenge tests and predictive modelling are complementary tools. In fact, when predictive models are used, usually common environmental parameters are sometimes sufficient to predict the behaviour of the microorganism investigated in the food matrix. However, sometimes other parameters, not yet accounted for in the models, make the observed behaviour different from the model predictions. Thus, when studying a product, challenge tests are an important preliminary step if no qualitative knowledge on the behaviour (growth, inactivation, and survival) of a microorganism in the product is available in the scientific literature. However, proper use of predictive modelling requires personnel with enough knowledge and expertise and thus resources should be allocated for this purpose.

Overall, the performance of the cold chain is very important in assuring product quality and safety. The mean refrigerators' temperatures at consumers' home can be in the range of 8 to 10°C (James et al., 2008). Moreover, consumers do not always respect the storage time as indicated on the shelf-life label of food; thus, an abuse of storage time can also occur at consumer level. Under article 3 of Regulation (EC) 2073/2005, FBOs are obliged to ensure that food safety criteria applicable throughout the shelf-life of products can be met under reasonably conditions of distribution, storage and use.

Usually, for 'safety assessment' of food, it is suggested (EURL-*Lm*) to mimic the worst case scenario and thus temperatures between 12 to 14°C are used (as it was done in the case-study on challenge testing and durability testing in cheese, Chapter 4). However, taking 12°C might be overall conservative and lead to 'unrealistic' results. Moreover, concerning the storage time, consider the time to consumption as the end of shelf-life might be overall worst case too and lead to conservative estimates. In fact, according to literature data (Daelman, Jacxsens, Membré, et al., 2013) chilled food is likely to be consumed within a few days of being bought and thus the normal storage time as being the end of the use-by date might be better considered as 'unreasonably' foreseen storage time. Therefore, this approach, being conservative, could lead to an overestimation of the risks; as consequence

a high amount of food will be considered not fit for consumption with the result of throwing away too much food unneeded.

On the other side, the 'reasonable' temperature abuse could be integrated in shelf-life testing using the 75$^{th}$ percentile of the temperature distribution as recommended by the EURL document on shelf-life studies (EURL-Lm, 2014). In fact, when establishing the use-by date of chilled, ready-to-eat food or performing risk assessment, instead of single-point estimates it is preferable to use distributions, which characterize the full range of potential values and their likelihood of occurrence. However, the existence of a considerable variability in consumers' refrigerator temperatures among European countries, asks to apply different temperature storage conditions according to the country where the product is intended to be sold. This kind of approach could be laborious and time consuming.

In chapter 5, a general rule able to describe, in terms of probability density distributions the domestic refrigerator temperature for northern and southern European countries and the storage times of chilled food with long or short use-by-date has been provided.

Therefore, in the absence of data, which collection requires allocation of resources, these simple rules could be suggested, at least for the European market and for chilled food products:

- Normal (7.0, 2.7)°C for southern countries and Normal (6.1, 2.8)°C for northern countries for temperature in a consumer's refrigerator;
- Exponential (use-by-date divided by 4) for the time spent at a consumer's home.

The suggested rules could be used in order to incorporate the storage time and temperature in shelf-life studies and food safety risk assessment.

The proposed distributions were used in a case study applied to paté and *L. monocytogenes* (Membré, 2013). When performing shelf-life assessment using single point estimates *L. monocytogenes* grew above the limit of 100 CFU/g after 12 days of shelf-life while when using the 75$^{th}$ percentile of the domestic refrigerator temperature distribution, the product reached the end of shelf-life (19 days) without exceeding the limit of 100 CFU/g. In case of risk assessment, the use of a deterministic method showed that 9.05% of samples of paté contained 2 log CFU/g or more when consumed while if using stochastic distributions, 0.50% of products contained 2 log CFU/g or more when consumed.

Recently a quantitative microbiological risk assessment (QMRA) on *L. monocytogenes* in ready to eat foods was elaborated by EFSA (https://www.efsa.europa.eu/en/consultations/call/170724-0) (EFSA Panel on Biological Hazards (BIOHAZ), n.d.). This QMRA pointed out that among the different factors impacting

on estimated risk in different food categories, storage temperature (especially the domestic refrigerator temperature, which is highly variable) was the most important factor. In particular, when the mean storage temperature increases from 5.9°C to between 9 and 10°C, the risk of listeriosis is doubled. Moreover, based on the quantitative risk characterisation of *L. monocytogenes* in various RTE food categories, the food subcategory associated with the largest number of cases per year was cooked meat (863 cases), followed by sausage (541 cases), gravad fish (370 cases), cold-smoked fish (358 cases), pâté (158 cases), soft and semi-soft cheese (19 cases) and hot-smoked fish (7 cases). The QMRA model included data on the prevalence and concentration of *L. monocytogenes* in the food categories “heat-treated meat”, “smoked and gravad fish”, and “soft and semi-soft cheese”, sampled at retail level, mainly at supermarkets and shops, but not at local food markets. Therefore, artisanal food products as the ones considered in this PhD study and namely artisanal raw milk cheeses (from soft to semi-hard) and fermented raw meat products (i.e. salami and soppresse), where *L. monocytogenes* was able to survive or even grow during shelf-life, were not taken up in this survey or included in the EFSA QMRA model. Thus, it is a point of attention to also consider prevalence data on these type of other local artisanal foods, which are indeed sold locally only and in low volumes, in future risk assessment more dedicated to these type of foods, to estimate the risk for listeriosis also for those consumers with preference for buying these type of foods.

### 8.3 Effect of consumer practices in the domestic kitchen on microbial behaviour

As previously described, the farm-to-fork chain is largely covered by safety and quality regulations and food products that reach consumers are mostly compliant and of good quality. However, this final part of the food chain is not covered by any regulations and therefore a potential risk for illness due to negligence during storage and preparation of food can occur and can easily be prevented by consumers themselves.

Among food handling practices, improper cooking and time-temperature abuse are considered (besides cross-contamination) the most common contributory factors in food borne illnesses. As highlighted in the Introduction (Chapter 1), literature data shows that high percentages of consumers undercook meat and think that the visual inspection is the right way to determine the end of the cooking process and judge the doneness of meat products. The required time-temperature combination to eliminate or decrease food-borne pathogens to a level that guarantees food safety is affected by several factors, including the

initial numbers of the pathogen (or the pathogenic strain) present in the food, the heat resistance of the pathogen, the heat transfer rate, the distribution of the pathogen in the food, the composition of the food product and its geometry. Therefore, assessing the effectiveness of any heat treatment is challenging and cannot be theoretically assessed but could profit from experimental studies.

In Chapter 6 of the present PhD research, the effect of domestic-style cooking on the residual presence and numbers of *Salmonella* Typhimurium in different types of poultry-based meat preparations has been investigated. From the results obtained it was noted that cooking food products applying shorter cooking times compared to the ones recommended on the label and using visual inspection of the internal color as a tool to assess the doneness of the meat, is not a reliable method to guarantee its microbiological safety. In particular, if high initial levels (i.e. 1,000 CFU/g) of pathogen contamination are present in the food product, residual *Salmonella* Typhimurium cells were still detected and enumerated in several samples even at the end of some cooking treatments performed according to label's instructions. However, besides the above-mentioned result, the consideration could be made about the frequency of finding such elevated numbers of *Salmonella* in poultry meat intended to be cooked before consumption. Usually the available data of *Salmonella* detection are expressed as presence/absence per 25 gram instead of being quantitatively expressed as CFU per gram. The presence of *Salmonella* in poultry meat is reported on a regular basis as shown in chapters 6 and 7 and at EU-level the prevalence of *Salmonella*-positive broiler flocks of *Gallus gallus* was 23.7% according to the baseline survey conducted in 2005-2006 (EFSA, 2007). On the other side, in order to have data on the (usually low) numbers of *Salmonella* in food, the MPN method should be applied, as it has been done in chapter 4 of the present study (for the enumeration of *L. monocytogenes* in naturally contaminated cheese) and in chapters 6 and 7 (for the enumeration of *Salmonella* in poultry-based meat preparations). However, performing MPN is time consuming and cumbersome and therefore it is usually not done on a 'routine basis'.

Still, considering that effective cooking treatments at consumer level is an issue, resources and efforts should be allocated in order to provide to the consumer clear and safe cooking instructions, which have to take into account the wide range of conditions in which meat and meat preparations will actually be prepared in the kitchen and expected to be (thoroughly or not) heat-treated. Therefore, the scientific community should make available to the food industries the needed scientific information in order to allow FBO to provide clear

and science-based cooking instructions on food labels, thus giving consumers a proper risk management tool. At the same time, consumers have the responsibility, if they want to limit the risk of food borne infection, to follow the recommended temperatures and use thermometers to measure the final temperature of food after cooking. However, very few consumers (especially in EU) use a thermometer to assess the doneness of food and many consumers do like to have their meat not 'well-done' for sensorial reasons. Still, many consumers are probably not realizing that some meat types, such as poultry meat preparations, are quite amenable for pathogen contamination, if not by *Salmonella* spp., then by *Campylobacter* spp. (a pathogen which was not in the scope of the present PhD) (Habib, Sampers, Uyttendaele, Berkvens, & De Zutter, 2008).

Educational efforts, initiated by either FBO or CA, should aim to develop consumer's consciousness of the importance of a proper cooking treatment in order to eliminate or reduce microbiological hazards to acceptable levels. Compliance with label's instructions and measurement of the internal temperature of food are the main tools to use in order to assess correctly the readiness for consumption and the safety of a food product. However, considering that many consumers think that thermometers are inconvenient and difficult to use, when performing educational campaigns it might be recommended to also include information on how to use visual indicators of adequate cooking. At the same time, the absence per 25 g of *Salmonella* and other food-borne pathogens in food may be of interest to maintain in case of consumer's culinary preferences for consuming meat or meat preparations not well-done (or even raw).

Another consumer's practice, which can heavily affect the safety of a food product, is the storage temperature. As shown in chapter 5, the majority of domestic refrigerators are running at higher than the recommended temperature and the temperature can vary according to the internal position measured. Moreover, consumers are not familiar with the concept of temperature abuse. In fact as reported in the introduction (Chapter 1), high percentages of consumers do not know the recommended refrigerator temperature, most of them transport the food items purchased at retail stores in the car trunk and rarely use an insulated bag to maintain the temperature when carrying meat or perishable food at home. In addition, when thawing food, the first choice is the kitchen countertop, followed by the refrigerator.

How such time-temperature fluctuating conditions affect the behavior of *Salmonella*, one of the main causative agent of food-borne outbreaks in the EU, has been investigated

in chapter 7. The temperature abuse of 25°C for 2 h on poultry-based meat preparation before refrigerated storage did not have any effect on the presence and numbers of *Salmonella* in either the naturally or the artificially contaminated food. This result is probably explained by the fact that the applied time of 2 h was still in the microorganism's lag phase and thus growth of *Salmonella* was not observed. Further, among the different refrigeration's temperatures tested (i.e. 4, 8 and 12°C), storage at 12°C facilitated a significant increase of *Salmonella* Typhimurium in artificially contaminated kebabs (10 CFU/g), thus confirming that improper refrigeration temperatures can have a substantial effect on the growth of the pathogen. With regard to the thawing method, it was highlighted that thawing overnight in the refrigerator (8°C) was effective in order to, if not reducing, at least maintain the level of contamination of *Salmonella* Typhimurium in burgers, sausages and kebabs. On the other side, when thawing overnight on the kitchen countertop (23°C), among the different types of tested poultry-based meat preparations (i.e. burgers, sausages and kebabs) significant increases of *Salmonella* Typhimurium numbers were recorded only in kebabs.

It is to be noted, that in Chapter 7, among the different time-temperature conditions and the poultry-based meat preparations tested, the growth of *Salmonella* was reported only in the artificially contaminated samples and not in the naturally contaminated ones. Using artificially contaminated foods, results of growth, and survival noted should be reflected upon and conclusions taken with care. The experiments used an 'abuse' temperature of 12°C (representing a 'worst-case scenario') and the artificial inoculation (using cells without prior 'stress') may have overestimated the survival and growth of the pathogen. With natural contaminated samples, the exact levels and history of the pathogen contamination is not controlled which make results also difficult to interpret. When performing studies with 'naturally' contaminated samples, the results could be more variable and unpredictable due to the these factors including also the non-homogenous distribution of the contamination. However, it is preferable to work with naturally contaminated food in order to reflect the actual situation occurring.

Still taken all into account, it cannot be ignored that, in particular for some meat types (kebabs in the present study), there was an issue of surviving *Salmonella* cells, and thus there is indeed a need for strategies in order to improve and reinforce simple messages as 'keep it cool', 'cook it well' (WHO, 2006). There is still space for improvement in order to make these educational campaigns more effective.

SafeConsume, a European project funded by Horizon2020 and coordinated by Nofima in Norway was recently initiated and aims to reduce health burden from food-borne illnesses through the change of consumers' behavior (http://safeconsume.eu/the-project). In order to meet that aim, an interdisciplinary team of natural scientists, technologists, microbiologists, sociologists, architects and designers are working together and will develop effective and convenient tools and products, information strategies, education and inclusive food safety policy in order to make it easier for people to do things correctly in the kitchen.

Thus, also here, there is the need of a collaborative effort, this time between 'food science' experts and 'communication experts'. The firsts can provide further data on consumer's behaviour that can be used by the latter to develop more tailored-made educational campaigns able to deliver the right message and thus providing the basis for behavioral changes of consumers.

Still, many of the educational programs are directed towards general population while the particular groups at risk (e.g. young, old, pregnant and immunocompromised) that need to be reached by the message might be missed. In addition, it has been shown that educational programs on food safety and hygiene matters since primary schools can be useful in order to increase the children's awareness on food related risks, leading to significant benefit for primary prevention (Losasso et al., 2014).

The importance to build and deliver a highly targeted message according to the different at risk consumer's groups is clearly highlighted by human food-borne listeriosis. In fact, it is reported that the notification rates of invasive listeriosis in the EU generally increase with increasing age, with the highest incidence rates observed in the age groups over 65 years old and in children below 1 year of age (mainly pregnancy-related cases). In addition to old age and increased susceptibility due to underlying conditions, medical practices and medications have been hypothesised as risk factors for human listeriosis (https://www.efsa.europa.eu/en/consultations/call/170724-0).

The food handling practices of the older population and other vulnerable groups are of particular interest. In fact, the study of Evans & Redmond (2015) on older adults' domestic kitchen storage practices in the United Kingdom, concluded that many older adults fail to adhere to recommended practices on the respect of use-by date and on refrigeration and storage practices, and this may increase the risks associated with listeriosis. Such data would inform targeted food safety education to improve food handling and storage practices among older adult consumers to reduce the risk factors associated with listeriosis in the domestic kitchen.

More recently, the importance to have separated campaigns addressing, besides the broad term risk group 'YOPI' (young, old, pregnant and immunocompromised), other 'vulnerable' groups[3] have been up taken by several European governmental food safety agencies. In fact, the Food Standard Agency (FSA) has provided a guidance for healthcare and social care organizations that prepare and supply food for people vulnerable to listeriosis (Food Standards Agency, 2016). The Belgian health council provided to the Belgian competent authorities specific recommendations regarding the risk-communication about listeriosis to some specific vulnerable groups (other than pregnant woman) (Hoge_Gezondheidsraad, 2016). Furthermore, educational campaigns targeting specific 'at risk' groups (e.g. cancer patients; adults above 65 years old) are becoming more frequent and several examples are reported in the Belgian health council recommendation.

As such, it is increasingly taken into consideration that groups at risk for contracting a food-borne infection are subjected to change because of changing composition of the European population in which, socio-economic and cultural trends are an important determinant for the definition of vulnerable groups. Therefore, the scientific experts and the competent authority (in the shape of public food safety agencies) have to identify these new risk groups in society and develop a targeted strategy to reach these groups. In this perspective, it is important to keep the educational background updated and be ready to extend the branches of knowledge especially towards assistive technology that can implement effective communication strategies. Finally, the efficacy of the educational campaigns and of other mitigation tools should be assessed in order to evaluate the effectiveness of any intervention strategies and improve the new campaigns on food safety promoted by governments.

## 8.4. Overall conclusions and recommendations

The development of a simplified FSMS for **small-scale FBOs**, based on the application of PRPs and the identification of few effective control points, which can be managed by the application of simple but science-based tools as being studied in this PhD, is shown to provide a valuable contribution to food safety within these small-scale artisanal

[3] Vulnerable groups: those individuals whose immune system is weakened in some way and may be more susceptible to developing infection from *L. monocytogenes* (listeriosis) and likely to suffer more severe symptoms. This includes but is not limited to cancer patients, patients undergoing immunosuppressive or cytotoxic treatment, unborn and newly delivered infants, pregnant women, people with diabetes, alcoholics (including those with alcoholic liver disease) and a variety of other conditions. Immune system capacity decreases progressively in the elderly, so elderly individuals are also included in this group (FSA, 2016).

food products production. In this perspective, the development of a cooperative approach between FBO and CA coupled with the support of food science skilled personnel is recommended. It leads to increase of food hygiene knowledge of FBO and the improvement of official controls of the CA, which could be seen not only as a punitive body but also as an important source of information and comparison.

It can also be concluded that **consumers** can heavily contribute to at home food safety. The effect of consumer's practices, as the ones investigated in our study (i.e. cooking and temperature abuse), on microbial behaviour have been clearly highlighted. Improper cooking and storage practices can allow food-borne pathogens as *Salmonella* to grow and reach levels, which can provoke food-borne infection. Informative and educative tools that target different risky behaviours and different at risk groups to create awareness about food safety at home should be set up. It is recommended that the delivered message is tailor-made in order to be taken up effectively. Therefore, an interdisciplinary approach is required where natural and social sciences are combined in order to develop a strategy able not only to increase the level of consumer's knowledge of food handling practices but also to affect the consumer's attitudes and behaviors in the domestic environment.

Finally, **competent authorities** based on the scenario in which they are operating determine the appropriate level of protection (ALOP) for public health and consequently affect the food safety objective that FBOs have to meet. However, governments do not only decide on what is or is not considered acceptable in terms of food safety but also have a task to provide support (e.g. tools, guidelines or information) and motivate both producers and consumers to meet and accept these objectives.

In addition, this PhD showed that **continuous work needs to be put in food safety assessment** and control as consumer preferences towards food are subjected to change (e.g. artisanal food). Therefore, the evolution of the market scenario and thus the typology of both consumer and FBO requires a continuous investment. At the same time, the CA has to be ready and prepared to follow this evolution. Nowadays, the EU legislation has put several food safety rules in place. However, the EU having a rich variety of foods and many regional specialties being part of the local 'food culture' also counts many small-scale companies, especially in rural areas, and this is an important income for these regions (along with tourism). Therefore, to keep these small-scale producers of artisanal food, but still ensuring food safety, guidelines and tools provided to improve food safety should be kept

simple as much as possible in order to be effectively up taken. Moreover, the controls on food safety performed by the CA should be not only a mere inspection of the compliance of food products and food safety management systems with EU regulations but also an opportunity of collaboration between FBO and CA. The interaction between these actors in the farm to fork continuum and the awareness of their role and responsibility in food safety can modulate the impact of consumer preferences and behaviour in microbial food safety assessment.

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# CURRICULUM VITAE

Anna Roccato is born in Rovigo (Italy) on the 25th of July 1979. She obtained her bachelor degree in veterinary medicine in 2007 at the University of Padova, discussing the thesis entitled “Evaluation of the managerial, structural and sanitary health characteristics of turkey farms in the densely populated avian areas of Veneto region”.

After the bachelor degree, from 2007 to 2008 she worked in the clinical medicine of pets. Afterwards, from February 2008 to March 2010 she obtained a scholarship at the Italian public health institute “Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe)” where she was involved in the research areas of surveillance of wildlife diseases and control of straying. In July 2010, she started to work as a scholarship holder at the Risk Analysis and Public Health Department of IZSVe (headed by dr. Antonia Ricci), focusing on food safety and food microbiology. In 2012, she followed the “International Training Programme on Food Safety, Quality Assurance Systems and Risk Analysis” at the department of food safety and food quality, faculty of bio-science engineering of Ghent University.

In 2013, she obtained, at the University of Padova, the specialization in “Animal health, breeding and livestock production” with a thesis on “*Salmonella* Enteritidis in laying hens of Veneto region: state of the art of cleaning and disinfection of sheds”. Still in 2013, she started the PhD at UGent on the “Impact of consumer preferences and behaviour in microbial food safety assessment” under the guidance of Prof. dr. ir. Mieke Uyttendaele and the approval of Dr. Antonia Ricci (co-promotor). During that period, she hold a research position at the IZSVe and obtained funding by the UGent promotor for a short research period at Ghent university. Moreover, in 2016 she took a short research period at ONIRIS under the supervision of Dr. Jeanne-Marie Membrè.

During the research, she participated in various national and international conferences and published in international peer reviewed journals. In the framework of this PhD research, she also cooperated with several food business operators and food companies for the collection of data.

## Publications in A1 peer-reviewed journals

**Roccato, A**., Uyttendaele, M., Membré, J. M. (2017). Analysis of domestic refrigerator temperatures and home storage time distributions for shelf-life studies and food safety risk assessment. Food Research International 96: 171–181.

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**Roccato A**., Uyttendaele, M., Cibin, V., Barrucci, F., Cappa, V., Zavagnin, P., Longo, A., 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.

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## Abstract or posters on symposia

**Roccato, A**., Barco, L., Patuzzi, I., Cristaudo, I., Ferrarini, S., Crema, M., Comin, D., Mioni, R., Ricci, A. (2016). Investigation of microbiological hazards and process hygiene indicators of street foods in Italy: preliminary results. *Poster presentation*. Foodmicro 2016. One health meets food microbiology, 19 - 22 July, Dublin, Ireland. Abstract book, page 545.

**Roccato, A.**, Cibin, V., Barrucci, F., Favretti, M., Piovesana, A., Pezzuto, A., Dal Cin, M., De Rui, S., Ricci, A. (2015). Risk-based control options to mitigate microbial risks associated to traditional fermented Italian salami and soppresse consumption. *Poster presentation*. Shaping the Future of Food Safety, Together, 14 – 16 October, Milan, Italy. Proceedings of the 2nd EFSA Scientific Conference. EFSA Journal 2015, 13(10):s1310, 137 pp., page 112.

**Roccato, A**., Barco, L., Lettini, A. A., De Pasquale, F., Ramon, E., Dalla Pozza, M. C., Sillo, S., Ricci, A. (2015). Isolation and characterization of *Yersinia Enterocolitica* from vegetables and pork meat. *Poster presentation*. XVI Congresso Nazionale S.I.Di.L.V, 30 of September - 2 of October, Montesilvano (PE), Italy. Abstract book, pages 300-301.

**Roccato, A**., Uyttendaele, M., Barrucci, F., Cibin, V., Favretti, M., Piovesana, A., Pezzuto, A., Dal Cin, M., De Rui, S., Ricci, A. (2015). Artisanal Italian Salami and Soppresse: Identification of Control Strategies to Manage Microbiological Risks. *Poster presentation*. IAFP Annual Meeting, 25-28 July, Portland, Oregon (U.S.). Journal of Food Protection, Supplement A, Volume 78, page 183.

**Roccato, A**., Uyttendaele, M., Cibin, V., Barrucci, F., Cappa, V., Zavagnin, P., Longo, A., Ricci, A. (2015). Domestic Consumer-style Storage and Thawing Practices: Effect on *Salmonella* in poultry-based Meat Preparations. *Poster presentation*. IAFP Annual Meeting, 25-28 July, Portland, Oregon (U.S.). Journal of Food Protection, Supplement A, Volume 78, page 184.

**Roccato, A**., Uyttendaele, M., Barco, L., Cibin, V., Barrucci, F., Patuzzi, I., Ricci, A. (2015). Domestic Handling of Chicken Carcasses: Quantification of *Campylobacter* Species Cross-contamination. *Oral presentation*. IAFP Annual Meeting, 25-28 July, Portland, Oregon (U.S.). Journal of Food Protection, Supplement A, Volume 78, page 62.

**Roccato, A**., Cibin, V., Zavagnin, P., Longo, A., Cappa, V., Ricci, A. (2012). Consumer's role in food safety: effect of storage and cooking on *Salmonella* spp. level in poultry meat food products. *Poster presentation*. 23rd International ICFMH Symposium, FoodMicro2012. Global Issues in Food Microbiology. Istanbul, 3-7 September. Abstract book, page 451.

Favretti, M., Pezzuto, A., **Roccato, A**., Piovesan, A., Cereser, A. Development of a simplified own check system for food micro-enterprises based on good hygiene practices. *Poster presentation*. 23rd International ICFMH Symposium, FoodMicro2012. Global Issues in Food Microbiology. Istanbul, 3-7 September 2012. Abstract book, page 452.

Cibin, V., **Roccato, A**., Ruffa, M., Barrucci, F., Ricci, A. (2012). Temperatures of Italian consumer's domestic refrigerators and factors affecting performances. *Poster presentation*. 23rd International ICFMH Symposium, FoodMicro2012. Global Issues in Food Microbiology. Istanbul, 3-7 September. Abstract book, page 467.

## Dissemination

**Roccato, A.** (2017). Predictive microbiology: general principles and practical cases. *Invited speaker (oral presentation of 30 mins)*. Predict and verify. Mathematical models and other innovative tools for prediction and verification of food safety, May 25th, Padova, Italy

**Roccato, A.** (2016). Latteria cheese: from production to consumption. Bacterial population and risk for the consumer. *Invited speaker (oral presentation of 30 mins)*. The production of raw milk Latteria cheese: results of monitoring in dairy factories of Friuli Venezia Giulia region. ERSA and AAFVG, November 12th, Gemona del Friuli, Italy.

**Roccato, A.** (2014). Traditional fermented Italian sausages: identification of control strategies to manage microbiological risks. *Invited speaker (oral presentation of 30 mins*). IAFP European Symposium on food safety, May 8th, Budapest, Hungary.

**Roccato, A.** (2013). Survey conducted in Italy on the consumer refrigeration temperatures, its impact on food safety illustrated with *Salmonella*. *Invited speaker (oral presentation of 30 mins*). IAFP European symposium on food safety, May 16th, Marseille, France.

## Doctoral schools

February/May 2015: Advanced Academic English: Writing Skills (Transferable skills, cluster Communication skills)

5-19 June 2014: Project management (Transferable skills, cluster Career management)

## Supervision of undergraduate students

Sara Sillo (Master dissertation: “Presence of potentially pathogenic *Yersinia enterocolitica* in raw and ready-to-eat vegetables”, University of Padova, Italy)

Maddalena Furlan (Master dissertation: “Food safety in the domestic environment: *Campylobacter* cross-contamination during handling of chicken in the kitchen”, University of Padova, Italy).

Rosanna Riello (Master dissertation: “Microbiological and structural aspects of shepherd's huts of Veneto region (Italy): analysis of monitoring plans 2012-2015”, University of Padova, Italy).