



## **EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on public health risks represented by certain composite products containing food of animal origin**

### **EFSA Publication**

*Link to article, DOI:*  
[10.2903/j.efsa.2012.2662](https://doi.org/10.2903/j.efsa.2012.2662)

*Publication date:*  
2012

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
EFSA Publication (2012). *EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on public health risks represented by certain composite products containing food of animal origin*. European Food Safety Authority. the EFSA Journal Vol. 10(5) No. 2662 <https://doi.org/10.2903/j.efsa.2012.2662>

---

#### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## SCIENTIFIC OPINION

### Scientific Opinion on Public health risks represented by certain composite products containing food of animal origin<sup>1</sup>

EFSA Panel on Biological Hazards (BIOHAZ)<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

This Opinion reviews the factors that affect microbial survival and growth in composite products, and in foods in general. It concludes that the main factors to be considered are: water activity, pH, temperature and duration of storage, processing, and intensity and duration of other non-thermal physical processes applied. Prevalence and concentration of the pathogens in food are important to determine the risk for consumers. The opinion presents a review of the quantitative microbiology models and databases that can be used to provide quantitative estimations of the impact of the above factors on the survival and growth of the main bacterial pathogens. In composite products, migration and diffusion of moisture and substances among the ingredients may change their physico-chemical parameters, particularly at the interfaces. Therefore, the assessment of the risk posed by composite products needs to consider the combinations of parameters most permissive to survival and growth of pathogens. Two complementary approaches are proposed for the identification and profiling of microbiological hazards in different specific composite products. The first one is based on past outbreaks and prevalence of hazards in the products and leads to the conclusion that the most frequent hazard-composite product combinations are *Salmonella* in cakes and bakery products. The second one consists in decision tools based on the impact on the pathogens of food composition and food processing. Categorisation of the risk for composite products requires information on their composition, processing and further handling, which can largely differ for foods belonging to the same category. Further conditions may influence the risk and should be verified, i.e. hygienic conditions during preparation of the composite products and their ingredients, shelf-life conditions, and reliability of cooking by consumers to inactivate pathogens. The decision tools developed apply to all composite products considered by the mandate, as well as to all other foods.

© European Food Safety Authority, 2012

#### KEY WORDS

Composite product, food-borne pathogens.

<sup>1</sup> On request from the European Commission, Question No EFSA-Q-2011-00235, adopted on 19 April 2012.

<sup>2</sup> Panel members: Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Havelaar, James Hope, Günter Klein, Kostas Koutsoumanis, James McLauchlin, Christine Müller-Graf, Christophe Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, John Threlfall, Ivar Vågsholm and Emmanuel Vanopdenbosch. Correspondence: biohaz@efsa.europa.eu

<sup>3</sup> Acknowledgement: The Panel wishes to thank the members of the Working Group on Public health risks represented by certain composite products: Olivier Cerf, Kostas Koutsoumanis, Christophe Nguyen-The, John Sofos, Antonio Valero and Marcel Zwietering for the preparatory work on this scientific opinion.

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on public health risks represented by certain composite products containing food of animal origin. EFSA Journal 2012;10(5):2662. [132 pp.] doi:10.2903/j.efsa.2012.2662. Available online: [www.efsa.europa.eu/efsajournal](http://www.efsa.europa.eu/efsajournal)

## SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ) was asked to deliver a Scientific Opinion on public health risks represented by certain composite products containing food of animal origin.

Currently, imports into the EU of composite products are subject to specific rules in relation to both public health and animal health aspects. Some derogations currently apply to those rules, and there is a need for the European Commission to develop harmonised risk-based public health rules for import of those composite products for which controls are not currently prescribed. Therefore, the European Commission requested EFSA to recommend/identify physico-chemical parameters for certain composite products containing no meat and/or less than 50% of products of animal origin that could be relevant for the growth/survival of pathogenic microorganisms of public health importance, so to assist the risk manager on deciding to carry out risk based controls. In addition, EFSA was requested to identify and profile the microbiological hazards for public health related to import of certain specific composite products.

The Opinion reviews the factors that affect microbial survival and growth in composite products, and in foods in general. It concludes that the main ones to be considered are: water activity ( $a_w$ ), pH, temperature and duration of storage, processing, and intensity and duration of other non-thermal physical processes applied. In general, foods with  $a_w$  below 0.88 or pH below 3.7 or stored frozen do not permit growth of or toxin formation by food-borne pathogenic bacteria. Considering only pathogens for which growth in food is usually required to cause illness, growth would occur only at pH above 4.3. In the case of heat-treated food without possibilities for recontamination, only spore-forming bacteria are considered, and thus growth would only occur in foods with  $a_w$  above 0.92 or pH above 4.3, or stored at temperatures above 3°C. Examples of the effect of heat treatments in the inactivation of biological hazards in food are provided, together with the consideration that combination between factors may be synergistic or antagonistic. The importance of determining at which steps of the food production chain treatments inactivating pathogens are applied is highlighted. The BIOHAZ Panel further indicates that prevalence and concentration of the pathogens in foods, which may be reduced by good hygienic practices, are important in determining the risk for consumers.

Specific estimations of growth and survival depend on the hazards considered. The Opinion presents a review of the quantitative microbiology models and databases that can be used to provide quantitative estimations of the impact of temperature, pH,  $a_w$  and their combinations on the survival and growth of the main bacterial pathogens.

Composite products contain several ingredients with different composition. It is concluded that migration and diffusion of moisture and substances among the ingredients may change their physico-chemical parameters, particularly at the interfaces. Therefore, the assessment of the risk posed by composite products needs to consider the combinations of parameters most permissive to survival and growth of pathogens.

With regard to identifying and profiling microbiological hazards in the different composite products listed in the mandate, the BIOHAZ Panel proposes two different approaches, which are complementary and should be applied in parallel.

The first approach is based on past outbreaks and prevalence of hazards in the composite products. Its application leads to the conclusion that the most frequent hazard-composite product combinations are *Salmonella* in cakes and bakery products. These results are based on the evidence available, and absence of confirmed outbreaks or low prevalence do not preclude potential risks. Most of the data used originate from EU databases and the distribution and prevalence of microbial pathogens in food, as well as sources and frequency of food-borne outbreaks, may differ from the ones related to potential exporting countries of composite products towards the EU.

The second approach consists in decision tools based on the impact on the pathogens of food composition and food processing. Three tools are developed in relation to hazards not needing growth, usually needing growth, and needing growth and toxin production to cause illness. They should be all applied to each composite product of interest, considering the behaviour of the hazard in the most sensitive components or ingredients or their interface in composite products. The decision tools lead to categorisation of risks into *low risk*, *moderate risk* (when inactivation of the hazards is expected by cooking practices by the consumer), and *qualified presumption of risk* (when the pathogens have the potential to cause disease via consumption of the composite product, if present in the food or its ingredients).

Bread, low moisture biscuits/cakes/chocolate, sweets, dry pasta and noodles, food supplements, and unfilled gelatine capsules in general do not permit growth of pathogens and therefore are of *low risk* with regard to hazards that need to grow in food to cause illness. They may pose *moderate risk* or *qualified presumption of risk* with regard to hazards that do not need to grow in food to cause illness. Soup stocks, flavourings, meat extracts, meat concentrates, and sterilised heat-treated foods without possible recontamination are in general of *low risk*. The other composite products considered, such as high moisture biscuits/cakes/chocolate/confectionery, fresh pasta and noodles, and olives with fish, may pose *moderate risk* or *qualified presumption of risk*.

Categorisation of the risk for composite products requires information on their composition, processing and further handling, which can largely differ for foods belonging to the same category. As proposed in the mandate, some of the categories of composite products include foods with different levels of risk (e.g. bakery products, cakes and confectionery), since the composition and processing of some of them may allow for the survival and growth of pathogenic microorganisms while others may not. Further conditions may influence the risk and should be verified, i.e. hygienic conditions during preparation of the composite products and their ingredients, shelf-life conditions, and reliability of cooking by consumers to inactivate pathogens.

The BIOHAZ Panel highlights that the conclusions above and the decision tools developed apply to all composite products considered by the mandate, as well as to all other foods.

## TABLE OF CONTENTS

Abstract .....	1
Summary .....	2
Table of contents .....	4
Background as provided by the European Commission.....	5
Terms of reference as provided by the European Commission.....	5
Assessment .....	7
1. Introduction .....	7
1.1. Legislative framework for imported composite products and impact on this Opinion.....	7
1.2. Composite products and public health biological hazards .....	9
2. Factors impacting on risks in composite products.....	12
3. Modelling of inactivation/growth of pathogenic microorganisms in foods.....	16
3.1. Tools available to predict inactivation/growth of pathogenic microorganisms in food.....	16
3.2. Inactivation data and models of hazards in food.....	18
3.2.1. Inactivation kinetics and primary inactivation models .....	18
3.2.2. Secondary inactivation models .....	21
3.2.3. Survival/inactivation of microorganisms in composite products .....	21
3.2.4. Concluding remarks.....	24
3.3. Growth/no growth models of pathogenic microorganisms in food .....	25
3.3.1. Concluding remarks.....	27
3.4. Growth models of pathogenic microorganisms in food.....	27
4. The dynamic environment of composite products.....	28
5. Identifying microbiological hazards for certain composite products .....	30
5.1. Data from the EU Summary Report.....	30
5.1.1. Prevalence data (2004-2009).....	30
5.1.2. Data and confirmed/verified food-borne outbreaks (2004-2009).....	31
5.2. Data from the Rapid Alert System for Food and Feed.....	33
5.3. Other data available in the literature .....	33
5.3.1. Biscuits, bread, cakes, chocolate, pasta and noodles .....	33
5.3.2. Confectionery (including sweets) .....	35
5.3.3. Gelatine capsules .....	36
5.3.4. Food supplements packaged for the final consumer, containing small amounts of animal product, and those including glucosamine, chondroitin, or chitosan.....	36
5.3.5. Olives with fish.....	36
5.3.6. Meat extracts, meat concentrates, soup stocks and flavourings packaged for final consumer, containing meat extracts, meat concentrates, animal fats, or fish oils, powders or extracts .....	36
5.4. Summary of available data concerning biological hazards-composite products associations.....	38
6. Profiling microbiological hazards for certain composite products .....	42
6.1. Decision trees for categorisation of risk .....	42
6.2. Example of the categorisation of risk for certain composite products.....	47
6.3. Notes and further considerations for Tables 12, 13 and 14.....	51
Conclusions .....	53
References .....	56
Appendices .....	68
A. Calculation of Dose-1% and P1 values reported in Table 1 .....	68
B. Chill chain of refrigerated food: temperature surveys of retail and domestic refrigerators.....	72
C. Mean <i>D</i> -values and <i>z</i> -values for vegetative bacteria and bacterial spores at given temperatures ..	78
D. Modelling of inactivation/growth of pathogenic microorganisms in food .....	81
E. Prevalence data of zoonotic agents in certain composite products in the EU (2004-2009).....	117
F. Confirmed food-borne outbreaks in certain composite products in the EU (2004-2009) .....	125
G. RASFF notifications .....	129
Glossary.....	131

## **BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION**

### Public health rules

Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin states that products of animal origin used in composite products are subject to the rules applicable to foodstuffs of animal origin.

Consequently, such products must come from an EU approved establishment located in an approved third country and they must be controlled at the Border Inspection Points (BIPs).

In order to avoid trade disruption, it was decided to provide for a temporary derogation (until 31/12/2013) from the above mentioned import rules in order to give time to food business operators to adjust to the new system. Composite products are, therefore, still subject to national controls.

After December 2013, if there will be no changes in the current legislation, the importers of composite products will need to comply with the rules specified in Regulation 853/2004 and provide guarantees through appropriate certificates and/or documentation for all composite products entering the Community regardless the quantity of processed food of animal origin they contain.

### Animal health rules

The situation for animal health rules is different. All composite products which are considered to be a risk from an animal health point of view (in general those containing any quantity of meat and those containing 50%, or more, of milk, fish and/or eggs) are covered by Commission Decision 2007/275/EC. However, the latter Decision provides for derogations for a number of composite products or foodstuffs exempting them from veterinary checks. Those products are listed in annex II of this Decision.

### Specific background

Taking into account the above mentioned temporary derogation (31/12/2013), the Commission needs to develop harmonised, risk-based public health rules for import of composite products containing no meat and less than 50% of milk, fish and/or eggs.

## **TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

In view of the above, and in accordance with Article 29(1) of Regulation No 178/2002, EFSA is requested to:

- Recommend/identify physico-chemical parameters for composite products containing no meat and/or less than 50% of products of animal origin that could be relevant for the growth/survival of pathogenic microorganisms of public health importance, taking into account the importance of other factors such as processing conditions, transport and/or storage conditions, and therefore assisting the risk manager on deciding to carry out risk based controls.
- Identify and profile the microbiological hazards for public health related to import of certain composite products containing no meat and/or less than 50% of products of animal origin. In the first instance the following list of products should be assessed:
  - biscuits;
  - bread;
  - cakes;

- chocolate;
- confectionery (including sweets);
- unfilled gelatine capsules;
- food supplements packaged for the final consumer, containing small amounts of animal product, and those including glucosamine, chondroitin, or chitosan;
- meat extracts and meat concentrates;
- olives stuffed with fish;
- pasta and noodles not mixed or filled with meat product;
- soup stocks and flavourings packaged for the final consumer, containing meat extracts, meat concentrates, animal fats, or fish oils, powders or extracts.

#### Clarification of the Terms of Reference

- The composite products to be covered by the current mandate are the ones currently excluded from importing controls at border inspection posts (BIPs) as foreseen by the relevant EU legislation. Products controlled at BIPs include composite products containing:
  - processed meat products, and
  - 50% or more of processed products of animal origin other than processed meat, and
  - less than 50% of processed milk products, unless the final products are shelf stable at ambient temperature or have undergone a complete cooking or heat treatment process throughout their substance, so that any raw product is denatured.

See also Figure 1 of the Opinion for further clarification of the products covered by the mandate.

- “Pathogenic microorganisms of public health importance” cover bacteria, viruses, parasites and fungi, as well as biogenic amines, when having a potential impact on public health. Agents of Transmissible Spongiform Encephalopathies as well as mycotoxins are excluded from the mandate.
- “Processing”, “unprocessed products” and “processed products” are defined by Regulation (EC) No 852/2004<sup>4</sup>. “Food supplements” are defined by Directive 2002/46/EC<sup>5</sup>.

---

<sup>4</sup> OJ L 139, 30.4.2004, p.55-205.

<sup>5</sup> OJ L 183, 12.7.2002, p.51-57.

## ASSESSMENT

### 1. Introduction

#### 1.1. Legislative framework for imported composite products and impact on this Opinion

The following definition of composite product is provided by European Union (EU) legislation: “*a foodstuff intended for human consumption that contains both processed products of animal origin and products of plant origin and includes those where the processing of primary product is an integral part of the production of the final product*” (Decision 2007/275/EC<sup>6</sup>). Definitions of processing, unprocessed products and processed products are also provided by EU legislation (see Glossary).

Currently, imports into the EU of composite products are subject to rules in relation to both public health and animal health aspects, as explained above in the Background of the mandate. As indicated there, the origin of the mandate is the need of the European Commission to develop harmonised risk-based public health rules for import of those composite products for which controls are not currently prescribed by the EU public health or animal health rules.

According to the above definition, in order to be considered a composite product, a food product must include among its components both processed products of animal origin and products, either processed or unprocessed, of plant origin. In order to give a few practical examples, the two following products can be therefore defined as composite products: pizza with raw tomatoes and mozzarella, sandwich with ham and raw/cooked vegetables. On the other hand, a product like sushi, when made of cooked rice and raw fish, or meat skewers, when made of raw meat and raw/cooked vegetables, cannot be considered composite products according to the above definition, since the product of animal origin (i.e. raw fish and raw meat in these cases) would be unprocessed.

The second part of the definition of Decision 2007/275/EC establishes that, in addition to the above products, also the foodstuffs for which processing is performed during the production process of the final product have to be considered composite products. According to that if the meat skewer of the above example (made of raw meat and raw/cooked vegetables) would be cooked and then placed on the market, it should then have to be considered a composite product.

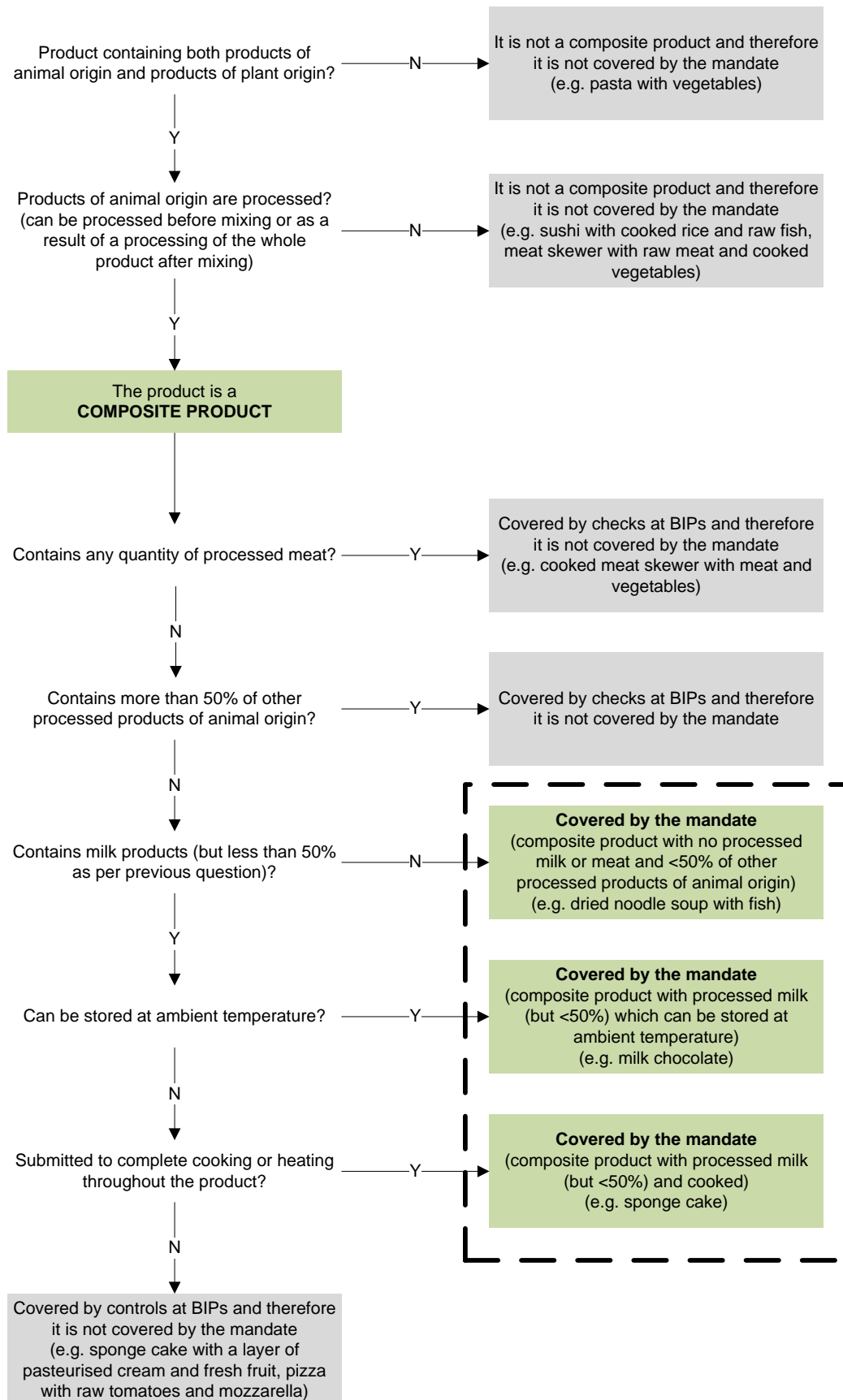
Figure 1 below provides a decision tree guiding through the definition of the composite products covered by the present mandate, including the classification of the products mentioned in the examples above.

Although some infant formulae may correspond to the definition of “composite products”, these are covered by specific EU legislation and therefore not considered in the framework of this mandate.

---

<sup>6</sup> OJ L 116, 4.5.2007, p.9-33.





**Figure 1:** Decision tree indicating composite products covered by the present mandate.

## 1.2. Composite products and public health biological hazards

Hazards which may be present in composite products are those carried by the ingredients as well as those which may contaminate the food during its preparation. Biological hazards from animal, human and environmental reservoir can therefore be present in composite products, representing all the microbial hazards commonly transmitted by foods. This includes, for example: (i) pathogens causing infections (*Salmonella*, food-borne pathogenic *E. coli*, *L. monocytogenes*, *Campylobacter*, food-borne pathogenic *Yersinia*, *C. perfringens*, diarrhoeic *B. cereus*, *C. botulinum* for infant botulism, food-borne viruses, food-transmitted parasites); (ii) pathogens producing toxins in the food (*C. botulinum*, *S. aureus*, emetic *B. cereus*); (iii) components of the food microbiota producing metabolites with negative impact on consumer's health (biogenic amines). However, some hazards have been more frequently associated with some ingredients or with some composite products. This will be further discussed in Chapter 5 for the specific composite products listed in Term of Reference 2 of the mandate for this Scientific Opinion.

Compared to other hazards, parasites in foods have received less attention, given the relatively low incidence in humans, in comparison to bacterial or viral infections. Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the vehicle for transmission of a range of parasites. Parasites that have been associated with vegetable- or fruit-borne outbreaks of infection, rather than individual cases, include the protozoan parasites *Giardia* (Mintz et al., 1993) and helminth parasites *Ascaris* (Raisanen et al., 1985). Fishery parasites are particularly relevant and their presence in composite products depends on the quality of the raw material, i.e. the catching or rearing of stock free from the parasites, the application of physico-chemical treatments to fishery products to ensure killing of any parasites which may be present, or the physical separation of parasite contaminated fishery products during processing. The main syndromes related to anisakiosis (*Anisakis simplex*) are allergic urticaria and anaphylactoid syndromes together with gastrointestinal infection. Furthermore, reports from Spain and from other countries (Alonso et al., 1997; Daschner et al., 1998; Foti et al., 2006; Pecquet et al., 2002) described syndromes combining simultaneous allergy and infection.

Growth or survival of bacteria in foods does not have the same impact on public health for all these hazards. For a given number of foods at consumption, some hazards have a higher probability to cause illness than others. In practice, this means that some hazards must grow in the food, or its ingredients, before consumption to reach numbers sufficient for a significant probability of causing illness. For other hazards, the numbers resulting from the initial contamination of the ingredient or from contamination during food handling are usually sufficient to cause illness. Table 1 classifies the main food-borne pathogens accordingly and provides examples of Dose-1% and P1 values (see Glossary). The values reported have been calculated based on data described in selected published risk assessments and scientific papers and a certain variation of the estimates is expected if calculating those values based on different experimental data.

Some bacterial hazards cause food-borne illness only after production of toxins in the foods, which also requires growth before food consumption. Similarly, biogenic amines result from growth of some components of the food microbiota.

Hazards do not have the same ability to survive or grow in foods. Food-borne viruses and parasites do not multiply in foods. The impact of the physico-chemical conditions of foods on growth and survival of food-borne pathogenic bacteria will be discussed in Chapter 2. Food-borne pathogenic bacteria producing spores (*C. botulinum*, *C. perfringens* and *B. cereus*) survive better under conditions prevailing in food and during food processing, compared to other food-borne vegetative pathogenic bacteria. Some bacterial toxins produced in foods are relatively resistant to food processing treatments (*S. aureus* enterotoxins, emetic toxin of *B. cereus*), as well as biogenic amines, while the botulinum toxin is relatively less heat stable.

As discussed in the EFSA Scientific Opinion on risk assessment of parasites in fishery products (EFSA Panel on Biological Hazards (BIOHAZ), 2010) allergenic compounds are highly resistant to heat and freezing (de Corres et al., 1996; Falcao et al., 2008). Therefore, treatments that kill *anisakidae* in fishery products may not protect the consumers against allergic reaction following consumption.

The consequences of infection or intoxication on consumer's health are not the same for all the hazards transmitted by foods. The proportion of deaths attributed to major pathogens in the EU between 2006 and 2010 (EFSA, 2007a, 2009, 2010, 2011a, 2012) are:

- 17-20% for *L. monocytogenes*;
- 0-5% for *C. botulinum* depending on the year (2.5% on average over the considered period);
- 0.1-0.2% for each *Salmonella*, *Campylobacter* spp., *S. aureus*, *Clostridium* spp. other than *C. botulinum*, and *Yersinia* spp.;
- less than 0.1% for pathogenic *E. coli* and *Trichinella*;
- no reported death for *Bacillus* spp. and viruses.

Illness due to some pathogens, such as *C. botulinum* intoxication, require long and intensive care, while severe cases may cause death of the patient. In France, between 2007 and 2009, 25% of diseased persons received artificial ventilation for up to 5 months (Mazuet et al., 2011). In the USA, the proportion of death among food-borne botulism cases was higher than in the EU, with an average of 6% fatality between 2001 and 2010 (from 0 to 25% depending on the year)<sup>7</sup>. Some pathogenic *E. coli* (EHEC) may cause the very severe haemolytic uremic syndrome (HUS) (EFSA, 2007b) and death, but this varies a lot with the strain. For instance, although no outbreaks caused by pathogenic *E. coli* led to death in the EU between 2006 and 2008, an O157:H7 strain in the USA in 2006 caused an outbreak with 13% HUS and 2.1% death (MMWR, 2009b). In 2011, in Germany, an O104:H4 strain caused an outbreak resulting in 27% HUS and 1.5% death (EFSA, 2011b).

---

<sup>7</sup> [www.cdc.gov/nationalsurveillance/botulism\\_surveillance.html](http://www.cdc.gov/nationalsurveillance/botulism_surveillance.html) (accessed on 19 April 2012)

**Table 1:** Examples of hazards classified according to the need of growth in foods for a significant probability of illness, with examples of Dose-1%<sup>§</sup> and P1<sup>¥</sup> values. Details on how Dose-1% and P1 values were calculated are included in Appendix A.

Pathogens		Population exposure	Dose-1% value <sup>§</sup>	P1 value <sup>¥</sup>	References
Illness may occur without growth of hazards in the food	Norovirus	no growth in food is possible highly infectious (probability of infection for a single particle of 0.5)			(Teunis et al., 2008)
	Parasites	no growth in food is possible ingestion of a few parasites may cause infection			
	<i>Salmonella</i> (salmonellosis)	any exposed people	4.1	$2.5 \cdot 10^{-3}$	(FAO/WHO, 2002)
	<i>Shigella</i>	any exposed people	8.8	$1.2 \cdot 10^{-3}$	(Cassin et al., 1998)
	<i>Campylobacter jejuni</i> (diarrheal disease)	adults	2.9	$3.5 \cdot 10^{-3}$	(FAO/WHO, 2009)
	EHEC (e.g. <i>E. coli</i> O157) (haemolytic uremic syndrome)	children < 6 years	8.4	$1.2 \cdot 10^{-3}$	(Delignette-Muller and Cornu, 2008)
		children 6-10 years	41.9	$2.4 \cdot 10^{-4}$	
<i>Yersinia enterocolitica</i>	no dose-response model available involved in water-borne infections and growth in water does not seem possible			(Eden et al., 1977; Han et al., 2003; Keet, 1974; Lund, 1996; Ostroff et al., 1994; Ramalho et al., 2001; Thompson and Gravel, 1986)	
Growth of hazards in the food is usually required to cause illness	<i>Listeria monocytogenes</i> (severe listeriosis)	more susceptible sub-population	$9.5 \cdot 10^9$	$1.1 \cdot 10^{-12}$	(FAO/WHO, 2004)
		less susceptible sub-population	$4.2 \cdot 10^{11}$	$2.4 \cdot 10^{-14}$	
	<i>Vibrio parahaemolyticus</i> (enterocolitis)	adults	$2.2 \cdot 10^4$	$4.6 \cdot 10^{-7}$	FDA <sup>8</sup>
	<i>Clostridium perfringens</i>		$1.5 \cdot 10^6$	$6.9 \cdot 10^{-9}$	(Golden et al., 2009; Jaloustre, 2011)
	<i>Bacillus cereus</i> (diarrhoeic)	no dose-response model available at least $10^5$ - $10^6$ cells per serving of foods causing illness			(EFSA, 2005)
Growth of hazards in the food is required for production of toxins or toxic metabolites that cause illness	<i>Clostridium botulinum</i>	no data available			
	<i>Staphylococcus aureus</i>				
	<i>Bacillus cereus</i> (emetic)				
	Bacteria producing biogenic amines				

<sup>§</sup>: Dose-1% value: estimated dose (total number of cells) that causes a certain effect in 1% of the individuals exposed.

<sup>¥</sup>: P1 value: estimated probability of a certain effect when the ingested dose is represented by one cell.

<sup>8</sup> [www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm185499.htm](http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm185499.htm) (accessed on 19 April 2012)

## 2. Factors impacting on risks in composite products

In general, survival, growth and multiplication of microorganisms in food depend on various factors which may be classified simply into those that are intrinsic or associated with the food material and those that are extrinsic or associated with the environment surrounding the food (Jay, 2000; Ray, 2004). In further considering these factors, Mossel et al. (1995), divided them even further, as follows: (i) intrinsic factors, including nutrients, water activity ( $a_w$ ), pH and buffering capacity, oxidation-reduction (redox) potential (Eh) and redox poisoning capacity, antimicrobial substances naturally present in foods, and biological intrinsic factors associated with the tissue structure of foods; (ii) processing factors, including physical treatments resulting in death such as heating and irradiation, as well as adding chemicals and changes in pH with addition of acids or generation of fermentation products, adding antimicrobials, and changes associated with microbial count increases from environmental contamination during processing; (iii) extrinsic or environmental factors, including temperature of storage, humidity and gas composition of storage atmosphere; and (iv) implicit factors, such as effects depending on the particular dominant microbiota that initially develops according to intrinsic, processing and extrinsic factors. Examples of minimum limits for growth of pathogens when all other parameters are at optimum values are summarised in Table 2. Table 3 reports combinations of pH and  $a_w$  values that may allow growth of pathogenic bacteria. Table 4 reports growth prediction ranges of temperature, pH and  $a_w$  for pathogenic bacteria based on estimations determined with ComBase and Pathogen Modeling Program (see Chapter 3.1), which however do not necessarily represent growth limits for those bacteria in real foods.

**Table 2:** Minimum limits for growth of pathogens when all other parameters are at optimum values (extracted from several sources (FDA, 2011; ICMSE, 1996; IFT, 2003; NACMCF, 2010)).

Pathogens	Min. temp. (°C)	Min. pH	Min. $a_w$	Oxygen
Norovirus	NAp	NAp	NAp	NAp
Parasites	NAp	NAp	NAp	NAp
<i>Salmonella enterica</i>	5.2	3.7	0.94	facultative
<i>Shigella</i> spp.	6.1	4.8	0.96	facultative
<i>Campylobacter</i> spp. - thermotolerant	30.0	4.9	0.99	microaerophilic
Pathogenic <i>Escherichia coli</i>	6.5	4.0	0.95	facultative
<i>Vibrio parahaemolyticus</i>	5.0	4.8	0.94	facultative
<i>Listeria monocytogenes</i>	-0.4	4.39	0.92	facultative
<i>Yersinia enterocolitica</i>	-1.3	4.2	0.95	facultative
<i>Clostridium perfringens</i>	10.0	5.0	0.93	anaerobic
<i>Bacillus cereus</i> (diarrhoeic / emetic)	4.0	4.3	0.92	facultative
<i>Staphylococcus aureus</i> - growth	7.0	4.0	0.83	facultative
- toxin	10.0	4.5	0.88	facultative
<i>Clostridium botulinum</i> - proteolytic	10.0	4.6	0.93	anaerobic
- non-proteolytic	3.0	5.0	0.97	anaerobic
Biogenic Amine Producers	NAv	NAv	NAv	NAv

NAp: not applicable (the agents do not growth in food).

NAv: data not available.

**Table 3:** Combinations of pH and  $a_w$  values that may allow growth of pathogenic bacteria (source: NACMF (2010), reprinted with permission from the *Journal of Food Protection*).

*Potential pathogens<sup>a</sup> of concern for growth studies based on interaction of product pH and  $a_w$ <sup>b</sup>*

$a_w$ values	pH values:					
	<3.9	3.9 to <4.2	4.2–4.6	>4.6–5.0	>5.0–5.4	>5.4
<0.88	NG <sup>c</sup>	NG	NG	NG	NG	NG
0.88–0.90	NG	NG	NG	NG	<i>Staphylococcus aureus</i>	<i>S. aureus</i>
>0.90–0.92	NG	NG	NG	<i>S. aureus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i> <i>S. aureus</i>
>0.92–0.94	NG	NG	<i>L. monocytogenes</i> <i>Salmonella</i>	<i>Bacillus cereus</i> <i>Clostridium botulinum</i> <i>L. monocytogenes</i> <i>Salmonella</i> <i>S. aureus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> <i>Salmonella</i> <i>S. aureus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> <i>Salmonella</i> <i>S. aureus</i>
>0.94–0.96	NG	NG	<i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>Vibrio parahaemolyticus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>V. parahaemolyticus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>C. perfringens</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>V. parahaemolyticus</i>
>0.96	NG	<i>Salmonella</i>	Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>V. parahaemolyticus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>V. parahaemolyticus</i> <i>V. vulnificus</i>	<i>B. cereus</i> <i>C. botulinum</i> <i>C. perfringens</i> <i>L. monocytogenes</i> Pathogenic <i>E. coli</i> <i>Salmonella</i> <i>S. aureus</i> <i>V. parahaemolyticus</i> <i>V. vulnificus</i>

<sup>a</sup> *Campylobacter* spp., *Shigella*, and *Yersinia enterocolitica* do not appear here because they are typically controlled when the pathogens listed are addressed.

<sup>b</sup> Data are based on the PMP (106), ComBase predictor (50), ComBase database (49), or peer-reviewed publications (11, 17, 45).

<sup>c</sup> NG, no growth; when no pathogen growth is expected, but formulation or process inactivation studies may still be needed.

**Table 4:** Growth prediction ranges of temperature, pH and  $a_w$  for pathogenic bacteria as estimated using ComBase and Pathogen Modeling Programs (source: NACMCF (2010), reprinted with permission from the *Journal of Food Protection*).

*Pathogen growth ranges used in ComBase and Pathogen Modeling Programs<sup>a</sup>*

Pathogen	ComBase					PMP				
	Temp (°C)		pH		$a_w$ (minimum)	Temp (°C)		pH		$a_w$ (minimum)
	Minimum	Maximum	Minimum	Maximum		Minimum	Maximum	Minimum	Maximum	
<i>B. cereus</i>										
With CO <sub>2</sub>	5	34	4.9	7.5	0.974					
Aerobic						5	42	4.7	7.5	0.97
Anaerobic						10	42	5.0	9.0	0.97
<i>C. botulinum</i> (growth only)										
Proteolytic	14	40	4.7	7.2	0.954	15	34	5.0	7.2	0.977
Nonproteolytic	4	30	5.1	7.5	0.974	5	28	5.0	7.0	0.977
<i>C. perfringens</i>	15	52	5	8	0.971	19	37	6.0	6.5	0.983
<i>E. coli</i> O157:H7										
With CO <sub>2</sub>	10	30	4.5	7	0.961					
Aerobic						5	42	4.5	8.5	0.97
Anaerobic						5	42	4.5	8.5	0.97
<i>L. monocytogenes</i>										
With CO <sub>2</sub>	1	35	4.4	7.5	0.934					
Aerobic						4	37	4.5	7.5	0.928
Anaerobic						4	37	4.5	8.0	0.97
<i>S. aureus</i> (growth only)										
Not specified	7.5	30	4.4	7.1	0.907					
Aerobic						10	42	4.5	9.0	0.911
Anaerobic						12	42	5.3	9.0	0.872
<i>Salmonella</i>										
With CO <sub>2</sub>	7	30	3.9	7.4	0.973					
Aerobic						10	30	5.6	6.8	0.974

<sup>a</sup> Limits tested in ComBase (48) and the Pathogen Modeling Program (PMP) (106) do not necessarily represent limits for growth. See Table 5 for growth limits.

Temperature is one of the most important factors affecting growth of pathogens and thus safety of refrigerated composite products. Available data show that the first steps of the logistic chain are in most cases satisfactorily controlled (Afchain et al., 2005). In contrast, conditions at the retail level are out of manufacturers' direct control and often deviate from legislated temperature limits. Temperature control is the least reliable from the retail store to domestic storage, the temperature of which is entirely dependent on the diligence of the consumer. Therefore, the chill chain is critical in determining a pathogen's growth, and the detailed knowledge on the temperature conditions during retail and domestic storage is a prerequisite for evaluating the risk of refrigerated composite products. Appendix B summarises the available information in relation to temperature surveys in retail and domestic refrigerators in some European countries.

Alterations of pH, Eh,  $a_w$  or structure of a food, caused by microbial growth, may favour or inhibit growth of other organisms. The effects of interacting factors can be synergistic or antagonistic. Examples of synergistic effects are: one organism removing a substance or changing the pH that is inhibitory to another, or an organism producing growth factors required by another.

In general, dominating microbial types and their extent of survival and growth depend on the characteristics, or the intrinsic factors, of the food product considered. Microbial growth, however,

modifies the intrinsic factors through its metabolic action, which, consequently, can influence subsequent microbial growth in terms of types and levels of growth.

Combining materials or ingredients to form composite products also modifies intrinsic parameters, throughout or at the interface of components, depending on the type of product, resulting in new equilibria of intrinsic parameters that also influence microbial dominance and growth. In addition, combining food materials to form composite products also modifies the microbiota of the composite through cross-contamination, as microorganisms, including pathogens, from different food materials might be introduced in the composite product.

Processing factors involved in manufacture of various foods, e.g. heat treatments, drying, acidification, etc., modify the food material, as well as its dominant microbiota and its potential for development in the composite product. In addition, extrinsic environmental factors, associated with product storage, also play a major role in the selection of dominant microorganisms and their influence on the spoilage and safety of the products before consumption.

Microbial contamination, however, is also subject to change due to cross-contamination during various periods of exposure to the environment or handling and exposure to contamination sources. Such contaminants may also include pathogens not present in the original ingredients or products. Their fate will also depend on further handling, processing or preparation of the product before consumption. It should be noted that even if a product does not allow microbial growth, it may be bacteriologically unsafe if contaminated post-processing and before consumption with pathogens that can cause illness without growth in the food, and even at low doses. However, cross-contamination with toxigenic pathogens, or pathogens needing high numbers to cause illness, may not be of concern if the characteristics of the product do not allow cell multiplication and formation of the toxin as the pathogenic agent (e.g. *S. aureus*).

Multicomponent composite products present more complex situations, especially at the interface of the dissimilar components, where there will be an equilibrium established in properties that affect microbial growth, which may alter the expected behaviour of pathogens during storage in either of the food components or their interface. This might be especially of concern when a food component provides the contaminants (e.g. bacterial spores) and the other modifies the properties (e.g. pH,  $a_w$ , etc) at the interface of components allowing growth of contaminants. Further, microenvironments allowing growth of bacterial pathogens may be established within composite products through growth of non-pathogenic species of microorganisms (e.g. mould growth in acidic products raising the microenvironment pH through metabiosis and allowing subsequent bacterial spore growth).

In considering the microbial safety of composite products, it is advisable to identify sources and types of contamination at all stages of production and handling and from all components, the phase or component of the composite product most susceptible to microbial growth, and potential food component and microbial interactions such as metabiotic effects that may influence microbial selection and growth during storage. Presence of small numbers of certain pathogens, even on products not allowing proliferation, may be undesirable.

In summary:

- Microbial survival and growth in foods depend on intrinsic, extrinsic, processing and implicit factors including nutrients,  $a_w$ , pH or acidity, Eh, antimicrobials naturally present in foods, the properties of the structure of foods, temperature of storage or processing, non-thermal physical processes such as irradiation and high pressure, changes in pH due to fermentation or direct acidification, antimicrobial additives, gas composition of the storage atmosphere and its humidity, and food product changes associated with microbial growth. The effects of interacting factors can be synergistic or antagonistic.



- Producing composite products by combining materials or ingredients modifies their intrinsic parameters, especially at the interface of components, and results in new equilibria of intrinsic parameters that also influence microbial type selection and growth. Cross-contamination associated with combining food materials to form composite products also modifies the microbial flora of the composite. Being multicomponent, composite products may alter the behaviour of pathogens during their storage. When considering the microbial safety of composite products, it is useful to identify sources and types of contamination at all stages of production and handling.
- Other important considerations are the component or phase of the composite product most susceptible to microbial growth, as well as potential symbiotic or metabiotic interactions of the food components and microorganisms during storage. With the recognition of pathogen strains able to cause illness in low numbers, it is important to consider the presence of even small numbers of pathogen cells even on products not allowing proliferation.

Composite products with the same names may have undergone very different treatments and may represent very different risks. “Recipe dishes” types of food (e.g. a ready-to-eat meal containing rice and fish) could be packaged after the heat treatment, and consumed after a short refrigerated storage, some could be pasteurized in the final package and sold refrigerated with a longer shelf-life, and some could be sold as sterilised in the final package. The first product could contain a large range of hazards, whereas the second would contain only heat-resistant hazards and the last would presumably contain none of the hazards listed in Table 1. Some recipe dishes are also sold frozen and in this case growth of pathogenic bacteria is not possible during shelf-life. Cakes are also another type of composite product that may include products with very different  $a_w$ , from permissive to inhibitory of growth of pathogenic bacteria.

### 3. Modelling of inactivation/growth of pathogenic microorganisms in foods

#### 3.1. Tools available to predict inactivation/growth of pathogenic microorganisms in food

Predictive microbiology has been established itself as a scientific discipline that uses mathematical equations to summarize and make readily available quantitative information on the microbial responses in various foods under different conditions (McMeekin et al., 2008). Development of models to predict survival, growth or inactivation of microorganisms in foods has been a most active research area within food microbiology during the last 25 years (Ross and Dalgaard, 2004). Microbial growth and inactivation models are now sufficiently detailed and accurate to make important contributions since scientists and regulators can make reasonable predictions of the relative risk posed by a hazard in a particular food or by a food process. However, these models can be only used for comparative purposes and not to provide absolute predictions, as most of them have been established based on laboratory experiments. In addition, when these models are used for composite products, interactions between ingredients or components and time-varying conditions should be taken into account. Therefore the models should be validated in the target products before practical application.

Mathematical models have been incorporated into a considerable number of predictive microbiology software tools that are available to predict survival/growth of microorganisms in foods:

- **Pathogen Modeling Program (PMP):** The PMP is available for use free of charge (<http://portal.arserrc.gov/>) and, with more than 5000 downloads per year, it is probably the most widely used predictive microbiology application software. PMP has been available for close to 20 years and it is regularly being updated and expanded. The present version includes more than 40 models for different bacterial pathogens. The software allows growth or inactivation of pathogens to be predicted for different combinations of constant temperature, pH, NaCl/ $a_w$  and, in some cases, other conditions such as organic acid type and concentration, atmosphere, or nitrite. In addition, PMP includes models that predict the effect of cooling temperature profiles on growth of *C. botulinum* and *C. perfringens* after cooking. Predictions can be exported and the software

contains references to studies from which the models were developed. In 2007 PMP was integrated with the Predictive Microbiology Information Portal (PMIP).

- **Combined database on predictive microbiology information (ComBase):** ComBase ([www.combase.cc](http://www.combase.cc)) is a web-based resource for quantitative and predictive food microbiology. Its main components are: a database of observed microbial responses to a variety of food-related environments and a collection of relevant predictive models. ComBase is managed by the ComBase Consortium consisting of the Institute of Food Research (IFR) in the United Kingdom, the USDA Agricultural Research Service (USDA-ARS) in the United States, and the University of Tasmania Food Safety Centre (FSC) in Australia. The ComBase predictive models are a collection of software tools based on ComBase data to predict the growth or inactivation of microorganisms. Currently available predictive tools include the following on line applications:
  - *ComBase Predictor*, a set of 23 growth models and 6 thermal death models for predicting the response of many important food-borne pathogenic and spoilage microorganisms to key environmental factors. A Microsoft Excel version of this web application can also be found in the ComBase Excel Demo provided in the website.
  - *Perfringens Predictor*, an application specially designed for predicting the growth of *C. perfringens* during the cooling of cooked meats. A Microsoft Excel AddIn version of the program can also be found in the Downloads section of the web site.
- **Sym'previus:** Sym'previus ([www.symprevius.org](http://www.symprevius.org)) is an extensive decision support system developed in France that includes a database and simulation tools for growth, survival, inactivation and growth/no growth interface of pathogenic bacteria and some spoilage microorganisms. Evaluation of consumer exposure can be done by means of a probabilistic module. Information from Sym'previus is available on a commercial basis through contact centres as indicated on the homepage cited above.
- **Seafood Spoilage and Safety Predictor (SSSP):** The SSSP software has been developed by Danish Technical University ([http://sssp.dtuaqua.dk/HTML\\_Pages/Help/English/Index.htm](http://sssp.dtuaqua.dk/HTML_Pages/Help/English/Index.htm)) to facilitate the practical use of mathematical models to predict shelf-life as well as growth of spoilage and pathogenic bacteria in seafood. The SSSP v. 3.1 released August 2009 includes: four product-specific relative rate of spoilage (RRS) models, three generic RRS models, four product-specific microbial spoilage models, a generic model to predict microbial growth and shelf-life, modules to compare predictions from SSSP with users own data of shelf-life or growth of bacteria, models to predict growth and histamine formation by *M. psychrotolerans* and *M. morgani*, growth and growth boundary model for *L. monocytogenes* and a model to predict the simultaneous growth of *L. monocytogenes* and lactic acid bacteria in lightly preserved seafood.
- **Microbial Responses Viewer (MRV):** The MRV (<http://cbnfri.dc.affrc.go.jp>) is a new database consisting of microbial growth/no growth data of nineteen different microorganisms derived from ComBase. The specific growth rate of each microorganism is modelled as a function of temperature, pH and  $a_w$  using a Poisson log-linear model, which is a family of generalized linear models (GLMs). The specific growth rate is illustrated using a two-dimensional contour plot with growth/no growth data. The software allows the user to rapidly view growth/no growth contour plots superimposed by actual ComBase data. Contours of any two of three variables (temperature, pH and  $a_w$ ) can be visualized, while the third is held constant.
- **Refrigeration index (RI) calculator:** The RI calculator was developed by Meat & Livestock Australia Limited ([www.foodsafetycentre.com.au/refrigerationindex.php](http://www.foodsafetycentre.com.au/refrigerationindex.php)). It predicts the expected growth of *E. coli* on meat from temperature and other data. The model has values for pH,  $a_w$  and lactate concentration, which, in addition to temperature, all affect the growth rate of *E. coli*. The current RI model allows for the user to enter data on temperatures of the product over time. Choosing the type of product sets the other parameters.

- **Opti-Form@ Listeria control model 2007 (PURAC):** This software predicts the effect of organic acids, temperature, pH and moisture on growth of *L. monocytogenes* in meat products. It can be requested ([www.purac.com/purac\\_com/d9ed26800a03c246d4e0ff0f6b74dc1b.php](http://www.purac.com/purac_com/d9ed26800a03c246d4e0ff0f6b74dc1b.php)) from the PURAC company.
- **Websim-MILQ:** WebSim Milq is a web implementation of predictive models designed to optimise heat treatment processes in dairy companies. Information about the software can be obtained (<http://websim.milq.org/websim/milq/LoginForm.aspx>) through NIZO Food Research in the Netherlands.
- **Shelf Stability Predictor:** The software has been developed by the Center for Meat Process Validation at the University of Wisconsin - Madison ([http://meathaccp.wisc.edu/ST\\_calc.html/](http://meathaccp.wisc.edu/ST_calc.html/)) and provides a set of models for predicting the growth of *L. monocytogenes* and *S. aureus* on ready-to-eat meat products as a function of pH and  $a_w$ .
- **Temperature History Evaluation for Raw Meat (THERM):** Developed by the Center for Meat Process Validation at the University of Wisconsin – Madison (<http://meathaccp.wisc.edu/>). THERM is an online tool designed for evaluating the safety of meat or poultry at temperatures between 50°F and 115°F (10°C to 46°C).
- **Process Lethality Determination Spreadsheet:** Developed by AMI Foundation, USA ([www.amif.org/ht/d/sp/i/26870/pid/26870](http://www.amif.org/ht/d/sp/i/26870/pid/26870)). This tool provides processors with a science-based validation tool that can be used to demonstrate the effectiveness of a specific heat process to destroy a microorganism of concern. Specifically, the interactive model allows the user to input actual in-process data from a given cook cycle and determine if the process achieves the required log reduction for the microorganism of concern. The goal is to define or map the heating and cooling profile of the product by observing the temperature characteristics of the product during heating and cooling.

### 3.2. Inactivation data and models of hazards in food

Modelling inactivation of food-borne pathogens has been widely studied since the 1920s, being one of the first achievements in predictive microbiology. Inactivation models were initially focused on the destruction of *C. botulinum* spores in low acid canned foods (Hersom and Hulland, 1980; Jay, 1992).

#### 3.2.1. Inactivation kinetics and primary inactivation models

Theoretically the log reduction of vegetative bacteria vs treatment time follows a linear relationship, based on the calculation of the time to achieve one decimal or  $\log_{10}$  reduction ( $D$ -value), while specific increases in temperature ( $z$ -value) produce a reduction in the  $D$ -value by a factor of 10.

Table 5 reports a comparison of the calculated time ( $F_{ref}$ ) required to achieve an 8  $\log_{10}$  reduction of representative vegetative bacteria and bacterial spores at a given reference temperature ( $T_{ref}$ ). Details on the origin of the data, as well as  $D$ -values and  $z$ -values at specific temperatures are provided in Appendix C, Table 17.

Values for some spoilage bacteria are also included, which show that some are more resistant to sterilization treatments than the most resistant pathogenic bacteria. To obtain shelf stable foods, food processors may use heat treatments, which would largely inactivate pathogens.

Generally it is assumed that for a pasteurisation process (72°C, 15-20 sec) vegetative cells are inactivated by more than 5  $\log_{10}$  units. In Table 5 it can be seen that all vegetative pathogens are even reduced by 8  $\log_{10}$  units for these specific conditions within 15 sec (maximum time is 13.2 sec). Specific food products can have a protective effect, like for example chocolate, as it can be seen for *Salmonella* Typhimurium Chocolate syrup (5 min needed at 70°C for an 8  $\log_{10}$  reduction).

**Table 5:** Mean  $F_{ref}$  values (in minutes and seconds for a 8  $\log_{10}$  reduction) calculated at a given reference temperature ( $T_{ref} = 70^{\circ}\text{C}$  for vegetative cells and  $120^{\circ}\text{C}$  for spores) for various vegetative bacteria and spores in foods (for additional information see Appendix C, Table 17).

		<b>Food matrix</b>	<b><math>T_{ref}</math> (<math>^{\circ}\text{C}</math>)</b>	<b><math>F_{ref}</math> (min)</b>	<b><math>F_{ref}</math> (sec)</b>
<b>Food-borne pathogen (vegetative)</b>	<i>Salmonella</i> Typhimurium	Chocolate syrup	70	5.0	298
	<i>Salmonella</i> Typhimurium	Egg yolk	70	0.018	1.1
	<i>Listeria monocytogenes</i>	Chicken gravy	70	0.057	3.4
	<i>Clostridium perfringens</i>	Minced beef	70	0.079	4.8
	<i>Escherichia coli</i> O157:H7	Ground/minced beef	70	0.024	1.4
	<i>Escherichia coli</i> O157:H7	Pork sausage	70	0.027	1.6
	<i>Salmonella</i> spp.	Chicken broth	70	0.14	8.1
	<i>Listeria monocytogenes</i>	Ham	70	0.22	13
<b>Food-borne pathogen (spore-forming; refers to spores unless otherwise mentioned)</b>	<i>Bacillus</i> spp. (vegetative cells)	Milk	70	0.003	0.20
	<i>Clostridium botulinum</i> (proteolytic)	Mushroom extract	120	0.40	24
	<i>Bacillus cereus</i>	Infant formula (milk-based) (pH 6.3)	120	0.16	9.8
	<i>Bacillus cereus</i>	0.25M phosphate buffer (pH 7.0)	120	0.067	4.0
	<i>Clostridium botulinum</i> (proteolytic Type A)	Pea puree	120	0.99	59
	<i>Clostridium botulinum</i> (proteolytic Type A)	Spaghetti, tomato sauce and cheese	120	1.2	74
	<i>Clostridium botulinum</i> (proteolytic Type A)	Spanish rice	120	1.3	78
	<i>Clostridium botulinum</i> (proteolytic Type A)	Mackerel in oil	120	3.9	236
	<i>Clostridium botulinum</i> (proteolytic Type B)	Fresh corn puree (pH 6.9)	120	2.6	158
	<i>Clostridium botulinum</i> (proteolytic Type B)	Canned asparagus (pH 5.04)	120	0.82	49
	<i>Clostridium botulinum</i> (non proteolytic Type B)	Phosphate buffer	120	0.02	1.2
<i>Clostridium botulinum</i> (non proteolytic Type E)	Surimi	120	0.001	0.06	
<b>Food spoilage bacteria (spore-forming; refers to spores)</b>	<i>Geobacillus stearothermophilus</i>	Mushroom extract	120	15	886
	<i>Clostridium sporogenes</i>	Peas puree	120	20	1,199
	<i>Clostridium sporogenes</i>	Phosphate buffer (pH 7.0)	120	25	1,501
	<i>Clostridium sporogenes</i>	Natural asparagus (acidified) (pH 5.0)	120	11	675

Other studies intended to quantify microbial inactivation caused by the application of thermal and non-thermal processes describe more complex modelling approaches in which deviation from linearity is observed. These deviations from first-order kinetics may cause some underestimation of the survival of pathogenic bacteria (Bermúdez-Aguirre and Corradini, 2012).

A short description of the available primary inactivation models is provided in Appendix D.

Concerning food-borne viruses, most information on inactivation by heat was obtained on hepatitis A virus. In a neutral pH food such as milk heated at 71°C, 6.5 min to 12.6 min were needed to obtain a 4 log<sub>10</sub> reduction, depending on the fat content (Bidawid et al., 2000). In mussels, cooking for 9 min in boiling water (between 71°C and 76°C core mussel temperature) reduced hepatitis A virus by at least 3.6 log<sub>10</sub>, but failed to totally eliminate an initial level of 4.6 log<sub>10</sub> virus (Crocì et al., 2005). In an acid food such as a raspberry or strawberry purée, approximately 6 min at 70°C were needed for a 4 log<sub>10</sub> reduction (Deboosere et al., 2010). Human pathogenic Norovirus cannot be cultivated on cells and their heat resistance is not known. Surrogate viruses (feline caliciviruses) are more heat sensitive than hepatitis A virus, and 1.5 min at 70°C caused 6 log<sub>10</sub> reduction (Buckow et al., 2008). The z-values for hepatitis A virus found in strawberry purées (Deboosere et al., 2004; Deboosere et al., 2010) were between 20°C and 24°C, markedly different from those for bacteria listed in Table 17.

In order to prevent and control transmission of fishery product-borne parasites to humans, in particular *Anisakis* spp., Regulation (EC) No 853/2004<sup>9</sup> lays down provisions for fishery products to be consumed raw or almost raw, fishery products that are to undergo a cold smoking process, and marinated and/or salted fishery products, if the processing is insufficient to destroy nematode larvae. This requires freezing to a temperature of not more than -20°C in all parts of the product for not less than 24 hours for the following:

- composite foods in which fishery components will be consumed raw or almost raw;
- composite foods in which fishery components come from herring, mackerel, sprat, (wild) Atlantic and Pacific salmon, if they are to undergo a cold smoking process in which the internal temperature of the fishery product is not more than 60°C; and,
- composite foods including marinated and/or salted fishery components, such as olives stuffed with fish, if the processing is insufficient to destroy nematode larvae.

Among chemical treatments, salting and marinating (with vinegar, lemon juice, wine, soy sauce, or brine) have been traditionally used to inactivate anisakidae larvae. It has been estimated that 28 days of storage in brine with 6.3% salt and 3.7% acetic acid in the aqueous phase of the fish was the maximum survival time in herring (Karl et al., 1995). Other physical methods include high hydrostatic pressure, irradiation, drying, low voltage current and smoking treatments. A complete review of the effects of these preservation methods is provided in a recent EFSA Opinion (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

Among toxins that can be produced in foods, those of *C. botulinum* are the most heat labile. To obtain a 4-5 log<sub>10</sub> reduction of their activity, 1 to 20 min at 70°C were needed, depending on the toxin types and the food matrices (Hauschild, 1989). For instance milk pasteurization may not inactivate all toxin types (Rasooly and Do, 2010). z-values could be calculated for botulinum toxins and varied from 3 to 8°C depending on toxin types (Hauschild, 1989). In practice, 20 min at 79°C or 5 min at 85°C are required to safely inactivate botulinum toxins. *Staphylococcus* enterotoxin A is gradually inactivated by heating at 100°C, but the food may retain toxicity even after 30 min (Bergdoll, 1989). Emetic toxin from *B. cereus* (cereulide) is the most heat resistant. It was not affected by 120°C/15-20 min treatment at pH 7-8 (Rajkovic et al., 2008; Shinagawa et al., 1996). Being small molecules, biogenic amines a presumably heat stable.

<sup>9</sup> OJ L 226, 25.6.2004, p.22-82, as last amended.

### 3.2.2. Secondary inactivation models

The above models apply for the specified temperatures studied. To estimate survival curves at other temperatures, the common approach in predictive microbiology is to perform regression analyses of the inactivation parameters (most often the inactivation rate, or the  $D$ -value) as a function of temperature to obtain a secondary model. The secondary model can be extended to include other environmental conditions (i.e., pH,  $a_w$ ).

During the last 30 years an increased number of such models have been developed (see Appendix D, Table 20), while some of them have been incorporated in the predictive software described in Chapter 3.1. The use of these models can provide useful information on the inactivation of pathogens during processing and storage of foods.

Since isothermal survival parameters can also depend on factors such as pH, NaCl concentration or  $a_w$ , they too should be considered in model equations to account for their influence on the inactivation. Equations mostly used are referred to as polynomial, and include Davey (modified Arrhenius), Bigelow, Mafart or logistic models. Generally speaking, response surface models include interaction terms between environmental factors (referring to synergistic/antagonistic effects), while modified Arrhenius or Bigelow types do not.

More details and a brief description of some secondary inactivation models are provided in Appendix D, together with examples of death curves for different temperatures, pH and  $a_w$ , simulated with one of these models (see Appendix D, Figure 14).

As a summary of these simulations, at lethal temperatures an acid environment (low pH) tends to increase the rate of death of pathogenic bacteria. At lethal low pH values (acid environment), refrigeration temperatures tend to reduce the rate of death of pathogenic bacteria. The impact of  $a_w$  in combination with low pH and refrigeration is more dependent on the bacterial species considered.

Therefore, the effectiveness of inactivation treatments in composite products might differ depending on type of pathogen, food composition or intensity of the treatment, among other factors. The survival of most microorganisms is directly or indirectly influenced by the intrinsic and extrinsic properties of food products, which may act synergistically or antagonistically on inactivation. With respect to temperatures, pH and  $a_w$ , a first estimate of their combined action on pathogens inactivation can be obtained in currently available models and databases. Considered globally, heat treatments currently applied in food processing to kill microorganisms can achieve several  $\log_{10}$  reductions in a few seconds or minutes. In contrast, at storage (non-lethal) temperatures, low pH and/or  $a_w$  values currently found in foods may cause inactivation of pathogenic bacteria, but longer times may be required for several  $\log_{10}$  reductions.

### 3.2.3. Survival/inactivation of microorganisms in composite products

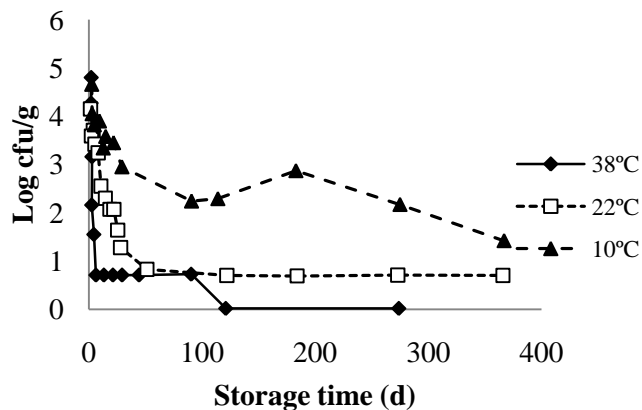
Composite products are often complex mixtures of several food components. The range of such foods is large, including such diverse products as oil-in-water emulsions, reconstituted meat and fish products and confectionery (e.g. chocolates containing fruits, nuts and sugar granules). To consider microbial safety aspects in the design of an inactivation treatment in composite products, one should be aware about the distribution of the microorganisms within the different food components. In composite products, although microorganisms are mainly located in the aqueous phase, the structural features of this phase could have different micro-architecture which might be uniform throughout the food, or particular regions may have a specific structure. Wilson et al. (2002) considered some micro-architecture states in structured foods which can be also applied to composite products, such as gel, oil-in-water and water-in-oil emulsions, liquid, surface or gelled emulsions. Especially, oil components and fat matter of composite foods are reported to have a protective effect in microbial cells against preservatives and heat treatments (Wilson et al., 2002).

Regarding the efficiency of temperature in heating treatments, Periago et al. (2004) studied the survival rate of *B. sporothermodurans* spores in pea (pH 6.1) and mushroom (pH 6.0) soups with different viscosities. Isothermal inactivation curves were obtained by fitting the Weibull model. They found that different inactivation behaviour in both soups, probably attributed to the viscosities of the food matrices. In pea soup, a 3 log<sub>10</sub> reduction can be achieved after 8.5 min at 121°C, while in mushroom soup it would be necessary to apply a longer treatment (11.3 min) to obtain the same reduction. Although not concerning pathogenic bacteria, this example illustrates an impact of the food matrix on heat resistance that cannot be explained by pH and a<sub>w</sub>.

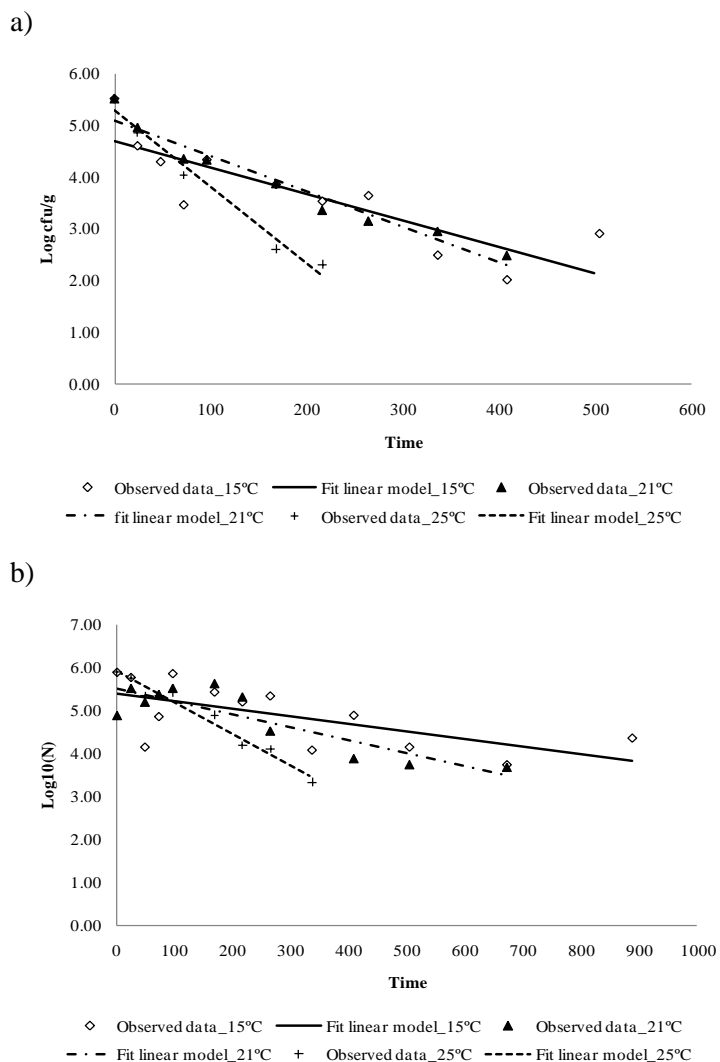
Generally, the structured matrix commonly found in composite products causes microorganisms to be immobilized and colonies to be formed. This constrained colony causes an additional stress and has been reported to result in narrower boundaries of the habitat domain (i.e. increase the sensitivity to other inactivation factors) (Brocklehurst et al., 1997; Wilson et al., 2002). However, this food matrix can also provide an additional resistance to inactivation treatments. Indeed, Lee et al. (1989) showed a higher resistance of *Salmonella* to heat treatments in milk chocolate than in aqueous media. This is also related to the low a<sub>w</sub> levels found in composite products, which is the factor that largely accounts for the protective action of carbohydrates or fats in heated foods. This low a<sub>w</sub> allows pathogens such as *Salmonella* to increase its thermal resistance and to survive processing conditions that would normally be expected to destroy this pathogen (Barrile et al., 1970; D'Aoust et al., 1975). Indeed, growth and survival of *Salmonella* on raw ingredients used for the manufacture of confectionery products, such as almonds or crushed shells has been studied (Uesugi and Harris, 2006). Danyluk et al. (2008) demonstrated that *S. Enteritidis* can migrate through the nut shell to the kernel in wet conditions. The extreme heat resistance of *Salmonella* in dry environments and reduction on adding moisture has been reported in cocoa liquor (Goepfert and Biggie, 1968) and in peanut butter (Ma et al., 2009). Mattick et al. (2001) reported on heat resistance in a variety of dry and semi-dry foods and developed surface response models. Izurieta and Komitopoulou (2012) provided quantitative data (*D* and *z* values) of various *Salmonella* strains in cocoa beans or hazelnut shells. It was proven that the addition of moisture of ca. 7% w/v markedly reduced *D*-values of *Salmonella* strains associated with outbreaks from consumption of confectionery ingredients. Other Gram positive microorganisms like *S. aureus* can survive and eventually grow in low-a<sub>w</sub> foods such as bread (Vora et al., 2003) independently from the contamination level.

Reduction of a<sub>w</sub> also markedly increased heat resistance of food-borne viruses. For instance, in strawberry purée, without sugar added, around 2 min at 75°C caused a 1 log<sub>10</sub> reduction of hepatitis A virus, whereas in presence of sugar, to achieve the same reduction at 85°C, 1 min and 6 min were needed for, respectively, 28°Bx and 52°Bx (Deboosere et al., 2004; Deboosere et al., 2010).

In some low-a<sub>w</sub> foods, the survival capacity is prolonged at low storage temperatures. Baylis et al. (2004) studied the survival of *E. coli* O157:H7, O111:H- and O26:H11 in chocolate (see Figure 2) and confectionery products such as biscuit cream and mallow during storage at 38°, 22° and at 10°C. A rapid decline was observed in products stored at 38°C and increased survival occurred in products stored at 10°C. Indeed, survival was longer in chocolate, which was the product with the lowest a<sub>w</sub> (0.40-0.52), whereas death occurred more rapidly in the biscuit cream and mallow which had higher a<sub>w</sub> (0.73-0.79). The same result was obtained in fruit malt loaf (pH 5.2, a<sub>w</sub> 0.89) when stored at 15°, 21° and 25°C. In this case, linear models were fitted to survival data of *Salmonella* and *L. monocytogenes* (obtained from ComBase) (see Figure 3 and Appendix D, Table 18).



**Figure 2:** Fate of *E. coli* O157:H7, O26:H11 and O111:H- in chocolate during storage at 10°, 22° and 38°C. Adapted from Baylis et al. (2004).



**Figure 3:** Fit of linear models to survival data obtained from ComBase for *Salmonella* spp. (graph "a") and *L. monocytogenes* (graph "b") in fruit malt loaf stored at 15°, 21° and 25°C.



Therefore, it is concluded that temperature is unlikely to be the only factor that affects the rate of decline of these bacteria. In addition to the impact of certain compounds in a food, survival will in particular be influenced by the  $a_w$  dynamics of the product. At higher storage temperatures (e.g. room temperature)  $a_w$  could be reduced because of desiccation of the food product.

Microbial survival in acidified composite foods such as egg, seafood or pasta salads has also been well described (Hwang and Marmer, 2007; Hwang and Tamplin, 2005).

Predictive models must take into account possible changes of the temperature during storage of composite products since they are exposed to temperature variations during storage, affecting both moisture transfer rate and their shelf-life. If a model is properly validated, predictions based on data obtained from broth systems can be applied to microorganisms present in structured foods.

#### 3.2.4. Concluding remarks

Temperature,  $a_w$  and pH are the main physico-chemical factors which permit assessment of survival and inactivation of pathogens in composite products. This implies knowledge of the treatment (time and temperature for heat) applied in all parts of the food, of its storage conditions (time and temperature), and its composition (pH and  $a_w$ ) for all components. This information permits use of the models and databases described in this chapter for a first estimation of the survival time or extent of inactivation. However, such estimates must be considered with caution in the case of composite products, due to potential impact of the food matrix and its heterogeneity. Challenge testing may be needed for an accurate assessment of inactivation. Effects of other factors, such as non-thermal treatments (e.g. high pressure, irradiation, presence of preservatives), are less documented.

Heating high  $a_w$  foods is the most efficient and well-documented pathogen inactivation process:

- Mild heat treatment (such as in the heart of a very lightly cooked food or pasteurization) of, for example, a few seconds at 70°C permits several  $\log_{10}$  units of inactivation of vegetative bacterial pathogens and parasites, but may not be sufficient for food-borne viruses and will not inactivate bacterial spores nor bacterial toxins formed in foods.
- Cooking of foods for several minutes at 90°C or 100°C practically eliminates vegetative bacterial pathogens, food-borne viruses, and parasites. It permits several  $\log_{10}$  units of inactivation for spores of non-proteolytic *C. botulinum* (psychrotolerant *C. botulinum*), but will not inactivate sufficiently spores of pathogenic *Bacillus* and other pathogenic *Clostridium*. Toxins from *C. botulinum* would be inactivated but not toxins from *S. aureus*, emetic toxin from *B. cereus*, or biogenic amines.
- Sterilization treatment such as a few minutes at 120°C will permit several  $\log_{10}$  unit reductions of all food-borne pathogens. Emetic toxin from *B. cereus* and biogenic amines will not be inactivated.
- Low  $a_w$  in parts of the composite products, or presence of an oil phase, may significantly reduce the efficacy of heat treatments, as illustrated in Table 5 for *Salmonella* in chocolate syrup. In contrast, low pH will increase the inactivation rate.
- Freezing (-20°C of all parts of the food for at least 24 hours) is commonly used to inactivate parasites in fish.
- Pathogens are inactivated over time at pH,  $a_w$  and temperature non-permissive for growth, but in many cases the decline is too slow (several days for 3  $\log_{10}$  reductions) to ensure absence of pathogens when the food is placed on the market. In particular, the prolonged survival of pathogens in low  $a_w$  foods (e.g. chocolate and some confectioneries) must be highlighted.

### 3.3. Growth/no growth models of pathogenic microorganisms in food

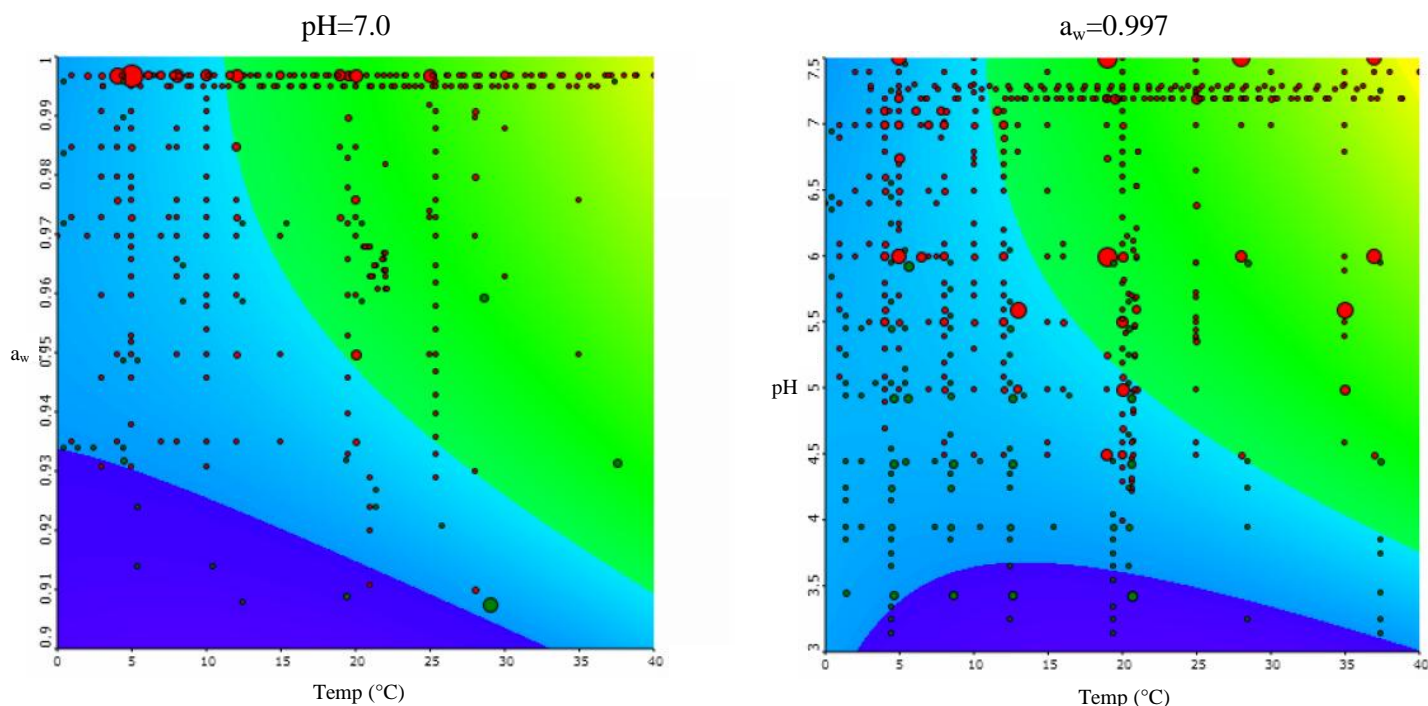
The ability of pathogenic bacteria to grow under certain environmental conditions is of great importance in risk assessment. Modelling the behaviour of microorganisms in the growth/no growth region has been recognized as an important component of “modern” predictive microbiology (McMeekin et al., 1997; McMeekin et al., 2002; McMeekin et al., 2000). Microbial growth/no growth models quantify the combined effect of various hurdles on the probability of growth and define combinations at which growth ceases. Consequently, such models may lead to a more realistic estimation of food safety risks, and can provide useful quantitative data for the development of processes that allow production of safer food products.

Prediction of microbial growth limits is based on probability models. Genigeorgis et al. (1971) introduced the concept of probability of pathogen outgrowth in order to predict the combination of conditions that prevented growth and toxin production of *S. aureus*. In that study, the probability of a single cell initiating growth was calculated by dividing the number of cells having initiated growth by the total number of cells and modelled using linear regression with polynomial terms. However, the use of linear regression for modelling bounded variables such as growth/no growth data presents several disadvantages and usually results in poor goodness of fit (Zhao et al., 2001). Ratkowsky and Ross (1995) proposed the application of logistic regression for modelling the boundary between growth and no growth. In the latter study, a kinetic model was modified such that it could predict the probability of growth using linear logistic regression with the cardinal parameters treated as fixed values. Later, Salter et al. (2000) used the non-linear logistic regression, which allows estimation of all parameters of the model. The advantages and problems of using non-linear logistic regression in predictive microbiology are extensively discussed in a publication by Ratkowsky (2002).

In recent years several models for microbial growth limits have been published and some of them have been incorporated in the predictive software tools described in Chapter 3.1. A list of available growth/no growth interface models of pathogenic bacteria is provided in Appendix D, Table 21. The use of these models can provide valuable information on the ability of pathogens to grow during distribution and storage of foods and the assessment of risk. Figure 4 presents an example of the growth/no growth region of *Listeria monocytogenes* at various environmental conditions predicted by the available tools and Table 6 shows the pH limits for growth ( $P = 0.5, 0.1, 0.01$ ) of the same pathogen at various  $a_w$  and temperature conditions. Further examples for other pathogens are provided in Appendix D, Figure 15 and Table 19.

In addition to temperature, pH and  $a_w$ , absence of oxygen is a condition determining *C. botulinum* growth and toxin production in foods. Vacuum packaging and some modified atmosphere packaging systems of composite products would create conditions favourable for toxin production whenever other factors permit growth.

**Figure 4:** Growth/no growth response of *L. monocytogenes* under various environmental conditions predicted by the Microbial Responses Viewer (MRV). Points represent observed growth (●) and no growth (○). The size of the points indicates the number of experiments. The different colours indicate the predicted growth rate by the MRV in h<sup>-1</sup>: ■: <-0.3; ■: -0.1 to 0.1; ■: 0.1 to 1 (growth); ■: >1 (growth).



**Table 6:** Predicted pH limits for growth (P = 0.5, 0.1, 0.01) of *L. monocytogenes* (Koutsoumanis et al., 2004) at various a<sub>w</sub> and temperature conditions.

a <sub>w</sub>	Predicted pH limits at various a <sub>w</sub> , temperature and P values											
	5°C			10°C			15°C			25°C		
	0.5	0.1	0.01	0.5	0.1	0.01	0.5	0.1	0.01	0.5	0.1	0.01
0.99	4.76	4.69	4.61	4.45	4.39	4.34	4.29	4.24	4.19	4.23	4.19	4.14
0.98	4.84	4.77	4.69	4.53	4.47	4.41	4.37	4.32	4.26	4.32	4.28	4.23
0.97	4.96	4.87	4.79	4.62	4.56	4.49	4.46	4.41	4.35	4.43	4.38	4.33
0.96	5.10	5.00	4.91	4.73	4.66	4.60	4.56	4.51	4.44	4.54	4.48	4.43
0.95	5.28	5.16	5.05	4.86	4.78	4.71	4.68	4.61	4.55	4.66	4.60	4.54

### 3.3.1. Concluding remarks

Temperature, pH and  $a_w$  are the main factors to consider in assessing whether pathogenic bacteria will grow in foods. Refrigeration alone will not ensure absence of growth of *L. monocytogenes* and *Y. enterocolitica* (see Table 2). The conditions specified in Regulation (EC) No 2073/2005<sup>10</sup>, on microbiological criteria for foodstuffs, for foods not permitting growth of *L. monocytogenes* (i.e.,  $\text{pH} \leq 4.4$  or  $a_w \leq 0.92$ , or  $\text{pH} \leq 5.0$  and  $a_w \leq 0.94$ ) will not inhibit growth of all pathogenic bacteria, since *S. aureus* grows at lower  $a_w$ . Considering each factor individually, with all others set at optimum, composite products will not permit growth of (or toxin production from) any of the pathogens listed in Table 1, if:

- they are kept frozen, or
- have a pH below 3.7, or
- have an  $a_w$  below 0.88.

Considering only pathogens for which growth in food is usually required in order to cause illness (see Table 1), the pH limit for growth would be 4.3.

The growth domain may be narrowed if only spore-forming bacteria are considered (i.e., food heated without possible recontamination) and no growth would occur if:

- they are kept below 3°C, or
- have a pH below 4.3, or
- have an  $a_w$  below 0.92.

Any combination of these factors, or addition of other factors (e.g. food preservatives, use-by date), must be assessed on a case-by-case basis, with the models and tools described in this Opinion providing a first estimation of the probability of growth.

### 3.4. Growth models of pathogenic microorganisms in food

For pathogens able to grow on a food product, the extent of growth during distribution and storage is among the most significant risk factors, in particular for those pathogens for which growth is usually required to cause illness (see Table 1). Mathematical models predicting the growth of pathogens in foods can provide valuable information for risk assessments.

The development of a predictive model for microbial growth includes the following steps: a) the experimental design and the collection of data, b) the description of the response of microorganisms to a single set of conditions over time using “primary” models and the estimation of the growth parameters (i.e., growth rate, lag phase), and c) the use of secondary models that describe the effect of environmental conditions, e.g. physical, chemical, and biotic features, on the values of the parameters of the primary model.

A primary model for microbial growth aims to describe the kinetics of the process with as few parameters as possible, while still being able to accurately define the distinct stages of growth. The most commonly used primary models are the logistic, the Gompertz, the Baranyi and Roberts, the Buchanan three phase linear, and the McKellar models.

A brief description of the various models is provided in Appendix D, together with an overview of the growth models for pathogenic bacteria published in the literature (see Appendix D, Table 22). These

<sup>10</sup> OJ L 338, 22.12.2005, p.1-26, as last amended.

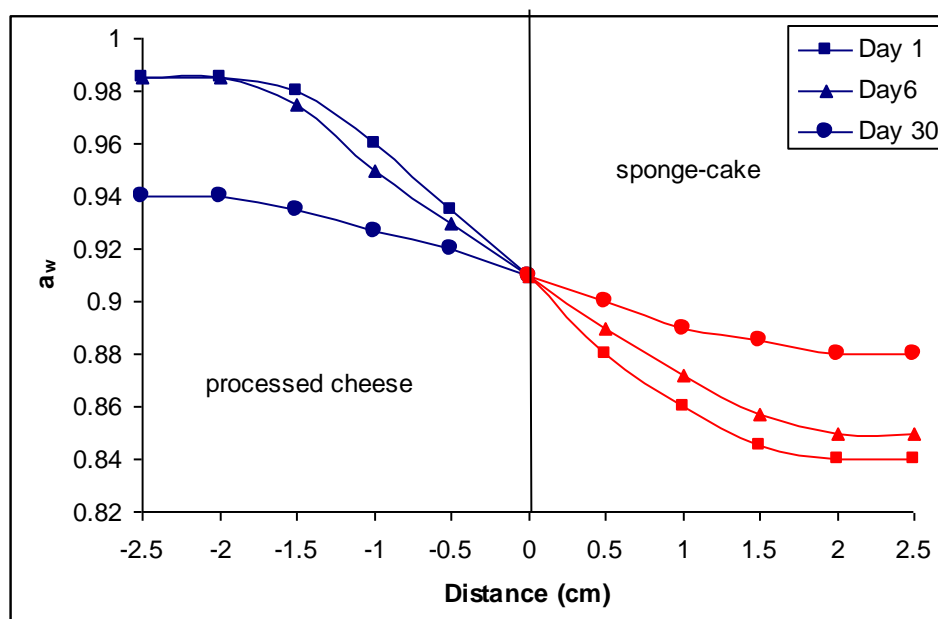
models can be used to estimate the extent of growth of pathogenic bacteria in a food based on the storage conditions (“extrinsic” factors) and product characteristics (“intrinsic” factors). Examples of the predicted growth rate of selected pathogens at various temperature, pH and  $a_w$  conditions are presented in Appendix D, Figures 16, 17 and 18.

#### 4. The dynamic environment of composite products

Many composite products are heterogeneous (e.g. composed of ingredients with different physico-chemical properties), and variable over time (e.g. physico-chemical properties changes over time). The most favourable physico-chemical conditions for survival and growth of pathogens must be considered in evaluating potential risks.

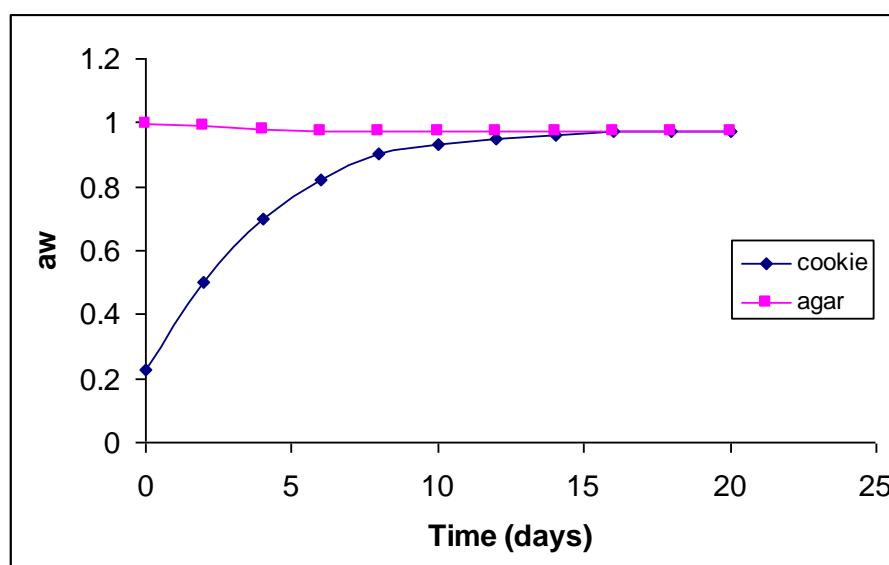
Composite products are multi-domain systems with dynamic environments. Loss or gain of moisture as well as migration of acids and other antimicrobial compounds from one region or food component to another region will continuously occur in order to reach equilibrium with the surrounding food components and the environment. Migration phenomena in composite products are influenced by several factors. For example, the two main factors affecting moisture migration are the  $a_w$  equilibrium (thermodynamics) and factors affecting the diffusion rate (dynamics of mass transfer). Multi-domain foods with regions formulated to different  $a_w$  cause the whole system to be in a non-equilibrium state. This will result in moisture migration from the higher  $a_w$  (higher chemical potential) to the lower  $a_w$  region. In this case both the equilibrium properties and the kinetics of water transport are of outmost importance for microbial stability.

The dynamic environment of composite foods has been investigated extensively in past years with particular emphasis on the kinetics of water transport between the different components. Hong et al. (1986) described a model for predicting moisture transfer in dried fruits and almonds mixture, Karathanos and Kostaropoulos (1995) discussed water diffusion in dough/raisin system, and Guillard et al. (2003a) validated a model for predicting moisture transfer in agar gel/sponge-cake composite product. Figure 5 shows the moisture dynamics in a real food composed of commercial processed cheese in contact with sponge cake, simulated based on data from Guillard et al. (2003b). As it is shown, there is a time-dependent decrease in the moisture content of the processed cheese of high initial  $a_w$  (0.985) and a respective rapid increase in the sponge cake of lower initial  $a_w$  (0.840). Closed to the interface (distance = 0) the  $a_w$  values of the two components at equilibrium are equal.



**Figure 5:** The  $a_w$  distribution in a processed cheese/sponge cake composite product at different time (simulation based on data from Guillard, et al. (2003b)).

Similar results were reported by Roca et al. (2008) for the dynamics of moisture equilibration in a two-components food systems, constituted of a cookie of initial  $a_w$  of 0.23 in direct contact with an agar gel of initial  $a_w$  of 0.999, simulating a high moisture cookie filling (see Figure 6).



**Figure 6:**  $a_w$  evolution in cookie (initial  $a_w=0.23$ ) in contact with agar gel (initial  $a_w=0.999$ ) at 20°C (simulation based on data from Roca et al. (2008)).

In a composite food system, an increase of water content in the dry, low  $a_w$  region, where no antimicrobial systems are present, could allow for microbial growth if  $a_w$  increases above the minimum limit for pathogen growth (see Table 2). Similar phenomena may occur in the case of migration of acids, preservatives and other compounds affecting microbial growth.

Based on the above, the dynamic environment of multi-domain foods should always be considered when evaluating their microbial stability and safety. For example, in evaluating the compliance of ready-to-eat composite products with Regulation (EC) No 2073/2005 safety criteria for *L. monocytogenes*, changes in product characteristics with time should not be ignored. For the purpose of this Regulation, ready-to-eat foods are divided into those that are able to support growth of *L. monocytogenes* and into those that are not. Products with  $\text{pH} \leq 4.4$  or  $a_w \leq 0.92$ , products with  $\text{pH} \leq 5.0$  and  $a_w \leq 0.94$  are automatically considered as unable to support growth of *L. monocytogenes*. For ready-to-eat foods that are able to support growth of *L. monocytogenes*, the Regulation demands the absence of the pathogen (in 25 g) “before the food has left the immediate control of the food business operator, who has produced it”, but allows up to 100 CFU/g for “products placed on the market during their shelf-life”. The Regulation indicates that predictive microbiology can be used for the estimation of pathogen’s growth. In the case of composite products, however, the environment ( $\text{pH}$ ,  $a_w$ ) may not be constant. When the dynamics of the environment and its effect on growth of *L. monocytogenes* is unknown, the most favourable conditions for growth must be used as a basis for evaluating compliance with safety criteria. Specific attention should be given to the interfaces between ingredients or components in a composite product, considering that combinations of components may create more favourable conditions for growth compared to those in the individual components. For example, contact between two ingredients, one with a low  $a_w$  and a neutral  $\text{pH}$ , and one with high moisture and low  $\text{pH}$  (e.g. sandwiches with some types of cheese slices and tomato slices), may permit neutralisation of the low  $\text{pH}$  and increase of the  $a_w$  at the interface. Both ingredients in isolation would not permit growth of bacterial pathogens for different limiting factors, but at the interface equilibration could occur and permit growth.

## 5. Identifying microbiological hazards for certain composite products

Data reported in the following sections are extracted from specific databases, as explained below. A description of the type of products for which data is reported in those databases and of their ingredients (including their relative amount) is normally not provided. This, together with the fact that precise definitions of the different categories of composite products discussed in this Opinion is not always available, makes it sometimes difficult to classify the products into one or another of those categories. For example, some products could be classified into “cakes” or “confectionery” when summarising the data. In addition, based on their ingredients and relative amounts, some of them could have been excluded from the remit of this specific Opinion (e.g. in case they were containing more than 50% of ingredients of animal origin).

Most of the information reported below originates from data collected at EU level. The distribution and prevalence of microbial pathogens in food, as well as sources and frequency of food-borne outbreaks may differ from the ones related to potential exporting countries of composite products towards the EU. This has to be borne in mind when considering the data reported below and any resulting conclusions, which are used only to inform the identification of microbiological hazards in certain composite products.

### 5.1. Data from the EU Summary Report

Data in relation to zoonoses, zoonotic agents, antimicrobial resistance and food-borne outbreaks are collected every year in the European Union (EU), as prescribed by Directive 2003/99/EC<sup>11</sup>. Those data are elaborated and published yearly by EFSA and ECDC in a summary report and include information on the zoonotic agents reported in food by the EU Member States.

A search among the published data available (years 2004-2009) has been performed in order to collect information on the prevalence of zoonotic agents and verified food-borne outbreaks reported in the composite products included in Term of Reference 2 of the mandate for this Scientific Opinion. The search was performed by looking at the relevant reporting food categories as well as at the free comments given by the reporting authorities when reporting information, so to maximise the probabilities of detecting the information on the composite products of interest.

Although information on many of those composite products is scarce, for some others data are available, and are summarised in this chapter.

#### 5.1.1. Prevalence data (2004-2009)

Globally, data in relation to the following composite products were reported by Member States: bread, cakes, other bakery products, chocolate, confectionery, sweets, gelatine (not specified if part of gelatine capsules), stuffed olives (not specified if stuffed with fish), pasta, noodles, dehydrated soups and flavourings. However, for several of the above-mentioned categories, a limited amount of data was available. No data at all were available in relation to the other composite products (food supplements, meat extracts and meat concentrates). Overall prevalence data were collected in relation to the following zoonotic agents: *Salmonella*, *Campylobacter* spp., pathogenic *E. coli*, *L. monocytogenes*, *Enterobacter sakazakii* (now *Cronobacter* spp.), *S. enterotoxins* and *Y. enterocolitica*.

When considering positively reported zoonotic agents in bakery products (bread, cakes and other bakery products), *Salmonella* and *L. monocytogenes* are the only agents reported, with prevalences lower than 1%: *Salmonella* 0.3% (out of a total of 27,153 samples), *L. monocytogenes* 0.6% (out of 5,244 samples).

In chocolate, *Salmonella* (0.1%, out of 1,120 samples), *Staphylococcus enterotoxins* (14.3%, out of 28 samples) and histamine (100%, out of 10 samples) were reported.

<sup>11</sup> OJ L 325, 12.12.2003, p.31-40, as last amended.

In confectionery and sweets, *Salmonella* (0.6%, out of 9,838 samples), *L. monocytogenes* (1.0%, out of 3,959 samples), *Y. enterocolitica* (1.7%, out of 180 samples) and *Staphylococcus* enterotoxins (13.5%, out of 37 samples) were reported.

In pasta and noodles, the following zoonotic agents were positively reported: *Salmonella* (0.7%, out of 3,557 samples), pathogenic *E. coli* (2.5%, out of 120 samples), *L. monocytogenes* (2.1%, out of 941 samples).

The detailed data available for the different composite products are reported in Appendix E, including combinations of composite products and zoonotic agents for which 0% positive samples were recorded. When looking at prevalence data, it should be borne in mind that the data are collected in the framework of surveillance and monitoring schemes which are not harmonised between EU Member States, and often also between different years in the same Member State, in terms of food categories sampled, number and type of samples collected, methodologies used etc. Therefore the data are clearly not representative of the situation in the EU with regards to the prevalence of zoonotic agents in the various food categories. The small number of samples taken from the composite products of interest compared to other types of food should also be considered.

### 5.1.2. Data and confirmed/verified food-borne outbreaks (2004-2009)

With regard to reporting of food-borne outbreaks in the EU, starting from 2007 new reporting specifications have been applied. Since then Member States need to report detailed data about the type of food involved in the outbreaks and the causal zoonotic agents only for those outbreaks where the link between human cases and implicated foodstuffs is established by laboratory analysis or epidemiological evidence (“verified outbreaks”), while detailed data on the other outbreaks (“possible outbreaks”) are not reported. This was not the case in previous years, when details were reported for both types of outbreaks (called, at the time, “confirmed outbreaks” and “suspected outbreaks” respectively). In order to increase harmonisation among the data reported along the years, only data in relation to confirmed outbreaks were considered for years 2004-2006 when extracting data for the composite products of interest.

Globally, data on verified outbreaks were found in relation to biscuits, bread, cakes and other bakery products, chocolate, confectionery, pasta and noodles. No data were available for the other categories of composite products (gelatine capsules, food supplements, meat extracts, meat concentrates, olives stuffed with fish, soup stocks and flavourings).

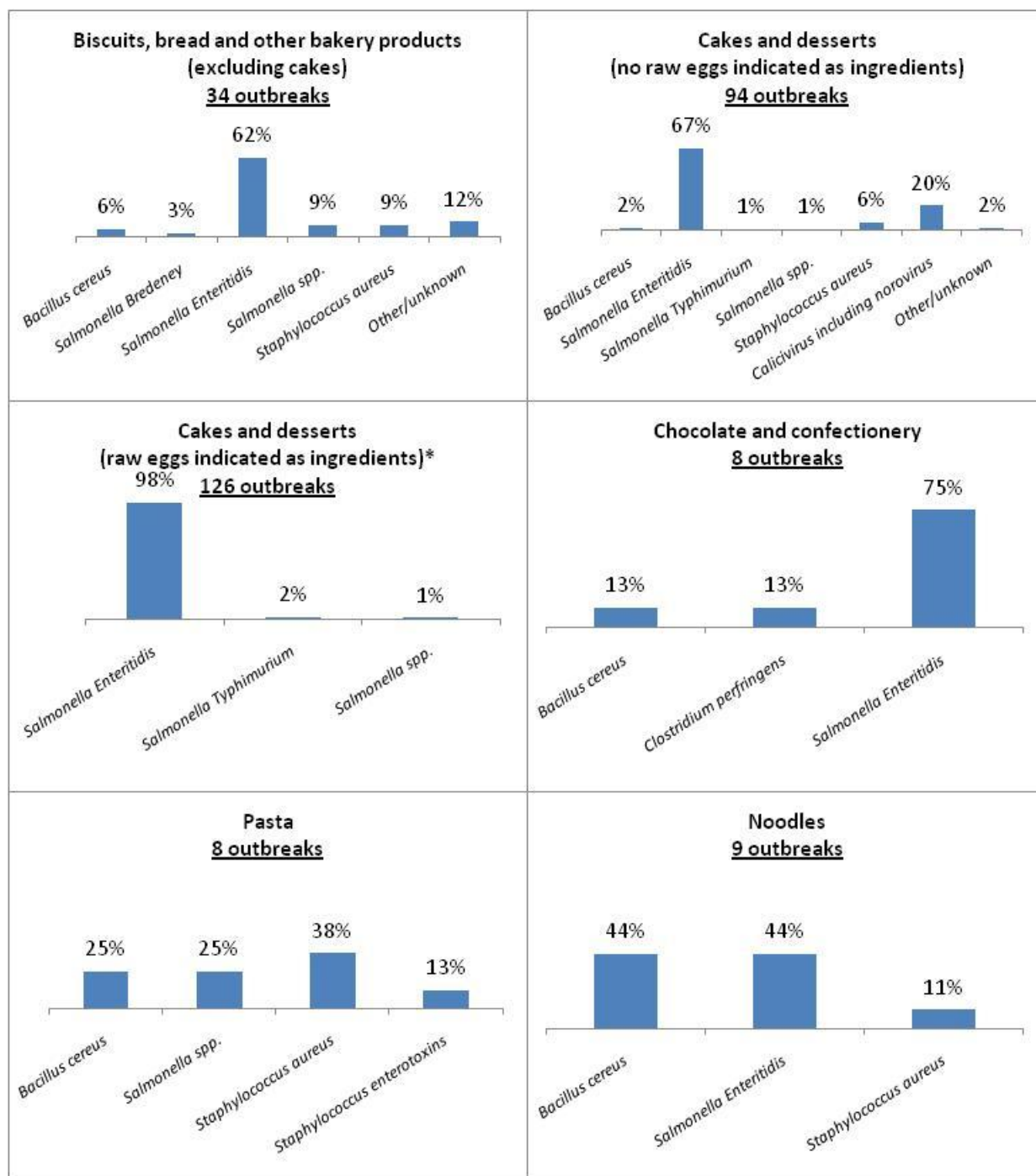
In those categories of composite products, a total of 279 outbreaks was reported, with 5,929 associated cases of human illness. It is noted that 79% of those outbreaks were linked to cakes (in more than half of those outbreaks, the source identified was cakes and desserts with presence among ingredients of raw eggs and that were not submitted to subsequent processing) and 91% to bakery products in general. Cakes containing raw eggs among their ingredients and that are not processed before consumption are not composite products as defined by Decision 2007/275/EC, since they contain unprocessed products of animal origin. However, it can be difficult to distinguish between those products and the ones containing processed eggs or processed after inclusion of raw eggs. In the case of outbreaks, the distinction is only possible according to the epidemiological information reported, and such details are not always available in the EU database considered. In addition, such distinction is not available with reference to the information provided in the other sections of this Chapter, in particular for prevalence and RASFF data. For this reason, available information on cakes containing raw eggs and not processed before consumption is described and further considered.

Zoonotic agents implicated include: *Salmonella*, *S. aureus*, *Staphylococcus* enterotoxins, *B. cereus*, Norovirus, *C. perfringens*. In particular, *Salmonella* Enteritidis was implicated in 78% of the verified outbreaks reported and in 70% of the cases of illness reported. Overall, 10 deaths were reported as linked to the reported outbreaks, 9 of which in *Salmonella*-related outbreaks and 1 in *Staphylococcus* enterotoxins-related ones.



Figure 7 summarises the proportion of outbreaks related to the different zoonotic agents in the composite products for which data were available. More detailed data are reported in Appendix F.

It is noted that the number of reported outbreaks depends not only on the real number of outbreaks occurring, but also on the efficiency of the national system for investigation, identification and reporting of the outbreaks in place in the different EU Member States.



\* Cakes and desserts containing raw eggs are not composite products since they contain unprocessed products of animal origin.

**Figure 7:** Number and proportion of reported verified outbreaks due to different zoonotic agents in certain composite products in the EU in 2004-2009.

## 5.2. Data from the Rapid Alert System for Food and Feed

Alerts notified by EU Member States and the European Commission through the Rapid Alert System for Food and Feed (RASFF) can also provide information about hazards identified in food and feed from local authorities while performing checks in food (and feed) products in several steps of the food chain (e.g. during the production process, in the market, at EU borders etc.). Alerts notified in the period 2001-2011 have been screened to find out information on hazards identified in the list of composite products provided in Term of Reference 2. However, very few data are available (see Appendix F for details) and only in relation to certain products, i.e. biscuits, cakes, chocolate, confectionery and sweets, food supplements, pasta and noodles. Hazards identified include bacterial agents already described in the prior sections for prevalence and outbreak data, and are qualitatively reported in Table 7. It should be also noted that data from RASFF are not exhaustive and suffer from a number of reporting limitations (EFSA, 2010) and are not representative of the real situation.

**Table 7:** Microbiological agents reported in RASFF alerts from 1 January 2001 to 16 August 2011 in certain composite products.

Agent	Cakes, biscuits and other bakery products (7 alerts)	Chocolate (8 alerts)	Confectionery including sweets (10 alerts)	Food supplements (8 alerts)	Pasta and noodles (6 alerts)
<i>Bacillus cereus</i>	√			√	√
<i>Clostridium</i> spp.	√				
<i>Escherichia coli</i>	√			√	√
<i>Listeria monocytogenes</i>	√	√			
<i>Staphylococcus</i> spp.	√	√	√	√	√
<i>Salmonella</i> spp.	√	√	√	√	√

## 5.3. Other data available in the literature

### 5.3.1. Biscuits, bread, cakes, chocolate, pasta and noodles

Few outbreaks are documented in these products (see Table 8). The most frequent hazard is *Salmonella* in bakery products such as cake, bread and chocolate, originating from raw eggs used for the icing<sup>12</sup> or from food handlers. Staphylococcal enterotoxin comes second, in cakes, chocolate, pasta or noodle, mostly due to contamination by food handlers. Other hazards seem to be anecdotal, except viruses in bread and cakes.

<sup>12</sup> In such case the product would not be a composite product, since it would contain unprocessed products of animal origin.

**Table 8:** Microbiological hazards reported in scientific literature in certain composite products (biscuits, bread, cakes, chocolate, pasta, noodles).

Hazards	Composite products					
	Biscuits	Bread	Cakes	Chocolate	Pasta	Noodles
<i>B. cereus</i>		(Agata et al., 2002; Bailey and von Holy, 1993; Rosenkvist and Hansen, 1995)	(Agata et al., 2002)	(Agata et al., 2002; Nelms et al., 1997)	(Agata et al., 2002; Delbrassinne et al., 2011; Dierick et al., 2005)	(Agata et al., 2002; Takabe and Oya, 1976)
<i>Escherichia coli</i> STEC			(O'Brien et al., 2001)			
<i>Streptococci</i> (Group A)					(Falkenhorst et al., 2008)	
<i>Listeria monocytogenes</i>				(Dalton et al., 1997)		
<i>Salmonella</i>	(Carbó Malonda et al., 2005)	(Kimura et al., 2005; Liu et al., 2006; Lu et al., 2004; Milazzo and Rose, 2001; Solhan et al., 2011)	(Ager et al., 1967; Cinquetti et al., 1991; D'Argenio et al., 1999; Evans et al., 1996; Fernández de la Hoz Zeitler et al., 1994; Fielding et al., 2003; Frank et al., 2007; Gill et al., 1983; Harvey et al., 1961; Mearin et al., 2005; Mürmann et al., 2008; Stephens et al., 2007; Tribe et al., 2003; Tsuji et al., 2000; Van de Giessen et al., 1992; Ward et al., 2002; Zhang et al., 2007)	(Cordier, 1994; Craven et al., 1975; D'Aoust et al., 1975; Kapperud et al., 1989; Werber et al., 2005)	(Hauri et al., 2004; Rohde et al., 1975)	(Tribe et al., 2001)
<i>Staphylococcus aureus</i>			(Corpening and Foxhall, 1935; Costanzo Anunciação et al., 1995; Coughlin and Johnson Jr, 1941; Escartín et al., 1998; Stewart et al., 2003)	(Evenson et al., 1988)	(Matějovská et al., 1972; Woolaway et al., 1986)	(de Jong et al., 2004)
<i>Yersinia enterocolitica</i>				(Black et al., 1978)		(Morse et al., 1984)
<i>Virus</i>		(Warburton et al., 1991)	(Deneen et al., 2000; Friedman et al., 2005; Le Guyader et al., 2004; Murao, 1991 )			

### 5.3.2. Confectionery (including sweets)

**Table 9:** Microbiological hazards reported in scientific literature in various confectionery products.

Hazards	Composite products			
	Ice-creams	Cream pastries	Custard	Miscellaneous (vanilla pudding, vanilla sauce)
<i>B. cereus</i>				(Kramer and Gilbert, 1989)
<i>E. coli</i> verotoxigenic	(De Schrijver et al., 2008)			
<i>Listeria monocytogenes</i>	(Andre et al., 1990)			
<i>Salmonella</i>	(Buckner et al., 1994; Djuretic et al., 1996; Dreher and Lehnbecher, 1981; Gunn and Markakis, 1978; Hennessy et al., 1996; Morgan et al., 1994)	(Wichmann-Schauer et al., 2006)	(Barnes and Edwards, 1992)	
<i>Staphylococcus aureus</i>		(MMWR, 1983; Pereira et al., 1994b)	(Beckers et al., 1980)	

The confectionery products implicated in the outbreaks summarised in Table 9 contained presumably more than 50% of foods of animal origin (milk and eggs) and are likely not within the Terms of Reference of the mandate for this Opinion. However, similar risk factors could lead to outbreaks for confectionery prepared under similar conditions but with lower content of foods of animal origin. Whenever identified, the origin of the contamination of the confectionery product was, in most *Salmonella* outbreaks, uncooked or lightly cooked eggs. Food-handler was the source of the contamination with *E. coli* (De Schrijver et al., 2008). Temperature abuse during food distribution (MMWR, 1983) was suspected to have caused one *S. aureus* outbreak, whereas in Pereira et al. (1994a) toxin production by *S. aureus* was likely due to the difficulty to refrigerate rapidly enough a large cake containing cream. Cross-contamination with containers having contained raw egg products presumably caused one *Salmonella* outbreak (Hennessy et al., 1996). The *Salmonella* and *E. coli* outbreaks linked to ice-creams, where no multiplication of the pathogen could occur, highlight the fact that very low amount of some bacteria can cause human infection. For instance, 25 cells of *Salmonella* Enteritidis were estimated per serving of ice-cream associated with an outbreak (Vought and Tatini, 1998).

An outbreak of *Salmonella* was linked to halva, a candy made from sesame seeds (Brockmann et al., 2004). Halva may contain some ingredients of animal origin in some recipes, but this was not specified in the publication.

Presence of *L. monocytogenes* (Miettinen et al., 1999) and *B. cereus* (Messelhauser et al., 2010; Zhou et al., 2010) was reported in ice-creams not associated to food-borne diseases. *Salmonella* was isolated from halva not associated with food-borne diseases (Brockmann et al., 2004).

Raw eggs, for *Salmonella*, and raw dairy product, for *L. monocytogenes*, verotoxigenic *E. coli* and *Campylobacter*, are a known source of food-borne diseases. These microbiological hazards can be reduced efficiently by pasteurization of egg and dairy products. Other ingredients used in confectionery have been the cause of food-borne outbreaks, in particular *Salmonella* outbreaks linked to dry nuts, dry seeds and nuts or seed preparations (Isaacs et al., 2005; Kirk et al., 2004; MMWR, 2007, 2009a; Müller et al., 2007; Unicomb et al., 2005).

A similar risk must be considered for confectioneries prepared with these ingredients without a sufficient bactericidal treatment.

Frozen soft fruits have caused several hepatitis A and Norovirus outbreaks (EFSA Panel on Biological Hazards (BIOHAZ), 2011). These food-borne viruses could be transmitted to confectionery if not inactivated by the process applied.

### **5.3.3. Gelatine capsules**

No reports of outbreaks or food-borne diseases linked to gelatine capsules or other gelatine products were found.

In one study, *B. cereus* was detected in 48% of the gelatine samples considered (Reekmans et al., 2009), without association to food-borne illness.

### **5.3.4. Food supplements packaged for the final consumer, containing small amounts of animal product, and those including glucosamine, chondroitin, or chitosan**

One case of listeriosis was caused by the consumption of alfalfa tablets (Farber et al., 1990) and some liver injury cases were attributed to the presence of toxigenic *B. subtilis* and *B. cereus* in a food supplement consumed by the patients (Stickel et al., 2009). The composition of the food supplement was not detailed in the reports, but they were likely mostly composed of botanicals.

No reports of food-borne outbreaks or diseases were linked to food supplements indicated as containing products of animal origin. No reports of disease linked to food supplements containing glucosamine, chondroitin, chitosan, or linked to these compounds alone were found. No reports on the prevalence of food-borne pathogens in these ingredients were found.

### **5.3.5. Olives with fish**

No reports of food-borne outbreaks or diseases for olives with fish were found.

However, processed anchovies, frequently used to stuff olives, have been associated once in a *Salmonella* outbreak (Ling et al., 2002). More generally, fish and processed fish have been the cause of outbreaks of salmonellosis (Guerin et al., 2004), of listeriosis (Elliot and Kvenberg, 2000; Rocourt et al., 2000; Tham et al., 2000) and of botulism (Cohen et al., 1988; Dine et al., 1981; French et al., 1992; MMWR, 1991; Sobel et al., 2007; Telzak et al., 1990; Weber et al., 1993).

Fish can also be a source of parasites for human whenever it is not adequately treated (EFSA Panel on Biological Hazards (BIOHAZ), 2010).

Preserved olives have caused a few outbreaks of botulism (Cawthorne et al., 2005; Fenicia et al., 1992; Horowitz, 2011). Biogenic amines (putrescine, histamine, tyramine) have been found in preserved olives, depending on the process and stage of processing or storage (Garcia-Garcia et al., 2000; Garcia-Garcia et al., 2001; Garcia et al., 2004).

### **5.3.6. Meat extracts, meat concentrates, soup stocks and flavourings packaged for final consumer, containing meat extracts, meat concentrates, animal fats, or fish oils, powders or extracts**

*Meat extracts* are pasty solids derived from high heat (130-140°C) liquid extraction of beef muscle or liver tissues or bones followed by concentration (Lund et al., 2000; Ockerman and Pellegrino, 1988). Meat extracts or extractives should not be confused with meat juice, a pressed fluid that contains constituents not found in meat extract (Ockerman and Pellegrino, 1988).

According to Ockerman and Pellegrino (1988), *broth* (beef or pork) or *stock* (beef or pork) are broths produced by simmering (100-140°C for 3-4 hours) meat and/or bones in water with seasonings.

*Beef concentrates* contain 3-4% solids and are derived from cooking fresh beef, which is followed by centrifuging and evaporating the stock under vacuum to 60% solids. The liquid portion is then salted to 25.5% of the water weight. Thus, 100 parts of concentrated (60% solids) stock contain 10.2 parts of salt or 9.3% added salt (Ockerman and Pellegrino, 1988).

Meat extracts, concentrates, broth and stock, are used as foundation and major flavouring ingredients in bouillon cubes, soups, sauces and gravies (Aberle et al., 2001; Lund et al., 2000).

*Lard* and edible *tallow* are produced by dry or moist (wet) rendering processes. Wet rendering involves heating fatty tissues in presence of water at temperatures (80-88°C), usually lower than those used in dry rendering (100-120°C). Wet rendering produces prime steam lard, while dry rendering is used for almost all inedible tallows and greases, and some lard (Gill, 1988; Morton et al., 1988). Tallow is produced by rendering animal fats usually in low temperature rendering systems, and the liquid material is then centrifuged to separate the oil, which is pumped through a horizontal filter press into an insulated and heated storage tank. Additional processing steps involved in lard and tallow productions are hydrogenation for removal of monoglyceride impurities, bleaching, and deodorization. Finally, lard is interesterified and tallow is fractionated (Morton et al., 1988).

Published information on the microbiology of meat extracts, meat concentrates, soup stocks and flavourings packaged for final consumer use, and containing meat extracts, meat concentrates, animal fats, or fish oils, powders or extracts is rather scarce and not very recent.

While heat treatment is adequate to render derived meat extracts sterile, subsequent handling and exposure to contaminated equipment may allow recontamination, which may increase during concentration through evaporation. Therefore, there may be considerable variation in microbiological contamination, which may be present in such products.

As recommended by Ockermann and Pellegrino (1988), proper cleaning and disinfection of equipment is very important for maintenance of the microbiological quality of meat extracts and related products. According to these authors, empty tanks and pipelines should be filled with 5 ppm chlorinated water and held until the next use. In addition, every 15 days, the complete system should be completely drained, rinsed with hot water, and filled with 0.3% caustic soda solution; this mixture should remain in the equipment for 2 hours. Then the system should be drained and rinsed with hot water, and filled with 5 ppm of cold chlorinated water until it is drained and rinsed immediately prior to reuse.

Moisture content of 16% allows meat extract to be usually stable, while at 20% moisture there is a need for higher salt levels to protect the extract. The thermophilic or thermoduric bacteria that may survive processing are not expected to grow during storage of 16% moisture product, but may serve as inoculum when combined with other foods of higher moisture content in composite products. Meat extract is an excellent microbial culture medium when diluted with water and exposed to air. Overall, increased moisture content and higher storage temperature accelerate bacterial growth in meat extract products (Ockerman and Pellegrino, 1988).

Typically meat extracts have an  $a_w$  of <0.80 and may contain 12-20% added salt (Lund et al., 2000). Other sources indicate that the composition of the meat extract is usually 18-20% moisture, 56-60% protein, 9-25% ash including 4-5% salt (when salt is added the total level increases to 9-10% in stocks and 12-18% in dried products), 1-2% water soluble materials, fat generally less than 2% and usually less than 1%, and pH of 6.0-6.5 (Ockerman and Pellegrino, 1988). According to these authors, commercial meat extractives such as beef stock, natural beef flavouring, pork stock, natural pork flavouring, natural ham flavouring, and chicken broth should have Standard Plate Count (SPC) <3,000/g and be *Salmonella* negative.

Meat extract is usually contaminated with spores of bacteria and mould spores. While pathogens are seldom found, the potential for their presence exists. It is important to exercise every cleaning and disinfection precaution to minimize contamination (Ockerman and Pellegrino, 1988).

As reported by Ockerman and Pellegrino (1988), average microbial counts (cfu/g) of meat extracts are:

- In Argentine product: soup going to evaporator: TPC, 1,300/g; concentrated soup out of evaporator: TPC, 2,000-30,000/g; thermophiles, 500/g; anaerobes, 100/g; coliforms, 0/g; *C. perfringens*, negative in 0.1 g.
- In meat extract out of a can: TPC, 100,000/g (30°C), 237,500/g (55°C); putrefactive anaerobes, 927/g; indole producers, absence (sometimes present); H<sub>2</sub>S producers, absence in 25g (sometimes present); *Staphylococcus* group, absence in 25g; *Salmonella-Shigella* group, absence in 25g.
- In American product: TPC, <1,000/g; coliforms, <10/g; *E. coli*, negative/g; *S. aureus*, <10/g; *Salmonella*, negative/25g; flat sour spores, <50/10g; total thermophilic spores, <12/10g.

Pressurized heating in tallow processing could result in sterilization, but the preceding removal of much of the water results in phase inversion, which incorporates the spores in the oil phase, protecting them from heat inactivation in the dry environment (Gill, 1988). Heating to 100°C may allow survival of thermoduric spores, while lower temperatures may lead to spore activation. In general, heating to 65-70°C or above for at least 10 min should destroy all vegetative microorganisms including pathogenic cells, while marginal under-processing may allow survival of thermoduric enterococci and lactobacilli when present in high numbers (Gill, 1988). Survivors in pasteurized tallow may include *Bacillus* species and very small numbers of *clostridia* and thermoduric *enterococci* (Gill, 1988).

Therefore, adequate heat treatments of edible by-products should destroy microorganisms with the exception of thermoduric spores, but the surfaces of cooked by-products become recontaminated from hands and equipment surfaces during subsequent handling. The microbial flora is variable, depending on sources of contamination, but is generally comparable with that of the corresponding raw materials (Gill, 1988).

In general, meat products produced by high temperature rendering may be considered as sterile. Contamination, if present, usually consists of factory environmental contaminants such as non-pathogenic halotolerant species of bacteria, yeasts and moulds. Some of these halophilic microorganisms can multiply at  $a_w$  of 0.71, much lower than pathogenic bacteria (see Table 2). In case of unintentional or intentional hydration during storage they are the most likely to multiply causing spoilage of the product (Gill, 1988; Lund et al., 2000).

Overall, commercial rendering operations are expected to produce tallows containing bacterial spores, at numbers that depend on the initial number of spores in the feedstock material. However, spores cannot grow in dry fat, but they may grow in compounded products or when included in formulations with moist materials in processed foods (Gill, 1988). It should be noted, however, that most tallows undergo further refining by treatment of molten fat with steam, acids, alkalis and absorbents. Such treatments reduce surviving spores numbers to levels negligible compared to contaminants present in foods where tallow is incorporated (Gill, 1988).

#### **5.4. Summary of available data concerning biological hazards-composite products associations**

The available information reported in the sections above was combined together in order to obtain an indication on the most important biological hazards to be considered when assessing the risk posed by certain composite products. The methodology developed in order to provide the hazard ranking included elicitation of experts.

Experts of the EFSA BIOHAZ Working Group on “Public health risks represented by certain composite products containing food of animal origin” were asked to independently provide criteria for judging the relevance of the data obtained from the four different sources of information available (EU prevalence data, EU outbreak data, EU RASFF alerts, papers published in the scientific literature) in the definition of the microbiological hazards of highest concern for the different composite products. The total scores resulting from the assessment of the individual experts for each combination hazard-composite product were normalised in a 1-100 scale and a global average score was calculated. The experts were then asked to provide thresholds to define the importance of the combination hazard-composite product (see results of the exercise in Table 10).

Very high importance of association can be illustrated for *Salmonella* and cakes (with and without raw eggs), for which there were 191 confirmed outbreaks (3,878 cases) in the EU from 2004 to 2009 (see Appendix F) and 17 citations in the scientific literature (see Table 8). The association of cakes with *S. aureus* (high importance of association) represented 6 outbreaks (46 cases) in the same period and 5 citations in the scientific literature. Cakes and *B. cereus* classified as low association represented 2 outbreaks (21 cases) and 1 citation in the scientific literature.

A number of limitations needs to be borne in mind when considering results from application of this methodology, such as:

- scores attributed and criteria used are by nature subjective;
- the number of experts involved was limited;
- in some cases interpretation of the data available is difficult, as already indicated in Chapters 5.1 to 5.3 above, for example:
  - prevalence data are not available or limited for a number of hazards and composite products, are not the result of a harmonised and representative sampling scheme;
  - reported outbreak data depend on the efficiency of the system in place in the different EU Member States;
  - RASFF alerts are a biased source of data; and
  - scientific papers were scored only by counting of the number of papers reporting evidence of an association between a hazard and a composite product, while a weight of the evidence provided by the different papers was not taken into account by the criteria.
- Some categories of composite products, in particular confectionery and cakes, encompass a wide range of processing conditions, and physico-chemical composition. Interpretation of the ranking should be done with caution as presumably it does not apply to all types of confectionery.
- Some composite products (e.g. olive with fish) may not be identified as such in the databases on prevalence and outbreaks. This may have caused a bias in the definition of the importance of the associations for those products. Past associations of pathogens with ingredients of such composite products are described in Chapter 5.3 and may be useful complementary information.
- In addition, few outbreaks linked to a composite product may be due to a low risk per serving of food or to a high risk per serving combined with a low consumption of this food.
- A high importance of a specific association does not mean that the composite product is always hazardous for consumers. It indicates that, due to its processing and composition, it can lead to food-borne illness in case of contamination. A good safety management system may nevertheless lead to safe composite products. Conversely, a low importance of the association may result from



good safety management in the past and this may change in case, for example, of new suppliers of the foods or ingredients.

- This exercise is not sufficient in itself to categorise composite products with respect to the risk of food-borne illness.

However, overall this exercise allowed combining the available data from different sources through a structured approach, based on the opinion of different experts, and is helpful when defining the relevance of microbiological hazards in certain composite products.

**Table 10:** Summary of the data available on the association between biological hazards and composite products according to EU prevalence and food-borne outbreak data (2004-2009), RASFF data (2001-2011) and scientific literature.

Hazards		Biscuits, bread and bakery products	Cakes (no raw eggs)*	Cakes (raw eggs)§	Chocolate	Confectionery and sweets	Pasta and noodles	Food supplements	Soup stocks and flavourings	Unfilled gelatine capsules	Olives with fish	Meat extracts and meat concentrates
Illness may occur without growth of hazards in food	viruses											
	<i>Salmonella</i>	some food types within these categories are not at high risk (see Chapter 6)				some food types within these categories are not at high risk (see Chapter 6)						
	<i>Campylobacter</i>											
	<i>Escherichia coli</i>											
	<i>Yersinia enterocolitica</i>											
Growth of hazards in food is usually required to cause illness	<i>Listeria monocytogenes</i>											
Growth of hazards in food is required for production of toxins or toxic metabolites that cause illness	<i>Clostridium</i>										olives stuffed with other product involved in outbreaks	
	<i>Bacillus cereus</i>											
	<i>Staphylococcus aureus</i>		no real higher risk than "cakes (no raw eggs)"									
	histamine											

No evidence of association

Evidence of medium importance of association

Evidence of high importance of association

Evidence of very high importance of association

\* Cakes (no raw eggs): includes several types of cakes in which the presence among ingredients of raw eggs associated with absence of subsequent processing was not explicitly indicated.

§ Cakes (raw eggs): includes cakes and desserts for which raw eggs were explicitly indicated among ingredients and that were not subsequently processed, and cakes and desserts for which this can be assumed. Cakes and desserts containing raw eggs among their ingredients and that are not subsequently processed are not composite products since they contain unprocessed products of animal origin.

## 6. Profiling microbiological hazards for certain composite products

### 6.1. Decision trees for categorisation of risk

Chapter 5 (see in particular Table 10) presents the hazards identified as associated with each category of composite products and an exercise to define the importance of the association. The information should be interpreted with caution because it is based on history, and results are influenced by many factors and circumstances, which are not known and may change in the future. In particular it integrates both prevalence of the pathogens and their past association with outbreaks with composite products, without attempting to determine which is more important.

A categorisation of risks based on the composite product parameters impacting on growth/survival of the hazards is therefore proposed in the present Chapter 6. This categorisation of the composite products should prevail over the results of the exercise presented in Chapter 5, because it is based on the food characteristics and can take into account the diversity of the composite products and possible future changes. This categorization is proposed to be based on developed decision trees (see Figures 8, 9 and 10) used to explain the approach, and on derived corresponding tables which are proposed for use in their implementation by risk managers (see Tables 12, 13 and 14).

The decision trees should be applied for all pathogens/hazards (see Table 1) that may be associated with a given composite product. Whenever, assuming presence of the pathogen, the trees indicate a low risk, this is due to the intrinsic composition or processing of the food, independently of information available in Table 10. In contrast, whenever the decision trees indicate a risk for a given pathogen, this risk can then be further qualified with the information of Table 10, such as prevalence and past association of outbreaks with the food, notwithstanding the limitations explained above, as well as based on additional information from questions included in each decision tree (see Figures 8, 9 and 10).

As explained in Chapter 1.2 of this Opinion, some pathogens may cause disease without growth in the food. The question on whether the composite product supports growth is not necessary for all pathogens. Some pathogens cause disease after growth and production of toxins in the food. In this case additional questions on the production of the toxins and their stability are needed to categorise the risk. Three decision trees are therefore proposed:

- one for pathogens which may not need to grow in food to cause illness (Figure 8);
- one for pathogens which usually need to grow in food to cause illness (Figure 9); and
- one for pathogens which need to grow in food to form toxins or toxic metabolites to cause illness (Figure 10).

The pathogens and hazards relevant for these three trees are those listed in Table 1. Each composite product must be assessed using the three trees, considering all these pathogens and hazards.

The questions in the decision trees are based on the treatments applied to the composite products that could inactivate pathogens, on the physico-chemical conditions of the composite products that influence pathogen growth, and on some of the characteristics of the pathogens considered (e.g. infectivity and production of toxins). The main factors and the domain permitting inactivation and growth of the relevant pathogens are those presented in Chapters 2 to 4 of this Opinion.

For any question, depending on the information available, the most conservative answers to the question must be used.

Answering the first question of the trees “*microbicidal treatment in package without recontamination?*”, requires unambiguous information on the processing conditions and their level of

control by the processor. Otherwise it should be assumed that the food has not received a microbiocidal treatment and/or could have been recontaminated.

With respect to the question “*supports growth?*” the following cases must be considered:

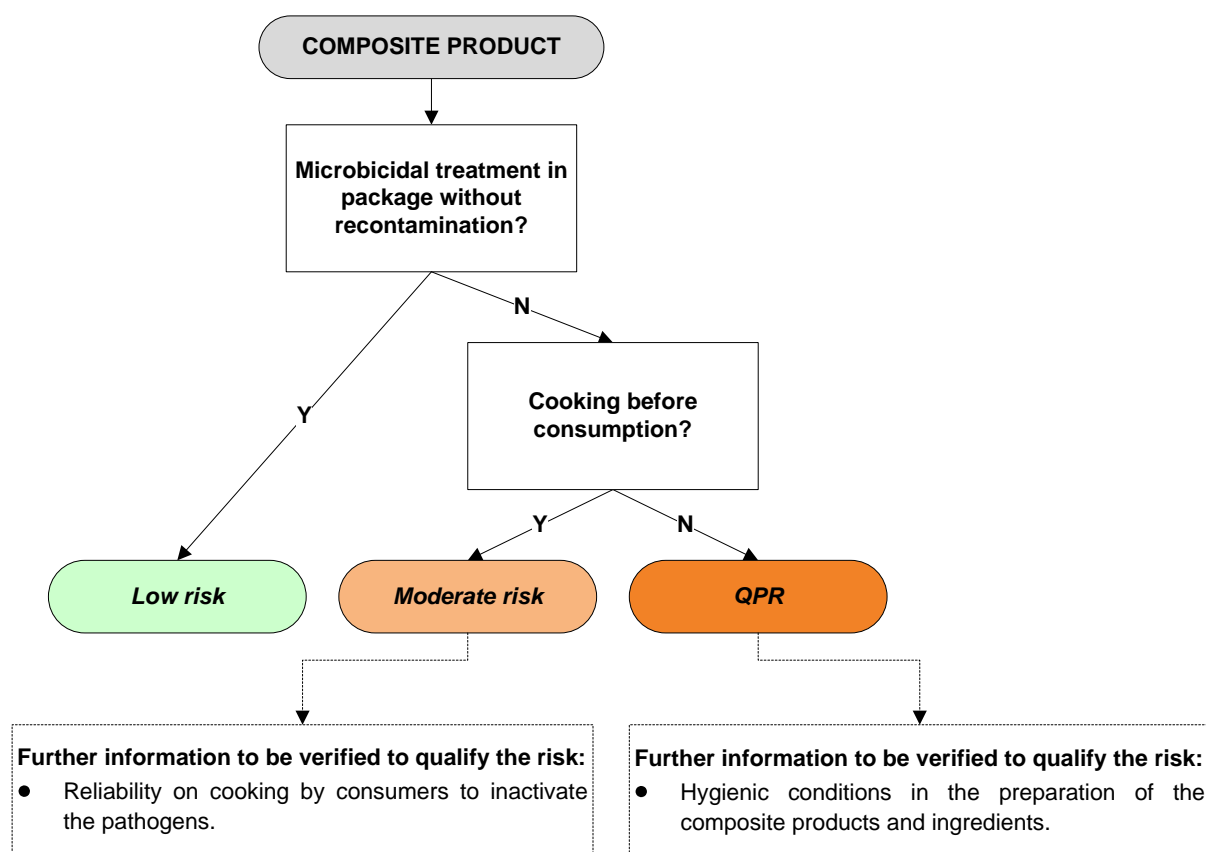
- In the absence of knowledge on the physico-chemical composition of the composite product and if it is not shelf-stable (i.e., permits growth of spoilage micro-organisms), growth of pathogenic bacteria must be assumed to be possible and the answer should be “yes”;
- If the food composition and storage temperature are known and within the growth domain of the pathogen, the answer should be “yes” in absence of other information. However, knowing other information may lead to a negative reply to this question. For instance, a short shelf-life or presence of additional antimicrobial hurdles, leading finally to a negative answer to the question “*supports growth?*”; and
- If the composite product consists of ingredients of contrasting physico-chemical characteristics, it may include interfaces between ingredients or components that allow growth (see Chapter 4) and in such case the answer should be “yes”.

In the absence of sufficient information on the food composition, the answer to the question “*toxin produced?*” will be “yes” if growth of the pathogen was assessed as possible in the previous question.

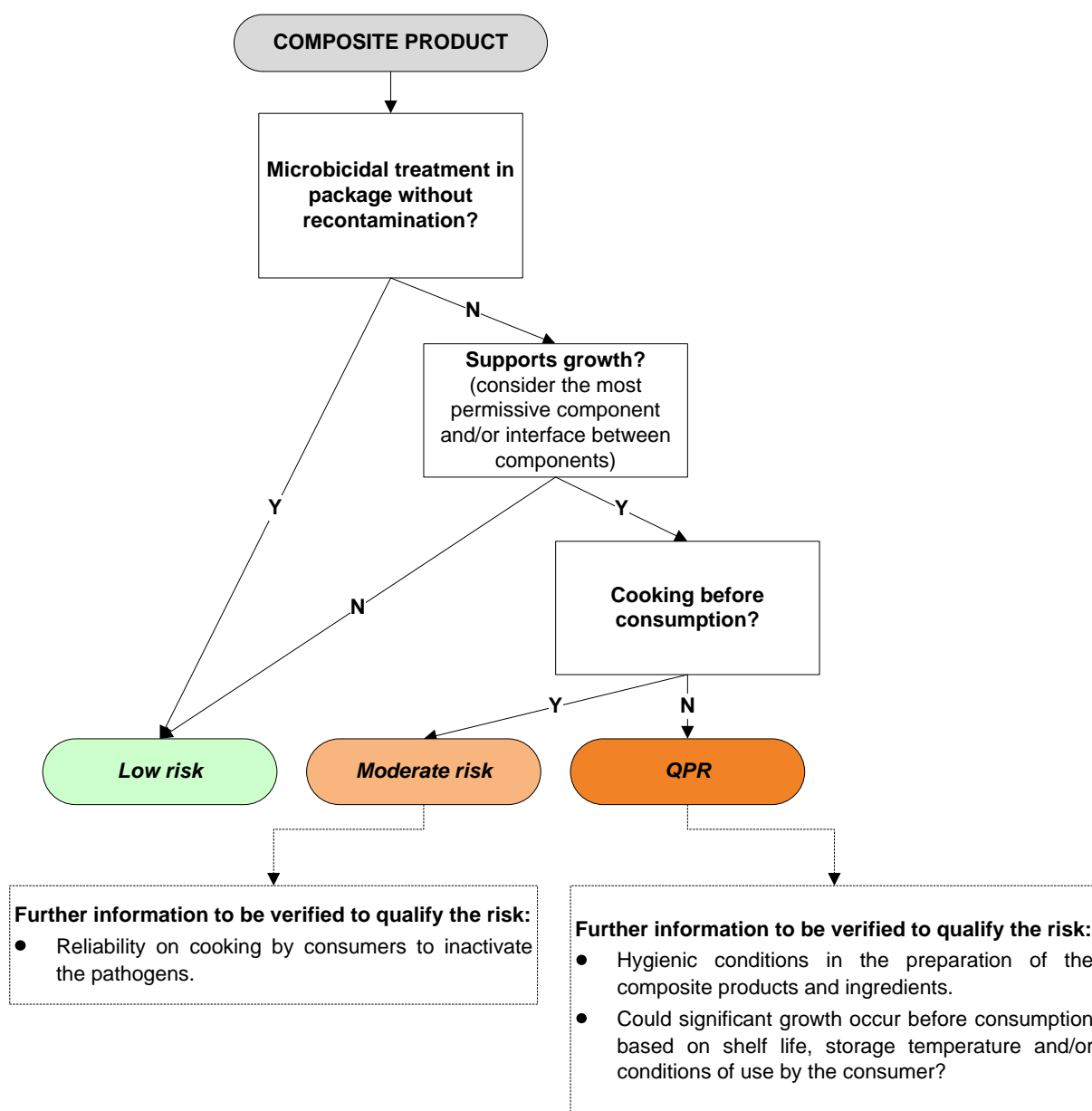
“Cooking” is accepted as a process modifying the structure and/or sensory properties of foods (different from reheating). Cooking practically inactivates food-borne viruses, parasites, vegetative bacteria and heat labile toxins such as botulinum toxins.

Clearly, the decision trees (Figures 8, 9 and 10) do not allow a full assessment of risks, but propose a categorization of the composite products into *low risk*, *moderate risk* and *qualified presumption of risk (QPR)*, for the hazards considered by each tree, based on information on the foods and their impact on the pathogens:

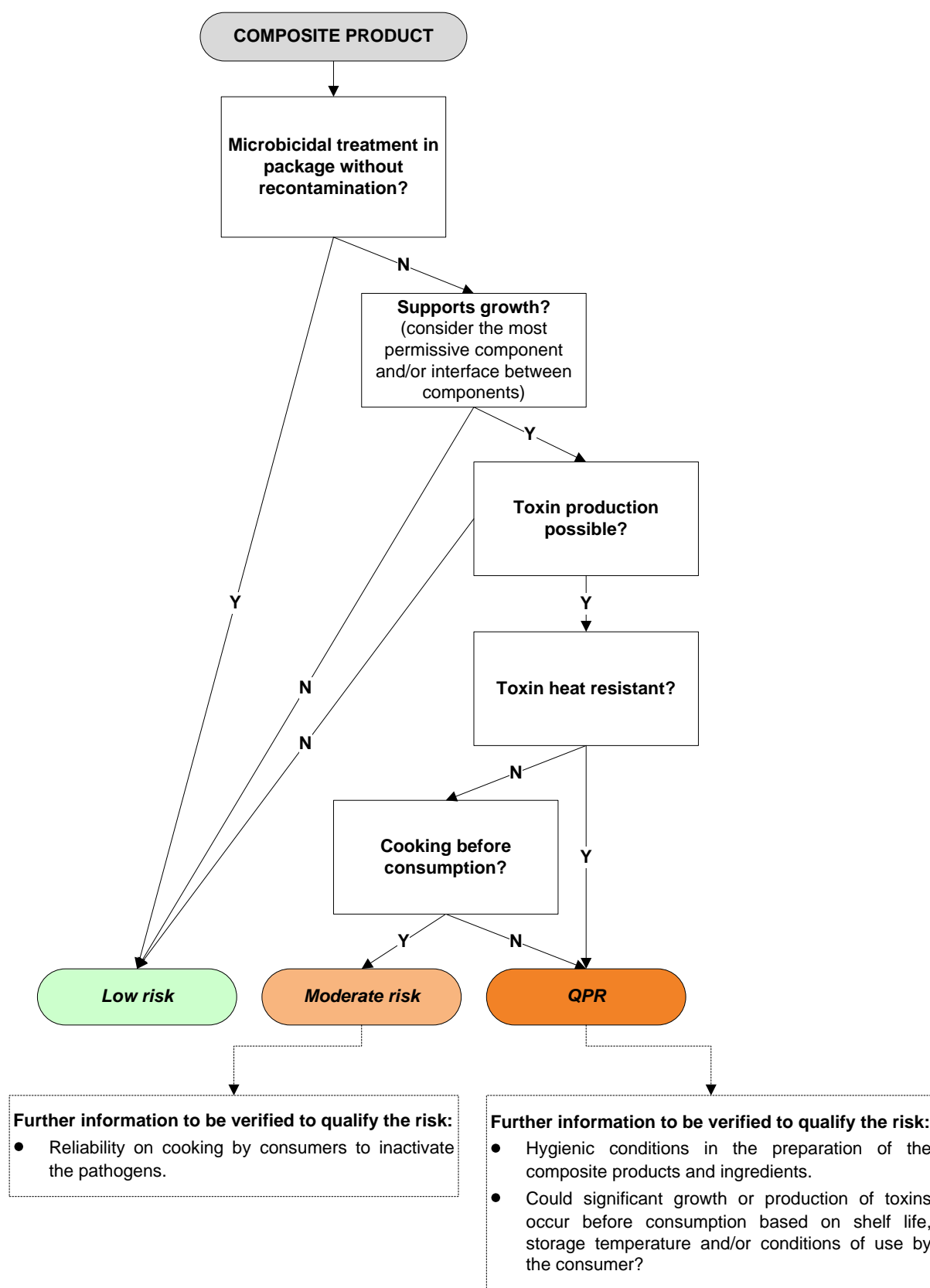
- *Qualified presumption of risk* means that the pathogens considered by this tree have the potential to cause disease via consumption of the composite product, if present in the food or its ingredients. *Qualified presumption of risk* must also be interpreted in light of the knowledge on the prevalence of the pathogen in the food concerned and its involvement in past outbreaks (see Table 10). It indicates that further information is needed for this type of products. This can be: more accurately defining food composition and shelf-life, better defining the potential of the pathogen to survive or grow (e.g. challenge test), or having more information on the level of hygiene during production and processing.
- *Low risk* means that the composition and processing of the food should cause inactivation of the pathogen or prevent the pathogen to reach hazardous levels at consumption.
- *Moderate risk* concerns foods cooked before consumption. It is not low risk, since the hazard may be still present at the moment of its preparation by the consumer. The possibility of cross-contamination in consumer’s kitchen of other foods consumed raw, or that the food is eaten without prior cooking, must be considered. In addition, the way of cooking will influence the level of inactivation of the pathogens.



**Figure 8:** Decision tree for categorisation of risk in composite products due to pathogens whose growth may not be needed in the food in order to cause illness.



**Figure 9:** Decision tree for categorisation of risk in composite products due to pathogens whose growth is usually required in the food in order to cause illness.



**Figure 10:** Decision tree for categorisation of risk in composite products due to pathogens whose growth is needed in the food for production of toxins or toxic metabolites that cause illness.

## 6.2. Example of the categorisation of risk for certain composite products

The decision trees (Figures 8, 9 and 10) have been applied to the composite products defined in Term of Reference 2 of the mandate for this Opinion, in order to obtain an indication on the possible level(s) of risk that they may pose. Table 11 summarises the results obtained through this exercise. Tables 12, 13 and 14 show the process leading to those results, including the answers to the different questions posed based on the decision trees. As explained in Chapter 6.1, it is often difficult to reply to all questions without having additional information on the characteristics of the composite products under consideration and therefore the results obtained need to be interpreted with caution and taken as examples of possible implementation of the decision trees. Further considerations related to the specific composite products are discussed under Chapter 6.3 on “Notes and further considerations for Tables 12, 13 and 14”.

In general, Tables 12, 13 and 14 represent possible tools to facilitate the practical application of the decision trees for the categorisation of risks. They could be also easily applied in other situations and for other composite products.

In the case of meat extracts and meat concentrates, there are no data available on pathogen prevalence or confirmed/verified outbreaks or cases of illness associated with consumption of contaminated meat extracts and concentrates. These products are usually contaminated with spores of bacteria and moulds, while pathogens are seldom found; the potential, however exists. Nevertheless, no growth or toxin production should be expected because of their low  $a_w$  (<0.80) and high salt content (9-18%). Similar considerations can be done for soup stocks and flavourings. Thus, soup stocks, flavourings, meat extracts and meat concentrates are to be considered products of *low risk*.

When it is concluded from the application of a decision tree that a composite product has a *qualified presumption of risk*, the risk manager should seek additional information in order to qualify the risk, as indicated in the decision trees and tables. In particular, if:

- the food manufacturer has no approval for proper hygienic and process controls

and/or

- based on shelf-life, storage temperature and/or conditions of use by the consumer, there is a possibility for growth of the pathogen or production of toxins before consumption,

then

- the risk should be re-classified from moderate to high, while the opposite situation would lead to qualifying the risk as low.



**Table 11:** Results obtained from the application of the decision trees to certain composite products as listed in Term of Reference 2 (see also Tables 12, 13 and 14 and Chapter 6.3).

Hazards		Biscuits and bread	Cakes (low a <sub>w</sub> , e.g. fruit loaf)	Cakes (high a <sub>w</sub> , e.g. with cream)	Chocolate	Confectionery and sweets				Pasta and noodles		Food supplements (dry powder)	Soup stocks and flavourings	Unfilled gelatine capsules	Olives with fish	Meat extracts and meat concentrates	
						st	ps	nh	sw	dry	fresh						
Illness may occur without growth of hazard in food	e.g.: Norovirus parasites <i>Salmonella</i> <i>Shigella</i> <i>Campylobacter</i> pathogenic <i>E.coli</i> <i>Y.enterocolitica</i>	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Low risk	Low risk	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	
		Low risk	Low risk	Qualified Presumption of Risk (QPR)	Low risk	Low risk	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Low risk	Qualified Presumption of Risk (QPR)	Low risk	Low risk	Low risk	Low risk	Qualified Presumption of Risk (QPR)	Low risk	Low risk
		Low risk	Low risk	Qualified Presumption of Risk (QPR)	Low risk	Low risk	Low risk	Qualified Presumption of Risk (QPR)	Qualified Presumption of Risk (QPR)	Low risk	Qualified Presumption of Risk (QPR)	Low risk	Low risk	Low risk	Low risk	Qualified Presumption of Risk (QPR)	Low risk

Low risk

Moderate risk

Qualified Presumption of Risk (QPR)

st: confectionery (sterilised)  
ps: confectionery (pasteurised)  
nh: confectionery (not heat treated)  
sw: confectionery (sweets)

**Table 12:** Table for categorisation of risk (from Figure 8) in composite products due to pathogens whose growth may not be needed in food in order to cause illness. The last column provides the example of the application of the table for certain composite products (see Chapter 6.3 for symbols and notes).

Microbiocidal treatment in package without recontamination?	Cooking before consumption?	Risk	Example of application to some composite products
Y	-	<i>Low risk</i>	confectionery (sterilised) <sup>a</sup> , confectionery (pasteurised) <sup>b</sup>
N	Y	<i>Moderate risk</i>	pasta and noodles
N	N	<i>QPR</i>	biscuits*, bread*, cakes (low $a_w$ , e.g. fruit loaf) <sup>o</sup> , cakes (high $a_w$ , e.g. with cream) <sup>o</sup> , chocolate, confectionery (not heat treated) <sup>c</sup> £, confectionery (sweets), food supplements (dry powder)¥, unfilled gelatine capsules¥, olives with fish <sup>e</sup> ¶

**Table 13:** Table for categorisation of risk (from Figure 9) in composite products due to pathogens whose growth is usually required in food in order to cause illness. The last column provides the example of the application of the table for certain composite products (see Chapter 6.3 for symbols and notes).

Microbiocidal treatment in package without recontamination?	Supports growth? (consider the most permissive component and/or interface between components)	Cooking before consumption?	Risk	Example of application to some composite products
Y	-	-	<i>Low risk</i>	confectionery (sterilised) <sup>a</sup>
N	N	-	<i>Low risk</i>	biscuits, bread, cakes (low $a_w$ , e.g. fruit loaf) <sup>o</sup> , chocolate, confectionery (sweets), pasta and noodles (dry) <sup>§</sup> , food supplements (dry powder) <sup>§</sup> , unfilled gelatine capsules
N	Y	Y	<i>Moderate risk</i>	pasta and noodles (fresh) <sup>§</sup>
N	Y	N	<i>QPR</i>	cakes (high $a_w$ , e.g. with cream) <sup>o</sup> ; confectionery (pasteurised) <sup>b</sup> @, confectionery (not heat treated) <sup>c</sup> £, olives with fish <sup>e</sup> †

**Table 14:** Table for categorisation of risk (from Figure 10) in composite products due to pathogens whose growth is needed in food for production of toxins or toxic metabolites that cause illness. The last column provides the example of the application of the table for certain composite products (see Chapter 6.3 for symbols and notes).

Microbiocidal treatment in package without recontamination?	Supports growth? (consider the most permissive component and/or interface between components)	Toxin production possible?	Toxin heat resistant?	Cooking before consumption?	Risk	Example of application to some composite products
Y	-	-	-	-	Low risk	confectionery (sterilised) <sup>a</sup>
N	N	-	-	-	Low risk	biscuits, bread, cakes (low $a_w$ , e.g. fruit loaf) <sup>o</sup> , chocolate, confectionery (sweets), pasta and noodles (dry) <sup>§</sup> , food supplements (dry powder), unfilled gelatine capsules
N	Y	N	-	-	Low risk	
N	Y	Y	N	Y	Moderate risk	
N	Y	Y	N	N	QPR	olives with fish <sup>e‡</sup>
N	Y	Y	Y	-	QPR	cakes (high $a_w$ , e.g. with cream) <sup>o</sup> , confectionery (pasteurised) <sup>b@</sup> , confectionery (not heat treated) <sup>c£</sup> , pasta and noodles (fresh) <sup>§</sup>

### 6.3. Notes and further considerations for Tables 12, 13 and 14

<sup>a</sup>: Confectionery (sterilised): For this purpose the following example of confectionery has been considered: theoretical *praliné-style preparation*, which could contain sugar, eggs, milk, starch, grounded nuts, in the form of a shelf-stable product (sterilised or UHT processed).

<sup>b</sup>: Confectionery (pasteurised): For this purpose the following example of confectionery has been considered: theoretical *praliné-style preparation*, which could contain sugar, eggs, milk, starch, grounded nuts, in the form of a pasteurised product (70°C for few minutes) kept refrigerated, or freshly prepared.

<sup>c</sup>: Confectionery (not heat treated): For this purpose the following example of confectionery has been considered: theoretical *praliné-style preparation*, which could contain sugar, eggs, milk, starch, grounded nuts, in the form of a product not submitted to further treatments and kept refrigerated.

<sup>e</sup>: Olives with fish: For this purpose, the following example has been considered: a product which is not heated in its final package and sold refrigerated, green olives (fermented in brine for their preparation) are stuffed with salted anchovies fillets.

\*: Biscuits and bread: Further consideration of the *QPR* status may lead to the conclusion that the risk is low, since the products are usually dry and recontamination is unlikely.

°: Cakes: For cakes  $a_w$  is an important information as high  $a_w$  cakes would permit growth to pathogenic bacteria. As shown in section 5, the use of raw eggs not further processed may be further information which could make *QPR* a high risk.

§: Pasta and noodles: The level of risk is usually low, but may depend on the  $a_w$  of the product (higher in fresh pasta than in dry pasta because growth of bacteria is possible if  $a_w$  is high), its temperature before cooking, and the cooking temperature.

@: Confectionery (pasteurised): In this example hazards for which answer to the question “*microbiological treatment in package without recontamination?*” is “no” are bacterial spores. Additional useful information when further considering the *QPR* status would be whether the shelf-life is long enough and in the case of *C. botulinum* if the product is stored under anaerobic conditions (e.g. vacuum packaged).

£: Confectionery (not heat treated): Additional useful information when further considering the *QPR* status would be the shelf-life duration. In the case of toxin-producing bacterium *C. botulinum*, a useful information would be whether the product is stored under anaerobic conditions (e.g. vacuum packaged). The knowledge on prevalence of hazards in the composite products and ingredients would be also useful. For instance, parasites are not relevant for this composite product and the risk related to *Salmonella* would be higher if the composite product was prepared with raw eggs and not further processed.

¥ Food supplements (dry powder) and unfilled gelatine capsules: Additional information when further considering the *QPR* status is that presence of, or outbreaks linked to, *Salmonella*, pathogenic *E. coli*, food-borne viruses, in gelatine capsule or food supplements have not been reported, and that parasites are not relevant hazards for these products.

¶ Olives with fish: Additional information useful when further considering the *QPR* status would be the possible presence of parasites (*Anisakis*) in the fish fillets. If the fish fillet were sufficiently frozen before processing, *Anisakis* could be inactivated but this will have no impact on bacterial and allergenic hazards.

† Olives with fish: When answering the various questions foreseen by the decision tree it is difficult to answer to the question “*supports growth?*” without an accurate knowledge of the pH and  $a_w$  (salt

content) at the interface between the fish and the olive. Each ingredient taken in isolation may not support growth of these pathogens (e.g. moderate salt content and low pH in olive, higher salt content in the fish fillets), but at the interface the olive might dilute the salt content of the fish, and the fish might buffer the acidity of the olive. The refrigerated storage is an additional hurdle to pathogen growth. If growth of some pathogens was possible, and if the answer would be “no” to the question “*cooking before consumption?*”, this would lead to *QPR* status for this combination of composite product/pathogens.

§: Food supplements (dry powder): One case of listeriosis has nevertheless been reported once with a food supplement (see Chapter 5.3), illustrating that *low risk* does not mean absence for risk. The case report did not give information on the numbers of *L. monocytogenes* in the food supplement, but a particularly high initial contamination of some ingredients used in the preparation of the food supplement might have occurred.

‡: Olives with fish: Similarly to what discussed in the above paragraph, the answer to the questions “*supports growth?*” and “*toxin production possible?*” would need an accurate knowledge of  $a_w$  and pH in the composite product. Due to the refrigerated storage, the only hazard forming toxin in the food would be psychrotrophic (non proteolytic) *C. botulinum* if storage conditions are anaerobic (e.g. vacuum packaging). If it was able to form toxin in this composite product, the answer to the question “*toxin heat resistant?*” would be “no” and if the answer to the question “*cooking before consumption?*” would be “no”, this would lead to a *QPR* status for this combination of composite product/pathogens. Additional useful information would be the presence of spores of psychrotrophic *C. botulinum* in fish and fish products, as well as past botulinum outbreaks linked to fish products.

## CONCLUSIONS

### General conclusions:

- The conclusions below apply to all composite products considered by the mandate, as well as to all other foods.
- Prevalence and concentration of the pathogens in foods, which may be reduced by good hygienic practices, are important in determining the risk for consumers. However, risks also depend on the physico-chemical conditions of the foods.
- Some pathogens need to grow in the food to reach sufficient numbers for a significant probability of illness with or without toxin production, whereas others may cause illness without growth in the food.
  - Hazards not needing growth in food to cause illness: Norovirus, parasites, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., enterohaemorrhagic *E. coli*, *Yersinia enterocolitica*.
  - Hazards usually needing growth in food to cause illness: *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, diarrhoeic *Bacillus cereus*.
  - Hazards needing growth and toxin production in food to cause illness: *Clostridium botulinum*, *Staphylococcus aureus*, emetic *Bacillus cereus*, bacteria producing biogenic amines.

### Conclusions in relation to Term of Reference 1:

**(Recommend/identify physico-chemical parameters for composite products containing no meat and/or less than 50% of products of animal origin that could be relevant for the growth/survival of pathogenic microorganisms of public health importance, taking into account the importance of other factors such as processing conditions, transport and/or storage conditions, and therefore assisting the risk manager on deciding to carry out risk based controls).**

- Among the factors affecting microbial survival and growth in foods, the main ones to be considered are water activity ( $a_w$ ), pH, temperature and time of storage, processing, as well as intensity and time of other non-thermal physical processes applied.
- In general, foods with  $a_w$  below 0.88 or pH below 3.7 or stored frozen do not permit growth of or toxin formation by food-borne pathogenic bacteria, leaving only potential risks of hazards not needing growth in food to cause illness.
- Considering only pathogens for which growth in food is usually required to cause illness, growth would occur only above pH 4.3. In the case of heat-treated food without possibilities for recontamination, only spore-forming bacteria are considered, and thus growth would only occur in foods with  $a_w$  above 0.92 or pH above 4.3, or stored at temperatures above 3°C.
- Heat treatments equivalent to 70°C for 15 sec for high  $a_w$  foods reduce vegetative cells of food-borne pathogenic bacteria and parasites of relevance for this mandate by more than five  $\log_{10}$  cycles. The same treatment may not achieve the same reduction of all food-borne viruses. Re-heating or warming foods cannot consistently and adequately inactivate pathogenic agents.
- Reliable inactivation of spores of pathogenic bacteria in high  $a_w$  foods can be achieved by sterilisation treatments (e.g. 3 min at 120°C).

- Some microbial toxins or toxic metabolites (e.g. emetic toxin of *B. cereus*), once produced in foods, cannot be inactivated by current food processing treatments, including thermal processing.
- Specific estimations of growth and survival depend on the hazards considered. Databases and models, using predictive microbiology, provide such quantitative estimations of the impact of temperature, pH,  $a_w$  and their combinations on survival and growth of the main bacterial pathogens. These are summarised in the Opinion.
- Combination of factors applied in the form of multiple hurdle technology can achieve inactivation or inhibition of growth at levels milder than those needed to be individually effective. For example, a combination of low  $a_w$  and pH is more effective in inhibiting growth than pH or low  $a_w$  alone. However, interaction between factors may be antagonistic. For example, low  $a_w$  can decrease the efficacy of heat in inactivating pathogens.
- Composite products contain several ingredients with different composition. Migration and diffusion among the ingredients may change the physico-chemical parameters, particularly at the interfaces. Therefore, the combinations of parameters most permissive to survival and growth of pathogens should be considered when assessing the risk posed by composite products.
- It is important to know at which steps of the food production chain treatments inactivating pathogens are applied. The risk will be low if no recontamination with a pathogenic agent can occur after the inactivating treatment (e.g. heat applied in the final package).

#### Conclusions in relation to Term of Reference 2:

**(Identify and profile the microbiological hazards for public health related to import of certain composite products containing no meat and/or less than 50% of products of animal origin. In the first instance the following list of products should be assessed: *see mandate*).**

- It is not always possible to determine whether a food meets the definition of composite product, unless the food composition and processing conditions are available.
- The Opinion presents two approaches for identifying and profiling microbiological hazards in the different composite products listed in the mandate. One approach is based on past outbreaks and prevalence of hazards in the composite products, mainly using EU-related information. The other approach is based on the impact of food composition and food processing on the pathogens and is presented as decision trees. The two approaches are complementary and should be applied in parallel to identify and profile the hazards.
- Approach 1 (association of biological hazards-composite products based on available data):
  - *Salmonella* caused most of the outbreaks associated with the composite products considered.
  - The most frequent hazard-composite product combinations are *Salmonella* in cakes and bakery products.
  - Identification of a high association of a composite product with a hazard through this approach is based on evidence that some foods in that category of composite products have been implicated in outbreaks or had higher prevalence of certain hazards. However, absence of confirmed outbreaks or low prevalence do not preclude potential risks.
  - Most of the outbreak and prevalence data on which this approach is based originate from EU databases. The distribution and prevalence of microbial pathogens in food, as well as sources and frequency of food-borne outbreaks, may differ from the ones related to potential exporting countries of composite products towards the EU.

- Approach 2 (decision tools):
  - The three decision trees and corresponding tables developed in this Opinion in relation to the three categories of hazards identified (not needing growth, usually needing growth, needing growth and toxin production to cause illness) should be all applied to each composite product of interest.
  - When using the decision tools presented in this Opinion, behaviour of the hazard in the most sensitive components or ingredients or their interfaces in a composite product must be considered.
  - Categorisation of the risk for composite products requires information on their composition, processing and further handling, which can largely differ for foods belonging to the same category of composite products.
  - Bread, low moisture biscuits/cakes/chocolate, sweets, dry pasta and noodles, food supplements, and unfilled gelatine capsules in general do not permit growth of pathogens. These products are therefore of *low risk* with regard to hazards that need to grow in food to cause illness. They may pose *moderate risk* or *qualified presumption of risk* with regard to hazards that do not need to grow in food to cause illness.
  - Soup stocks, flavourings, meat extracts, meat concentrates, and sterilised heat-treated foods without possible recontamination are in general of *low risk*.
  - The other composite products considered, such as high moisture biscuits/cakes/chocolate/confectionery, fresh pasta and noodles, and olives with fish, may pose *moderate risk* or *qualified presumption of risk*.
  - If the approach proposed identifies a *moderate risk*, further information should be verified, i.e. the reliability of consumer preparation to inactivate pathogens.
  - If the approach proposed identifies *qualified presumption of risk*, further information should be verified, i.e. hygienic conditions in the preparation of the composite products and ingredients, and shelf-life conditions.
- As proposed in the mandate, some of the categories of composite products include foods with different levels of risk (e.g. bakery products, cakes and confectionery), since the composition and processing of some of them may allow for the survival and growth of pathogenic microorganisms while others may not.
- The decision tools provided in this document are applicable to all composite products considered by the mandate, as well as to all other foods.



**REFERENCES**

- Aberle ED, Forrest JC, Gerrard DE, Mills EW, Hedrick HB, Judge MD and Merkel RA, 2001. Principles of Meat Science. Fourth Edition. Kendall/hunt Publishing Company, Dubuque, Iowa, USA.
- Afchain AL, Derens E, Guilpart J and Cornu M, 2005. Statistical modelling of cold-smoked salmon temperature profiles for risk assessment of *Listeria monocytogenes*. *Acta Horticulturae*, 674, 383-388.
- Agata N, Ohta M and Yokoyama K, 2002. Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. *International Journal of Food Microbiology*, 73, 23-27.
- Ager EA, Nelson KE, Galton MM, Boring JR and Jernigan JR, 1967. Two outbreaks of egg-borne salmonellosis and implications for their prevention. *Journal of the American Medical Association*, 199, 372-378.
- Alonso A, Daschner A and MorenoAncillo A, 1997. Anaphylaxis with *Anisakis simplex* in the gastric mucosa. *New England journal of medicine*, 337, 350-351.
- Andre P, Roose H, Vannoyen R, Dejaegher L, Uyttendaele I and Deschrijver K, 1990. Neuromeningeal listeriosis associated with an ice-cream. *Medecine Et Maladies Infectieuses*, 20, 570-572.
- Bailey CP and von Holy A, 1993. *Bacillus* spore contamination associated with commercial bread manufacture. *Food Microbiology*, 10, 287-294.
- Barnes GH and Edwards AT, 1992. An investigation into an outbreak of *Salmonella* Enteritidis phage-type 4 infection and the consumption of custard slices and trifles. *Epidemiology and Infection*, 109, 397-403.
- Barrile JC, Cone JF and Keeney PG, 1970. A study of *salmonellae* survival in milk chocolate. *Manufacturing Confectioner*, 50, 34-39.
- Baylis CL, MacPhee S, Robinson AJ, Griffiths R, Lilley K and Betts RP, 2004. Survival of *Escherichia coli* O157 : H7, O111 : H- and O26 : H11 in artificially contaminated chocolate and confectionery products. *International Journal of Food Microbiology*, 96, 35-48.
- Beckers HJ, Coutinho RA, Jansen JT and Vanleeuwen WJ, 1980. Staphylococcal food poisoning by consumption of sterilized vanilla custard. *Antonie Van Leeuwenhoek Journal of Microbiology*, 46, 224-225.
- Bergdoll MS, 1989. *Staphylococcus aureus*. In: Foodborne bacterial pathogens. MP Doyle. Marcel Dekker, New-York, 463-524.
- Bermúdez-Aguirre D and Corradini MG, 2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: A review. *Food Research International*, 45, 700-712.
- Bidawid S, Farber JM, Sattar SA and Hayward S, 2000. Heat inactivation of hepatitis A virus in dairy foods. *Journal of Food Protection*, 63, 522-528.
- Black RE, Jackson RJ, Tsai T, Medvesky M, Shayegani M, Feeley JC, MacLeod KIE and Wakelee AM, 1978. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *New England journal of medicine*, 298, 76-79.
- Brocklehurst TF, Mitchell GA and Smith AC, 1997. A model experimental gel surface for the growth of bacteria on foods. *Food Microbiology*, 14, 303-311.
- Brockmann SO, Piechotowski I and Kimmig P, 2004. *Salmonella* in sesame seed products. *Journal of Food Protection*, 67, 178-180.

- Buckner P, Ferguson D, Anzalone F, Taylor J, Hlady WG and Hopkins RS, 1994. Outbreak of *Salmonella* Enteritidis associated with homemade ice-cream - Florida, 1993 (reprinted from MMWR, vol 43, pg 669-671, 1994). *Jama-Journal of the American Medical Association*, 272, 1490-1492.
- Buckow R, Isbarn S, Knorr D, Heinz V and Lehmacher A, 2008. Predictive model for inactivation of feline Calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Applied and Environmental Microbiology*, 74, 1030-1038.
- Carbó Malonda RM, Miralles Espí MT, Sanz Bou R, Mañas Gimeno F, Guiral Rodrigo S and Pérez Pérez E, 2005. Outbreak of *Salmonella* Enteritidis food poisoning at a dining hall facility. *Revista española de salud pública*, 79, 47-57.
- Cassin MH, Lammerding AM, Todd EC, Ross W and McColl RS, 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int J Food Microbiol*, 41, 21-44.
- Cawthorne A, Celentano LP, D'Ancona F, Bella A, Massari M, Anniballi F, Fenicia L, Aureli P and Salmaso S, 2005. Botulism and preserved green olives. *Emerging Infectious Diseases*, 11, 781-782.
- Cinquetti S, Collareta A, Mazzer R, Scagnelli M, Raris M, Paludetti P and De Rui S, 1991. An outbreak of *Salmonella* Enteritidis infection associated with consumption of home-made cake. *Igiene Moderna*, 96, 333-340.
- Cohen A, Grinberg K, Slater P, Kotev P, Lucas J and Hasis U, 1988. Type-E botulism outbreak caused by white fish consumption. *Israel Journal of Medical Sciences*, 24, 385-386.
- Cordier JL, 1994. HACCP in the chocolate industry. *Food Control*, 5, 171-175.
- Corpening A and Foxhall EP, 1935. Outbreak of food poisoning, probably due to *Staphylococcus aureus*. *American Journal of Public Health*, 25, 938.
- Costanzo Anunciaçao LL, Linardi WR, do Carmo LS and Bergdoll MS, 1995. Production of staphylococcal enterotoxin A in cream-filled cake. *International Journal of Food Microbiology*, 26, 259-263.
- Coughlin FE and Johnson Jr B, 1941. Gastroenteritis outbreaks from cream-filled pastry. *American Journal of Public Health*, 31, 245.
- Craven P, Baine W, Mackel D, Barker W, Gangarosa E, Goldfield M, Rosenfeld H, Altman R, Lachapelle G, Davies J and Swanson R, 1975. International outbreak of *Salmonella* Eastbourne infection traced to contaminated chocolate. *The Lancet*, 305, 788-792.
- Croci L, De Medici D, Di Pasquale S and Toti L, 2005. Resistance of hepatitis A virus in mussels subjected to different domestic cookings. *International Journal of Food Microbiology*, 105, 139-144.
- D'Aoust JY, Aris BJ, Thisdele P, Durante A, Brisson N, Dragon D, Lachapelle G, Johnston M and Laidley R, 1975. *Salmonella* Eastbourne outbreak associated with chocolate. *Canadian Institute of Food Science and Technology Journal*, 8, 181-184.
- D'Argenio P, Romano A and Autorino F, 1999. An outbreak of *Salmonella* enteritidis infection associated with iced cake. *Eurosurveillance*, 4, 24.
- Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, Swaminathan B, Proctor ME and Griffin PM, 1997. Listeriosis from chocolate milk: linking of an outbreak of febrile gastroenteritis and "sporadic" invasive disease. *New England Journal of Medicine*, 336, 100-105.
- Danyluk MD, Brandl MT and Harris LJ, 2008. Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. *Journal of Food Protection*, 71, 397-401.
- Daschner A, Alonso-Gomez A, Caballero T, Barranco P, Suarez-De-Parga JM and Lopez-Serrano MC, 1998. Gastric anisakiasis: an underestimated cause of acute urticaria and angio-oedema? *British Journal of Dermatology*, 139, 822-828.

- de Jong JT, ten Brinke JM, van Ouwerkerk IM, Siebbeles MF and Fitz-James IA, 2004. Large-scale, acute, bacterial gastroenteritis caused by the enterotoxin of *Staphylococcus aureus* after a barbecue. *Nederlands tijdschrift voor geneeskunde*, 148, 2136.
- De Schrijver K, Buvens G, Possé B et al., 2008. Outbreak of verocytotoxin-producing *E. coli* O145 and O26 infections associated with the consumption of icecream produced at a farm, Belgium, 2007. *Eurosurveillance*, 13, 1-4.
- Deboosere N, Legeay O, Caudrelier Y and Lange M, 2004. Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. *International Journal of Food Microbiology*, 93, 73-85.
- Deboosere N, Pinon A, Delobel A, Temmam S, Morin T, Merle G, Blaise-Boisseau S, Perelle S and Vialette M, 2010. A predictive microbiology approach for thermal inactivation of Hepatitis A virus in acidified berries. *Food Microbiology*, 27, 962-967.
- de Corres LF, Audicana M, DelPozo MD, Munoz D, Fernandez E, Navarro JA, Garcia M and Diez J, 1996. *Anisakis simplex* induces not only anisakiasis: Report on 28 cases of allergy caused by this nematode. *Journal of Investigational Allergology & Clinical Immunology*, 6, 315-319.
- Delbrassinne L, Andjelkovic M, Rajkovic A, Bottledoorn N, Mahillon J and Van Loco J, 2011. Follow-up of the *Bacillus cereus* emetic toxin production in penne pasta under household conditions using liquid chromatography coupled with mass spectrometry. *Food Microbiology*, 28, 1105-1109.
- Delignette-Muller ML and Cornu M, 2008. Quantitative risk assessment for *Escherichia coli* O157:H7 in frozen ground beef patties consumed by young children in French households. *International Journal of Food Microbiology*, 128, 158-164.
- Deneen VC, Hunt JM, Paule CR, James RI, Johnson RG, Raymond MJ and Hedberg CW, 2000. The impact of foodborne calicivirus disease: the Minnesota experience. *Journal of Infectious Diseases*, 181, S281-S283.
- Dierick K, Van Coillie E, Swiecicka I, Meyfroidt G, Devlieger H, Meulemans A, Hoedemaekers G, Fourie L, Heyndrickx M and Mahillon J, 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *Journal of clinical microbiology*, 43 4277.
- Dine G, Soibinet C and Croix JC, 1981. 2nd case in France of botulism due to consumption of freshwater-fish. *Nouvelle Presse Medicale*, 10, 339-339.
- Djuretic T, Wall PG and Nichols G, 1996. An outbreak of Salmonella infection from ice cream. *New England journal of medicine*, 335, 824-824.
- Dreher M and Lehrnbecher M, 1981. Ice-cream contaminated with *Salmonella* as the cause of food poisoning. *Offentliche Gesundheitswesen*, 43, 142-145.
- Eden KV, Rosenberg ML, Stoopler M, Wood BT, Highsmith AK, Skaliy P, Wells JG and Feeley JC, 1977. Waterborne gastrointestinal illness at a ski resort - Isolation of *Yersinia enterocolitica* from drinking-water. *Public Health Reports*, 92, 245-250.
- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on biological hazards (BIOHAZ) on *Bacillus cereus* and other *Bacillus* spp in foodstuffs *The EFSA Journal*, 1-48.
- EFSA (European Food Safety Authority), 2007a. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. *The EFSA Journal*, 130, 1-352.
- EFSA (European Food Safety Authority), 2007b. Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. *The EFSA Journal*, 579, 1-61.

- EFSA (European Food Safety Authority), 2009. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal, 223, 1-312.
- EFSA (European Food Safety Authority), 2010. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. The EFSA Journal, 1496, 1-288.
- EFSA (European Food Safety Authority), 2011a. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009. The EFSA Journal, 9(3):2090, 378pp.
- EFSA (European Food Safety Authority), 2011b. Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Germany and France.
- EFSA (European Food Safety Authority), 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. The EFSA Journal, 10(3):2597, 442pp.
- EFSA Panel on Biological Hazards (BIOHAZ), 2010. Scientific Opinion on risk assessment of parasites in fishery products. The EFSA Journal, 8, 91pp.
- EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses. The EFSA Journal, 9, 96pp.
- Elliot EL and Kvenberg JE, 2000. Risk assessment used to evaluate the US position on *Listeria monocytogenes* in seafood. International Journal of Food Microbiology, 62, 253-260.
- Escartín EF, Saldaña-Lozano J and Montiel-Falcón A, 1998. Poisoning by enterotoxin from *Staphylococcus aureus* associated with mocha pastry. Microbiology and epidemiology. Revista latinoamericana de microbiología, 40, 25.
- Evans MR, Tromans JP, Dexter ELS, Ribeiro CD and Gardner D, 1996. Consecutive *Salmonella* outbreaks traced to the same baker. Epidemiology and Infection, 116, 161-167.
- Evenson ML, Ward Hinds M, Bernstein RS and Bergdoll MS, 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. International Journal of Food Microbiology, 7, 311-316.
- Falcao H, Lunet N, Neves E, Iglesias I and Barros H, 2008. *Anisakis simplex* as a risk factor for relapsing acute urticaria: a case-control study. Journal of Epidemiology and Community Health, 62, 634-637.
- Falkenhorst G, Bagdonaite J, Lisby M, Madsen SB, Lambertsen L, Olsen KEP and MoLbak K, 2008. Outbreak of group A streptococcal throat infection: don't forget to ask about food. Epidemiology and Infection, 136, 1165-1171.
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2002. Risk assessments of *Salmonella* in eggs and broiler chickens: Interpretative summary. Microbiological Risk Assessment Series 1 (ISBN 92-5-104873-8).
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2004. Risk assessment of *Listeria monocytogenes* in ready to eat foods: Interpretative summary. Microbiological Risk Assessment Series 4 (ISBN 92-5-105126-7).
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2009. Risk assessment of *Campylobacter* spp. in broiler chickens: Technical Report. Microbiological Risk Assessment Series No. 12 (ISBN 978-92-5-105879-4).
- Farber JM, Carter AO, Varughese PV, Ashton FE and Ewan EP, 1990. Listeriosis traced to the consumption of alfalfa tablets and soft cheese. New England journal of medicine, 322, 338-338.

- FDA (Food and Drug Administration), 2011. Fish and fisheries products hazards and controls guidance, 4th edition.
- Fenicia L, Ferrini AM, Aureli P and Padovan MT, 1992. Botulism outbreak from black olives. *Industrie Alimentari*, 31, 307-308.
- Fernández de la Hoz Zeitler K, Carpintero Redondo J, Puchades Belenguer J, Verde López C and García Colmenero C, 1994. Study of 2 food poisoning outbreaks in Mora (Toledo) with the same source of infection]. *Revista de sanidad e higiene pública*, 68, 589.
- Fielding JE, Snell P, Milazzo A, Del Fabbro L and Raupach J, 2003. An outbreak of *Salmonella* Typhimurium phage type 4 linked to cold set cheesecake. *Communicable diseases intelligence*, 27, 513-514.
- Foti C, Fanelli M, Mastrandrea V, Buquicchio R, Cassano N, Conserva A and Nettis E, 2006. Risk factors for sensitization to *Anisakis simplex*: A multivariate statistical evaluation. *International Journal of Immunopathology and Pharmacology*, 19, 847-851.
- Frank C, Buchholz U, Maaß M, Schröder A, Bracht KH, Domke PG, Rabsch W and Fell G, 2007. Protracted outbreak of *S. Enteritidis* PT 21c in a large Hamburg nursing home. *BMC public health*, 7, 243.
- French G, Pavlick A, Felsen A, Gross P, Brook J, Paul S and Genese C, 1992. Outbreak of type E botulism associated with an uneviscerated, salt-cured fish product - New-Jersey, 1992 (reprinted from *MMWR*, Vol 41, pp.521-522, 1992). *Jama-Journal of the American Medical Association*, 268, 963-963.
- Friedman DS, Heisey-Grove D, Argyros F, Berl E, Nsubuga J, Stiles T, Fontana J, Beard RS, Monroe S and McGrath ME, 2005. An outbreak of norovirus gastroenteritis associated with wedding cakes. *Epidemiology and Infection*, 133, 1057-1063.
- Garcia-Garcia P, Brenes-Balbuena M, Hornero-Mendez D, Garcia-Borrego A and Garrido-Fernandez A, 2000. Content of biogenic amines in table olives. *Journal of Food Protection*, 63, 111-116.
- Garcia-Garcia P, Brenes-Balbuena M, Romero-Barranco C and Garrido-Fernandez A, 2001. Biogenic amines in packed table olives and pickles. *Journal of Food Protection*, 64, 374-378.
- Garcia PG, Barranco CR, Quintana MCD and Fernandez AG, 2004. Biogenic amine formation and "zapatera" spoilage of fermented green olives: Effect of storage temperature and debittering process. *Journal of Food Protection*, 67, 117-123.
- Genigeorgis C, Martin S, Franti CE and Riemann H, 1971. Initiation of staphylococcal growth in laboratory media. *Applied microbiology*, 21, 934-939.
- Gill CO, 1988. Microbiology of edible meat by-products (Chapter 3). In: *Edible Meat By-Products, Advances in Meat Research, Volume 5*. Elsevier Applied Science, London, United Kingdom. AMaD Pearson, T.R. 47-82.
- Gill ON, Bartlett CLR, Sockett PN, Vaile MSB, Rowe B, Gilbert RJ, Dulake C, Murrell HC and Salmaso S, 1983. Outbreak of *Salmonella* Napoli infection caused by contaminated chocolate bars. *The Lancet*, 321, 574-577.
- Goepfert JM and Biggie RA, 1968. Heat resistance of *Salmonella* Typhimurium and *Salmonella* Senftenberg 775W in milk chocolate. *Applied microbiology*, 16, 1939-1940.
- Golden NJ, Crouch EA, Latimer H, Kadry A-R and Kause J, 2009. Risk Assessment for *Clostridium perfringens* in Ready-to-Eat and Partially Cooked Meat and Poultry Products. *Journal of Food Protection*, 72, 1376-1384.
- Guerin PJ, De Jong B, Heir E, Hasseltvedt V, Kapperud G and Styrm K, 2004. Outbreak of *Salmonella* Livingstone infection in Norway and Sweden due to contaminated processed fish products. *Epidemiology and Infection*, 132, 889-895.

- Guillard V, Broyart B, Bonazzi C, Guilbert S and Gontard N, 2003a. Evolution of moisture distribution during storage in a composite food modelling and simulation. *Journal of Food Science*, 68, 958-966.
- Guillard V, Broyart B, Bonazzi C, Guilbert S and Gontard N, 2003b. Preventing moisture transfer in a composite food using edible films: Experimental and mathematical study. *Journal of Food Science*, 68, 2267-2277.
- Gunn RA and Markakis G, 1978. Salmonellosis associated with homemade ice-cream - Outbreak report and summary of outbreaks in United States in 1966 to 1976. *Jama-Journal of the American Medical Association*, 240, 1885-1886.
- Han TH, Paik IK and Kim SJ, 2003. Molecular relatedness between isolates *Yersinia pseudotuberculosis* from a patient and an isolate from mountain spring water. *Journal of Korean Medical Science*, 18, 425-428.
- Harvey RWS, Price TH, Davis AR and Morley-Davies RB, 1961. An outbreak of *Salmonella* food poisoning attributed to bakers' confectionery. *J Hyg (Lond)*, 59, 105-108.
- Hauri AM, Saehrendt M, Spangenberg B and Roggentin P, 2004. A foodborne outbreak of *Salmonella enterica* subsp. *enterica* serovar Madelia at a silver anniversary reception. *European Journal of Clinical Microbiology & Infectious Diseases*, 23, 841-843.
- Hauschild AHW, 1989. *Clostridium botulinum*. In: Foodborne bacterial pathogens. MP Doyle. Marcel Dekker, New-York, 111-190.
- Hennessy TW, Hedberg CW, Slutsker L, White KE, BesserWiek JM, Moen ME, Feldman J, Coleman WW, Edmonson LM, MacDonald KL, Osterholm MT, Belongia E, Boxrud D, Boyer W, Danila R, Korlath J, Leano F, Mills W, Soler J, Sullivan M, Deling M, Geisen P, Kontz C, Elfering K, Krueger W, Masso T, Mitchell MF, Vought K, Duran A, Harrell F, Jirele K, Krivitsky A, Manresa H, Mars R, Nierman M, Schwab A, Sedzielarz F, Tillman F, Wagner D, Wieneke D and Price C, 1996. A national outbreak of *Salmonella enteritidis* infections from ice cream. *New England journal of medicine*, 334, 1281-1286.
- Hersom AC and Hulland ED, 1980. *Canned Foods. Thermal processing and microbiology*, 7th edn. Editor. Churchill Livingstone, Edinburgh, London and New York.
- Hong YC, Bakshi AS and Labuza TP, 1986. Finite element modeling of moisture transfer during storage of mixed multicomponent dried foods. *Journal of Food Science*, 51, 554-558.
- Horowitz BZ, 2011. The ripe olive scare and hotel Loch Maree tragedy: Botulism under glass in the 1920's. *Clinical Toxicology*, 49, 345-347.
- Hwang C-A and Marmer BS, 2007. Growth of *Listeria monocytogenes* in egg salad and pasta salad formulated with mayonnaise of various pH and stored at refrigerated and abuse temperatures. *Food Microbiology*, 24, 211-218.
- Hwang CA and Tamplin ML, 2005. The influence of mayonnaise pH and storage temperature on the growth of *Listeria monocytogenes* in seafood salad. *International Journal of Food Microbiology*, 102, 277-285.
- ICMSF (International Commission on Microbiological Specifications for Food), 1996. *Microorganisms in foods 5. Characteristics of microbial pathogens*. Blackie Academic and Professional, London.
- IFT (Institute of Food Technologists), 2003. Chapter III, Factors that influence microbial growth. *Comprehensive Reviews in Food Science and Food Safety Volume 2 (Supplement)*, pages 21-32.
- Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris LJ, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A and *Salmonella* Enteritidis PTO, 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *Journal of Food Protection*, 68, 191-198.

- Izurieta WP and Komitopoulou E, 2012. Effect of moisture of *Salmonella* spp. heat resistance in cocoa and hazelnut shells. *Food Research International*, 45, 1087-1092.
- Jaloustre S 2011. [Quantitative risk assessment as a mean for evaluating hazard control measures in a food sector. Application to *Clostridium perfringens* in hospital catering] (in French). Ph.D. Thesis: Paris Institute of Technology for Life, Food and Environmental Sciences (AgroParisTech) (France).
- Jay JM, 1992. *Modern Food Microbiology*. 4th Ed. Chapman & Hall, New York,
- Jay JM, 2000. *Modern Food Microbiology*. Aspen Publishers, Inc., Gaithersburg, Maryland, USA.
- Kapperud G, Lassen J, Dommarsnes K, Kristiansen BE, Caugant DA, Ask E and Jahkola M, 1989. Comparison of epidemiological marker methods for identification of *Salmonella* Typhimurium isolates from an outbreak caused by contaminated chocolate. *Journal of clinical microbiology*, 27, 2019.
- Karathanos VT and Kostaropoulos AE, 1995. Diffusion and equilibrium of water in dough/raisin mixtures. *Journal of Food Engin*, 25, 113-121.
- Karl H, Roepstorff A, Huss HH and Bloemsmas B, 1995. Survival of *Anisakis* larvae in marinated herring fillets. *International Journal of Food Science & Technology*, 29, 661-670.
- Keet EE, 1974. *Yersinia enterocolitica* septicemia: source of infection and incubation period identified. *New York State Journal of Medecine*, 74, 2226-2229.
- Kimura AC, Palumbo MS, Meyers H, Abbott S, Rodriguez R and Werner SB, 2005. A multi-state outbreak of *Salmonella* serotype Thompson infection from commercially distributed bread contaminated by an ill food handler. *Epidemiology and Infection*, 133, 823-828.
- Kirk MD, Little CL, Lem M, Fyfe M, Genobile D, Tan A, Threlfall J, Paccagnella A, Lightfoot D, Lyi H, McIntyre L, Ward L, Brown DJ, Surnam S and Fisher IST, 2004. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport - sharing molecular information to solve international outbreaks. *Epidemiology and Infection*, 132, 571-577.
- Koutsoumanis KP, Kendall PA and Sofos JN, 2004. Modeling the boundaries of growth of *Salmonella* Typhimurium in broth as a function of temperature, water activity, and pH. *Journal of Food Protection*, 67, 53-59.
- Kramer JM and Gilbert RJ, 1989. *Bacillus cereus* and other *Bacillus* species. In: *Foodborne bacterial pathogens*. MP Doyle. Marcel Dekker, New York, 21-70.
- Le Guyader FS, Mittelholzer C, Haugarreau L, Hedlund K-O, Alsterlund R, Pommepuy M and Svensson L, 2004. Detection of noroviruses in raspberries associated with a gastroenteritis outbreak. *International Journal of Food Microbiology*, 97, 179-186.
- Lee BH, Kermasha S and Baker BE, 1989. Thermal, ultrasonic and ultraviolet inactivation of *Salmonella* in thin films of aqueous media and chocolate. *Food Microbiology*, 6, 143-152.
- Ling ML, Goh KT, Wang GCY, Neo KS and Chua T, 2002. An outbreak of multidrug-resistant *Salmonella enterica* subsp *enterica* serotype Typhimurium, DT104L linked to dried anchovy in Singapore. *Epidemiology and Infection*, 128, 1-5.
- Liu L, He HF, Dai CF, Liang LH, Li T, Li LH, Luo HM and Fontaine R, 2006. Salmonellosis outbreak among factory workers—Huizhou, Guangdong Province, China, July 2004. *Morbidity and Mortality Weekly Report*, 55, 35-38.
- Lu P-L, Hwang I-J, Tung Y-L, Hwang S-J, Lin C-L and Siu LK, 2004. Molecular and epidemiologic analysis of a county-wide outbreak caused by *Salmonella enterica* subsp. *enterica* serovar Enteritidis traced to a bakery. *BMC Infectious Diseases*, 4, 48.
- Lund BM, Baird-Parker TC and Gould GW, 2000. *The Microbiological Safety and Quality of Food*. Volume I. Aspen Publisher, Inc. Gaithersburg, Maryland, USA.

- Lund V, 1996. Evaluation of *E. coli* as an indicator for the presence of *Campylobacter jejuni* and *Yersinia enterocolitica* in chlorinated and untreated oligotrophic lake water. *Water Research*, 30, 1528-1534.
- Ma L, Zhang G, Gerner-Smidt P, Mantripragada V, Ezeoke I and Doyle MP, 2009. Thermal Inactivation of *Salmonella* in Peanut Butter. *Journal of Food Protection*, 72, 1596-1601.
- Matějovská V, Ticha J and Cernovský J, 1972. Findings of staphylococci in pasta products. *Journal of hygiene, epidemiology, microbiology, and immunology*, 16, 148.
- Mattick KL, Jorgensen F, Wang P, Pound J, Vandeven MH, Ward LR, Legan JD, Lappin-Scott HM and Humphrey TJ, 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Applied and Environmental Microbiology*, 67, 4128-4136.
- Mazuet C, Bouvet P, King LA and Popoff M, 2011. Le botulisme humain en France, 2007-2009. *Bulletin Épidémiologique Hebdomadaire*, 22 février 2011, 6, 49-53.
- McMeekin T, Bowman J, McQuestin O, Mellefont L, Ross T and Tamplin M, 2008. The future of predictive microbiology: Strategic research, innovative applications and great expectations. *International Journal of Food Microbiology*, 128, 2-9.
- McMeekin TA, Brown J, Krist K, Miles D, Neumeyer K, Nichols DS, Olley J, Presser K, Ratkowsky DA, Boss T, Salter M and Soontranon S, 1997. Quantitative microbiology: A basis for food safety. *Emerging Infectious Diseases*, 3, 541-549.
- McMeekin TA, Olley J, Ratkowsky DA and Ross T, 2002. Predictive microbiology: towards the interface and beyond. *International Journal of Food Microbiology*, 73, 395-407.
- McMeekin TA, Presser K, Ratkowsky D, Ross T, Salter M and Tienungoon S, 2000. Quantifying the hurdle concept by modelling the bacterial growth/no growth interface. *International Journal of Food Microbiology*, 55, 93-98.
- Mearin F, Pérez-Oliveras M, Perelló A, Vinyet J, Ibañez A, Coderch J and Perona M, 2005. Dyspepsia and irritable bowel syndrome after a *Salmonella* gastroenteritis outbreak: one-year follow-up cohort study. *Gastroenterology*, 129, 98-104.
- Messelhauser U, Kampf P, Fricker M, Ehling-Schulz M, Zucker R, Wagner B, Busch U and Holler C, 2010. Prevalence of emetic *Bacillus cereus* in different ice creams in Bavaria. *Journal of Food Protection*, 73, 395-399.
- Miettinen MK, Bjorkroth KJ and Korkeala HJ, 1999. Characterization of *Listeria monocytogenes* from an ice cream plant by serotyping and pulsed-field gel electrophoresis. *International Journal of Food Microbiology*, 46, 187-192.
- Milazzo A and Rose N, 2001. An outbreak of *Salmonella* Typhimurium phage type 126 linked to a cake shop in South Australia. *Communicable diseases intelligence*, 25, 73-73.
- Mintz ED, Hudsonwragg M, Mshar P, Cartter ML and Hadler JL, 1993. Foodborne giardiasis in a corporate office setting. *Journal of Infectious Diseases*, 167, 250-253.
- MMWR, 1983. Staphylococcal food poisoning on a cruise ship. *MMWR*, 32, 294-295.
- MMWR, 1991. Fish botulism - Hawaii, 1990, (reprinted from *MMWR*, Vol 40, pp.412-414, 1991). *Jama-Journal of the American Medical Association*, 266, 324-&.
- MMWR, 2007. Multistate outbreaks of *Salmonella* serotype Tennessee infections associated with peanut butter - United States, 2006-2007. *MMWR*, 56, 521-524.
- MMWR, 2009a. Multistate outbreak of *Salmonella* infections associated with Peanut butter and peanut butter - containing products - United States, 2008-2009. *MMWR*, 58, 85-90.
- MMWR, 2009b. Surveillance for Foodborne Disease Outbreaks - United States, 2006. *MMWR*, 58, 609-615.



- Morgan D, Mawer SL and Harman PL, 1994. The role of home-made ice-cream as a vehicle of *Salmonella* Enteritidis phage type 4 infection from fresh shell eggs. *Epidemiology and Infection*, 113, 21-29.
- Morse DL, Shayegani M and Gallo RJ, 1984. Epidemiologic investigation of a *Yersinia* camp outbreak linked to a food handler. *American Journal of Public Health*, 74, 589-592.
- Morton ID, Gray JI and Tybor PT, 1988. Edible tallow, lard and partially defatted tissues (Chapter 12). In: *Edible Meat By-Products, Advances in Meat Research, Volume 5*. Elsevier Applied Science, London, United Kingdom. AMaD Pearson, T.R. 275-302.
- Mossel DAA, Cory JEL, Struijk CB and Baird RM, 1995. *Essentials of the Microbiology of Foods, A Textbook for Advanced Studies*. John Wiley and Sons, New York, New York, USA. Editor.
- Müller L, Hjertqvist M, Payne L, Pettersson H, Olsson A, Plym Forshell L and Andersson Y, 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005-2006 - suspected source: almonds. *Eurosurveillance*, 12, 153-155.
- Murao M, 1991 [Food-borne outbreak of gastroenteritis caused by small round structured virus. 2. An outbreak associated with bakery product consumption] (Jap.). *Kansenshogaku Zasshi*, 65, 1600-1605.
- Mürmann L, Santos MC, Longaray SM, Both JMC and Cardoso M, 2008. Quantification and molecular characterization of *Salmonella* isolated from food samples involved in salmonellosis outbreaks in Rio Grande do Sul, Brazil. *Brazilian Journal of Microbiology*, 39, 529-534.
- NACMCF (National Advisory Committee on Microbiological Criteria for Foods - Executive Secretariat, U.S. Department of Agriculture, Food Safety and Inspection Service, Office of Public Health Science), 2010. Parameters for determining inoculated pack/challenge study protocols. *Journal of Food Protection*. 73, 140-202.
- Nelms PK, Larson O and Barnes-Josiah D, 1997. Time to *B. cereus* about hot chocolate. *Public Health Reports*, 112, 240.
- O'Brien SJ, Murdoch PS, Riley AH, King I, Barr M, Murdoch S, Greig A, Main R, Reilly WJ and Thomson-Carter FM, 2001. A foodborne outbreak of Vero cytotoxin-producing *Escherichia coli* O157:H-phage type 8 in hospital. *Journal of Hospital Infection*, 49, 167-172.
- Ockerman HW and Pellegrino JM, 1988. Meat extractives (Chapter 15). In: *Edible Meat By-Products, Advances in Meat Research, Volume 5*. Elsevier Applied Science, London, United Kingdom. AMaD Pearson, T.R. 303-339.
- Ostroff SM, Kapperud G, Hutwagner LC, Nesbakken T, Bean NH, Lassen J and Tauxe RV, 1994. Sources of sporadic *Yersinia enterocolitica* infections in Norway - a prospective case-control study. *Epidemiology and Infection*, 112, 133-141.
- Pecquet C, Danis M and Leynadier F, 2002. *Anisakis simplex* and immediate hypersensitivity reactions. *Annales De Dermatologie Et De Venereologie*, 129, 303-305.
- Pereira ML, Docarmo LS, Dossantos EJ and Bergdoll MS, 1994a. Staphylococcal food poisoning from cream-filled cake in a metropolitan-area of South-Eastern Brazil. *Revista De Saude Publica*, 28, 406-409.
- Pereira ML, Docarmo LS, Dossantos EJ and Bergdoll MS, 1994b. Staphylococcal food poisoning from cream-filled cake in a metropolitan area of South-Eastern Brazil. *Revista De Saude Publica*, 28, 406-409.
- Periago PM, van Zuijlen A, Fernandez PS, Klapwijk PM, ter Steeg PF, Corradini MG and Peleg M, 2004. Estimation of the non-isothermal inactivation patterns of *Bacillus sporothermodurans* IC4 spores in soups from their isothermal survival data. *International Journal of Food Microbiology*, 95, 205-218.

- Raisanen S, Ruuskanen L and Nyman S, 1985. Epidemic ascariasis-evidence of transmission by imported vegetables. *Scandinavian journal of primary health care*, 3, 189-191.
- Rajkovic A, Uyttendaele M, Vermeulen A, Andjelkovic M, Fitz-James I, in't Veld P, Denon Q, Verhe R and Debevere J, 2008. Heat resistance of *Bacillus cereus* emetic toxin, cereulide. *Letters in Applied Microbiology*, 46, 536-541.
- Ramalho R, Afonso A, Cunha J, Teixeira P and Gibbs PA, 2001. Survival characteristics of pathogens inoculated into bottled mineral water. *Food Control*, 12, 311-316.
- Rasooly R and Do PM, 2010. *Clostridium botulinum* neurotoxin type B is heat-stable in milk and not inactivated by pasteurization. *Journal of Agricultural and Food Chemistry*, 58, 12557-12561.
- Ratkowsky DA, 2002. Some examples of, and some problems with, the use of nonlinear logistic regression in predictive food microbiology. *International Journal of Food Microbiology*, 73, 119-125.
- Ratkowsky DA and Ross T, 1995. Modeling the bacterial growth no growth interface. *Letters in Applied Microbiology*, 20, 29-33.
- Ray B, 2004. *Fundamental Food Microbiology*. Third Edition. CRC Press, Boca Raton, Florida, USA.
- Reekmans R, Stevens P, Vervust T and De Vos P, 2009. An alternative real-time PCR method to detect the *Bacillus cereus* group in naturally contaminated food gelatine: a comparison study. *Letters in Applied Microbiology*, 48, 97-104.
- Roca E, Adeline D, Guillard V, Guilbert S and Gontard N, 2008. Shelf Life and Moisture Transfer Predictions in a Composite Food Product: Impact of Preservation Techniques. *International Journal of Food Engineering*, 4.
- Rocourt J, Jacquet C and Reilly A, 2000. Epidemiology of human listeriosis and seafoods. *International Journal of Food Microbiology*, 62, 197-209.
- Rohde R, Aleksic S, Müller G, Plavsic S and Aleksic V, 1975. Profuse fimbriae conferring O-inagglutinability to several strains of *S. Typhimurium* and *S. Enteritidis* isolated from pasta products. Cultural, morphological, and serological experiments. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Erste Abteilung Originale. Reihe A: Medizinische Mikrobiologie und Parasitologie*, 230, 38.
- Rosenkvist H and Hansen Å, 1995. Contamination profiles and characterisation of *Bacillus* species in wheat bread and raw materials for bread production. *International Journal of Food Microbiology*, 26, 353-363.
- Ross T and Dalgaard P, 2004. Secondary models. In: *Modeling microbial responses in food*. RC McKellar and X. Lu. CRC Press, pp.63-150.
- Salter MA, Ratkowsky DA, Ross T and McMeekin TA, 2000. Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using nonlinear logistic regression. *International Journal of Food Microbiology*, 61, 159-167.
- Shinagawa K, Ueno Y, Hu DL, Ueda S and Sugii S, 1996. Mouse lethal activity of a HEp-2 vacuolation factor, cereulide, produced by *Bacillus cereus* isolated from vomiting-type food poisoning. *Journal of Veterinary Medical Science*, 58, 1027-1029.
- Sobel J, Malavet M and John S, 2007. Outbreak of clinically mild botulism type E illness from home-salted fish in patients presenting with predominantly gastrointestinal symptoms. *Clinical Infectious Diseases*, 45, E14-E16.
- Solhan S, Chan PP, Kurupatham L, Foong BH, Ooi PL, James L, Phua L, Tan AL, Koh D and Gohe KT, 2011. An outbreak of gastroenteritis caused by *Salmonella enterica* serotype Enteritidis traced to cream cakes. *Western Pacific Surveillance and Response*, 2.

- Stephens N, Sault C, Firestone SM, Lightfoot D and Bell C, 2007. Large outbreaks of *Salmonella* Typhimurium phage type 135 infections associated with the consumption of products containing raw egg in Tasmania. *Communicable diseases intelligence*, 31, 118-124.
- Stewart CM, Cole MB and Schaffner DW, 2003. Managing the risk of staphylococcal food poisoning from cream-filled baked goods to meet a food safety objective. *Journal of Food Protection*, 66, 1310-1325.
- Stickel F, Droz S, Patsenker E, Bogli-Stuber K, Aebi B and Leib SL, 2009. Severe hepatotoxicity following ingestion of Herbalife (R) nutritional supplements contaminated with *Bacillus subtilis*. *Journal of Hepatology*, 50, 111-117.
- Takabe F and Oya M, 1976. An autopsy case of food poisoning associated with *Bacillus cereus*. *Forensic Science*, 7, 97-101.
- Telzak EE, Bell EP, Kautter DA, Crowell L, Budnick LD, Morse DL and Schultz S, 1990. An international outbreak of type-E botulism due to uneviscerated fish. *Journal of Infectious Diseases*, 161, 340-342.
- Teunis PFM, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J and Calderon RL, 2008. Norwalk virus: How infectious is it? *Journal of Medical Virology*, 80, 1468-1476.
- Tham W, Ericsson H, Loncarevic S, Unnerstad H and Danielsson-Tham ML, 2000. Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. *International Journal of Food Microbiology*, 62, 173-175.
- Thompson JS and Gravel MJ, 1986. Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype O:3 from well water. *Canadian Journal of Microbiology*, 32, 700-701.
- Tribe IG, Hart S, Ferrall D and Givney R, 2003. An outbreak of *Salmonella* Typhimurium phage type 99 linked to contaminated bakery piping bags. *Communicable diseases intelligence*, 27, 389-390.
- Tribe IG, Tsimogiannis H, Mmolawa P and Davos D, 2001. An outbreak of *Salmonella* Typhimurium phage type 29 linked to a noodle restaurant in South Australia. *Communicable diseases intelligence*, 25, 72-72.
- Tsuji H, Shimada K, Hamada K and Nakajima H, 2000. Outbreak of *Salmonella* Enteritidis caused by contaminated buns peddled by a producer using traveling cars in Hyogo and neighboring prefectures in 1999: an epidemiological study using pulsed-field gel electrophoresis. *Japanese Journal of Infectious Disease*, 53 23-24.
- Uesugi AR and Harris LJ, 2006. Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. *Journal of Food Protection*, 69, 712-718.
- Unicomb LE, Simmons G, Merritt T, Gregory J, Nicol C, Jelfs P, Kirk M, Tan A, Thomson R, Adamopoulos J, Little CL, Currie A and Dalton CB, 2005. Sesame seed products contaminated with *Salmonella*: three outbreaks associated with tahini. *Epidemiology and Infection*, 133, 1065-1072.
- Van de Giessen AW, Dufrenne JB, Ritmeester WS, Berkers P, Van Leeuwen WJ and Notermans SHW, 1992. The identification of *Salmonella* Enteritidis-infected poultry flocks associated with an outbreak of human salmonellosis. *Epidemiology and Infection*, 109, 405-411.
- Vora P, Senecal A and Schaffner DW, 2003. Survival of *Staphylococcus aureus* ATCC 13565 in intermediate moisture foods is highly variable. *Risk Analysis*, 23, 229-236.
- Vought KJ and Tatini SR, 1998. *Salmonella* Enteritidis contamination of ice cream associated with a 1994 multistate outbreak. *Journal of Food Protection*, 61, 5-10.
- Warburton ARE, Wreghitt TG, Rampling A, Buttery R, Ward KN, Perry KR and Parry JV, 1991. Hepatitis A outbreak involving bread. *Epidemiology and Infection*, 106, 199-202.

- Ward B, Andrews R, Gregory J and Lightfoot D, 2002. The use of sequential studies in a salmonellosis outbreak linked to continental custard cakes. *Epidemiology and Infection*, 129, 287-293.
- Weber JT, Hibbs RG, Darwish A, Mishu B, Corwin AL, Rakha M, Hatheway CL, Elsharkawy S, Elrahim SA, Alhamd MFS, Sarn JE, Blake PA and Tauxe RV, 1993. A massive outbreak of type-E botulism associated with traditional salted fish in Cairo. *Journal of Infectious Diseases*, 167, 451-454.
- Werber D, Dreesman J, Feil F, van Treeck U, Fell G, Ethelberg S, Hauri A, Roggentin P, Prager R, Fisher I, Behnke S, Bartelt E, Weise E, Ellis A, Siitonen A, Andersson Y, Tschape H, Kramer M and Ammon A, 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases*, 5, 7.
- Wichmann-Schauer H, Kirschfeld R, Alpers K, Malorny B, Krokotsch B, Geuss-Fosu U, Laarmann S and Sinn G, 2006. Investigation of a foodborne outbreak caused by *Salmonella* Enteritidis in confectionery during a sports event in Berlin, July 2006. *Archiv Fur Lebensmittelhygiene*, 57, 172-179.
- Wilson PDG, Brocklehurst TF, Arino S, Thuault D, Jakobsen M, Lange M, Farkas J, Wimpenny JWT and Van Impe JF, 2002. Modelling microbial growth in structured foods: towards a unified approach. *International Journal of Food Microbiology*, 73, 275-289.
- Woolaway MC, Bartlett CLR, Wieneke AA, Bilbert RJ, Murell HC and Aureli P, 1986. International outbreak of staphylococcal food poisoning caused by contaminated lasagne. *Journal of Hygiene, Cambridge*, 96, 67-73.
- Zhang G, Ma L, Patel N, Swaminathan B, Wedel S and Doyle MP, 2007. Isolation of *Salmonella* Typhimurium from outbreak-associated cake mix. *Journal of Food Protection*, 70, 997-1001.
- Zhao L, Chen Y and Schaffner DW, 2001. Comparison of logistic regression and linear regression in modeling percentage data. *Applied and Environmental Microbiology*, 67, 2129-2135.
- Zhou GP, Zheng DS, Dou L, Cai QX and Yuan ZM, 2010. Occurrence of psychrotolerant *Bacillus cereus* group strains in ice creams. *International Journal of Food Microbiology*, 137, 143-146.

## APPENDICES

### A. CALCULATION OF DOSE-1% AND P1 VALUES REPORTED IN TABLE 1

Dose-response parameters of pathogens were collected from generic sources to classify their virulence potential. Either the exponential dose parameter  $r$  was reported, or the Beta-Poisson parameters  $\alpha$  and  $\beta$ .

#### Formulae used for calculation of Dose-1% and P1 values

$Pill=1-\exp(-rD)$	$Pill=1-(1+D/\beta)^{-\alpha}$
$P_{D=1}=1-\exp(-r)$ (is about $r$ )	$P_{D=1}=1-(1+1/\beta)^{-\alpha}$ (this is about $\alpha/\beta$ )
$D_{1\%}: 0.01=1-\exp(-r*D_{1\%})$	$D_{1\%}: 0.01=1-(1+D_{1\%}/\beta)^{-\alpha}$
$0.99= \exp(-r*D_{1\%})$	$0.99=(1+D_{1\%}/\beta)^{-\alpha}$
$\ln(0.99)= -r*D_{1\%}$	$0.99^{(-1/\alpha)}=1+D_{1\%}/\beta$
$D_{1\%}=-\ln(0.99)/r$	$D_{1\%}/\beta=0.99^{(-1/\alpha)}-1$
	$D_{1\%}=[0.99^{(-1/\alpha)}-1] \beta$

#### Salmonella

Beta-Poisson dose response parameters reported in FAO/WHO (2002):

$$\alpha = 0.1324$$

$$\beta = 51.45$$

Results:

$$\text{Dose-1\% value} = [0.99^{(-1/\alpha)}-1]\beta = 4.06$$

$$\text{P1 value} = 1-(1+1/\beta)^{-\alpha} = 2.54 \cdot 10^{-3}$$

Recently Teunis et al. (2010) even reported higher virulence of *Salmonella*.

#### Shigella

In the FAO/WHO (2011), reference is made to the Cassin et al. (1998) Beta-Poisson model for *Shigella* that is used as surrogate for *E. coli*.

The parameters from Cassin et al. (1998) are:

$$\alpha = 0.267$$

$$\beta = 229 \text{ (the average is taken)}$$

Results:

$$\text{Dose-1\% value} = [0.99^{(-1/\alpha)}-1]\beta = 8.8$$

$$\text{P1 value} = 1-(1+1/\beta)^{-\alpha} = 1.2 \cdot 10^{-3}$$

### **Campylobacter**

Beta-Poisson dose response parameters reported in FAO/WHO (2009):

$$\alpha = 0.21$$

$$\beta = 59.95$$

Results:

$$\text{Dose-1\% value} = [0.99^{(-1/\alpha)} - 1]\beta = 2.94$$

$$\text{P1 value} = 1 - (1 + 1/\beta)^{(-\alpha)} = 3.47 \cdot 10^{-3}$$

### **Escherichia coli**

r-values reported in Delignette-Muller et al. (2008):

$$r \text{ for children of less than 6 years} = 1.2 \cdot 10^{-3}$$

$$r \text{ for children of 6-10 years} = 2.4 \cdot 10^{-4}$$

Results for children of less than 6 years:

$$\text{Dose-1\% value} = -\ln(0.99)/r = 8.38$$

$$\text{P1 value} = 1 - \exp(-r) = 1.20 \cdot 10^{-3}$$

Results for children of 6-10 years:

$$\text{Dose-1\% value} = -\ln(0.99)/r = 41.87$$

$$\text{P1 value} = 1 - \exp(-r) = 2.40 \cdot 10^{-4}$$

### **Listeria monocytogenes**

r-values reported in FAO/WHO (2004):

$$r \text{ for more susceptible subpopulation} = 1.06 \cdot 10^{-12}$$

$$r \text{ for less susceptible subpopulation} = 2.37 \cdot 10^{-14}$$

Results for more susceptible population:

$$\text{Dose-1\% value} = -\ln(0.99)/r = 9.5 \cdot 10^9$$

$$\text{P1 value} = 1 - \exp(-r) = 1.1 \cdot 10^{-12}$$

Results for less susceptible population:

$$\text{Dose-1\% value} = -\ln(0.99)/r = 4.24 \cdot 10^{11}$$

$$\text{P1 value} = 1 - \exp(-r) = 2.36 \cdot 10^{-14}$$

**Vibrio parahaemolyticus**

Beta-Poisson dose response parameters reported by FDA<sup>13</sup>:

$$\alpha = 0.6$$

$$\beta = 1.31 \cdot 10^6$$

Results:

$$\text{Dose-1\% value} = [0.99^{(-1/\alpha)} - 1] \beta = 22.13 \cdot 10^4$$

$$\text{P1 value} = 1 - (1 + 1/\beta)^{(-\alpha)} = 4.58 \cdot 10^{-7}$$

**Clostridium perfringens**

D50% value reported in Jaloustre (2011):

$$D_{50\%} = 10^8$$

Results:

$$r = -\ln(0.50)/D_{50\%} = 6.93 \cdot 10^{-9}$$

$$\text{Dose-1\% value} = -\ln(0.99)/r = 1.45 \cdot 10^6$$

$$\text{P1 value} = 1 - \exp(-r) = 6.93 \cdot 10^{-9}$$

---

<sup>13</sup> [www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm185499.htm](http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm185499.htm)  
(accessed 2012-04-19)

## REFERENCES OF APPENDIX A

- Cassin MH, Lammerding AM, Todd EC, Ross W and McColl RS, 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int J Food Microbiol*, 41, 21-44.
- Delignette-Muller ML, Cornu M and AFSSA STEC study group, 2008. Quantitative risk assessment for *Escherichia coli* O157:H7 in frozen ground beef patties consumed by young children in French households. *International Journal of Food Microbiology*, 128, 158-164.
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2002. Risk assessments of *Salmonella* in eggs and broiler chickens: Interpretative summary. Microbiological Risk Assessment Series 1 (ISBN 92-5-104873-8).
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2004. Risk assessment of *Listeria monocytogenes* in ready to eat foods: Interpretative summary. Microbiological Risk Assessment Series 4 (ISBN 92-5-105126-7).
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2009. Risk assessment of *Campylobacter* spp. in broiler chickens: Technical Report. Microbiological Risk Assessment Series No. 12 (ISBN 978-92-5-105879-4).
- FAO/WHO (Food And Agriculture Organization, World Health Organization), 2011. Enterohaemorrhagic *Escherichia coli* in raw beef and beef products: approaches for the provision of scientific advice. Meeting report. Microbiological Risk Assessment Series 18. (ISBN 978-92-5-107010-9).
- Jaloustre S, 2011. [Quantitative risk assessment as a mean for evaluating hazard control measures in a food sector. Application to *Clostridium perfringens* in hospital catering] (in French). Ph.D. Thesis: Paris Institute of Technology for Life, Food and Environmental Sciences (AgroParisTech) (France).
- Teunis PFM, Kasuga F, Fazil A, Ogden ID, Rotariu O and Strachan NJC, 2010. Dose–response modeling of *Salmonella* using outbreak data. *International Journal of Food Microbiology*, 144, 243-249.



## B. CHILL CHAIN OF REFRIGERATED FOOD: TEMPERATURE SURVEYS OF RETAIL AND DOMESTIC REFRIGERATORS

The available data on the temperature of retail refrigerators in Europe are limited. Table 15 summarizes the results of three survey studies on retail storage temperature in France, Slovenia and Greece. The mean temperature in the three available surveys is very similar, ranging from 4 to 5 °C.

**Table 15:** Temperature survey data on retail refrigerators in three European countries.

Year	Country	n	T <sub>min</sub> °C	T <sub>mean</sub> °C	T <sub>max</sub> °C	Reference
1996	France	-	-	4.0	-	Pierre (1996)
2006	Slovenia	1,286	-2.2	4.6 (weighted)	12.2	Likar and Jevsnik (1996)
2010	Greece	110	0	4.98	11.7	Koutsoumanis et al. (2010)

The study of Koutsoumanis et al. (2010) was conducted in Greece and temperature was recorded with miniature dataloggers (COX TRACER™, Belmont, NC) in 60 retail cabinets from supermarkets. The distribution of the mean temperature for the tested retail refrigerators is presented in Figure 11. Temperature ranged from 0° to 11.7°C, with a mean of 4.98°C and a standard deviation of 2.9°C. In 70% of the tested refrigerators the temperature was above 4°C, while in 6.7% the temperature was above 10°C.

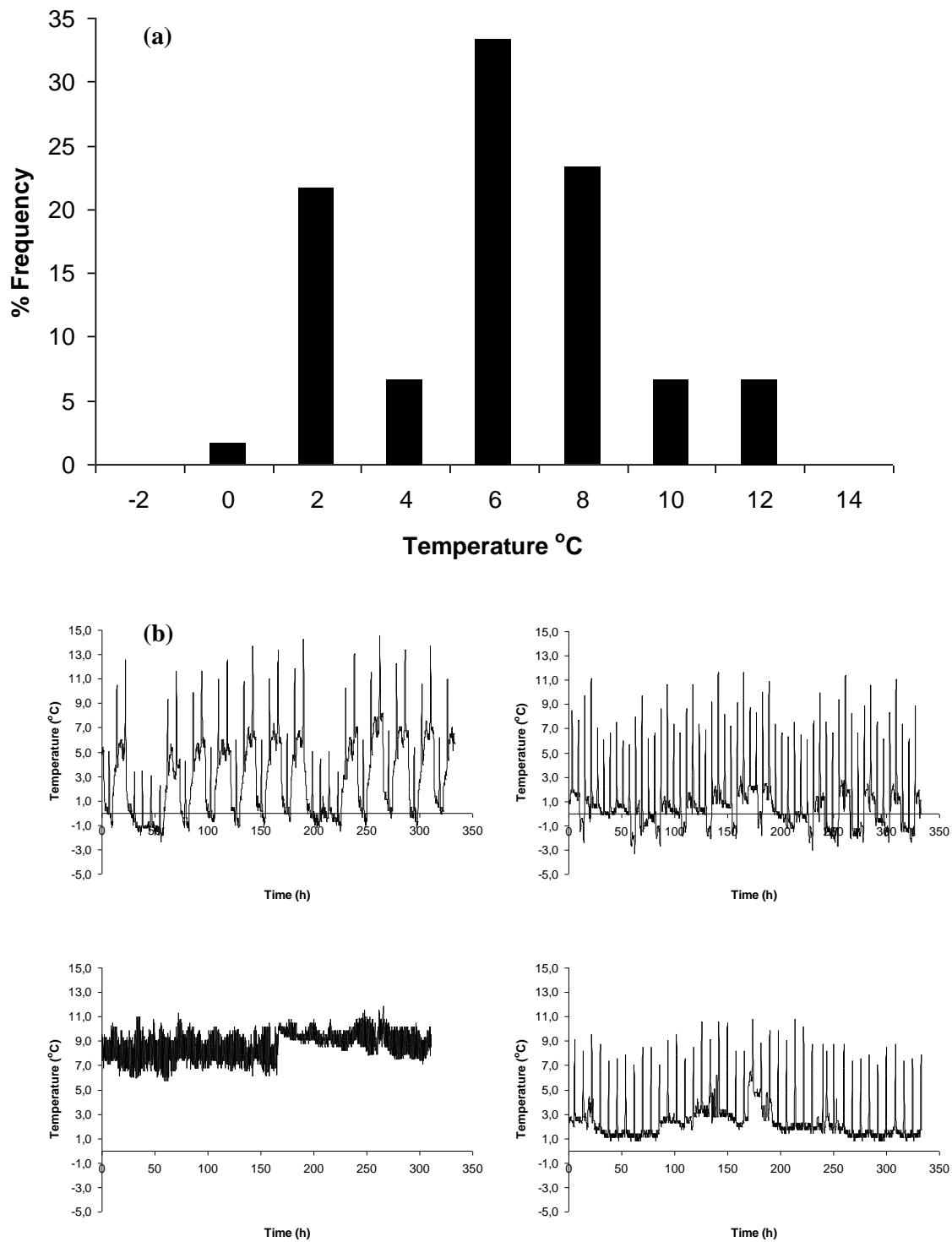
Refrigerated composite products may spend the majority of their shelf-life in a domestic refrigerator, rather than in a retail or industrial chiller. Domestic refrigerator temperatures can therefore have a significant effect on the safety of these products. Once a chilled food product has left the retailer from which it was purchased, it is beyond the reach of legislation, so correct handling and storage is entirely down to the motivation and knowledge of the purchaser.

Data from 17 surveys of domestic refrigerator temperatures from six European countries are presented in Table 16. The data are presented in such a manner as to facilitate comparison between surveys, although this is not always possible due to the use of different parameters and temperature ranges in the reporting of the data. Of the ten surveys (1,856 samples) for which a mean temperature was given, this ranged from 5° to 7.2°C. The weighted mean of the means was 6.5°C. No regional or temporal trends are evident from these data. The weighted mean percentage of domestic refrigerators running above the following temperatures (number of surveys in brackets) was: >4°C, 86.4% (2); >5°C, 53.9% (11); >6°C, 58.4% (5); >7°C, 41.8% (3); >8°C, 28.7% (2); >9°C, 26.5% (3); >10°C, 5.2% (4). In the latest survey, conducted in 100 Greek households, the mean temperature varied significantly between refrigerators, with a standard deviation of 2.58 °C. Another important fact confirmed in this survey was that, in all cases, there was major temperature variability recorded at the different locations of the refrigerators (see Figure 12). A significantly higher mean temperature was observed in the door shelf (8.4°C) compared to the upper (7.6°C) the middle (6.3°C) and the lower shelf (6.7°C). The latter observation is important, since some ready-to-eat foods such as pasteurized milk are usually stored in the door shelf of the domestic refrigerator (Xanthiakos et al., 2006). The above results concerning variation of temperature with time and location within the refrigerator are in agreement with other survey studies on domestic refrigerators (Laguerre et al., 2002; Taoukis et al., 2005).

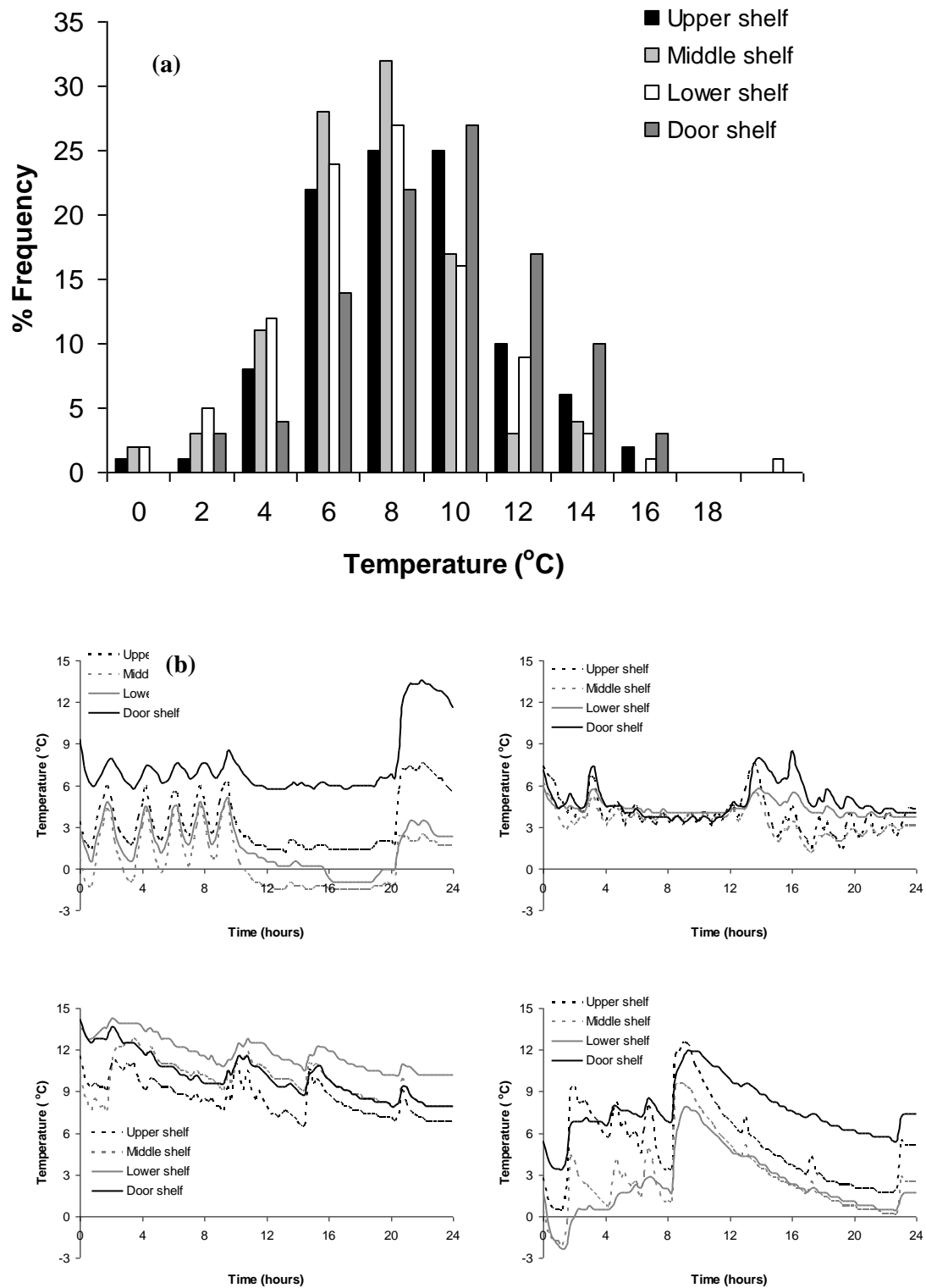
The above data clearly show that storage temperature at both retail and domestic level can vary significantly between refrigerators. This variability should be taken into account in the evaluation of the safety risks of refrigerated composite products.

**Table 16:** Temperature survey data on domestic refrigerators (modified from James et al.(2008)).

Year	Country	n	T <sub>min</sub> °C	T <sub>mean</sub> °C	T <sub>max</sub> °C	% refrigerators running at temp (°C)							Reference
						>4	>5	>6	>7	>8	>9	>10	
1990	UK	75		<5	15		6						Rose et al., 1990
1991	UK	252	0.9	6	11.4		70						Evans et al., 1991
1992	UK	150	0.8	6.5	12.6		71						Flynn et al., 1992
1993	France	102			14			70					Victoria, 1993
1994	Netherlands	125					70		28		2		Lezenne Coulander de, 1994
1997	Greece	136									50		Sergelidis et al., 1997
1997	UK	108	2	5.9	12		50						Worsfold and Griffith, 1997
1998	UK	645	-2	7	13		70						Johnson et al., 1998
2002	France	119	0.9	6.6	11.4		80						Laguerre et al., 2002
2003	UK	901					31					3	Ghebrehewet and Stevenson, 2003
2003	Greece	110				74		46		23		8	Bakalis et al., 2003
2004	Belgium	3,001	5.0	7.0	9.0			55.1					Devriese et al., 2006
2005	Ireland	100	-7.9	5.4	20.7		59						Kennedy et al., 2005
2005	Portugal	86						70					Azevedo et al., 2005
2005	Greece	258	-2	6.3				50				10	Taoukis et al., 2005
2005	Netherlands	31	3.8		11.5				68				Terpstra et al., 2005
2006	Greece	100	0.2	7.2	14.6	100	82	72	51	35	25	12	Koutsoumanis et al., 2010
2006	UK	24		5				33					Breen et al., 2006
<b>Sum / weighted mean</b>		<b>3,322</b>		<b>6.5</b>		<b>86.4</b>	<b>53.9</b>	<b>58.4</b>	<b>41.8</b>	<b>28.7</b>	<b>26.5</b>	<b>5.2</b>	



**Figure 11:** Distribution of mean temperature (a) and representative temperature profiles (b) in retail refrigerators (source: Koutsoumanis et al. (2010), reprinted with permission from *Applied and Environmental Microbiology*).



**Figure 12:** Distribution of mean temperature (a) and representative temperature profiles (b) in domestic refrigerators (source: Koutsoumanis et al. (2010), reprinted with permission from *Applied and Environmental Microbiology*).

**REFERENCES OF APPENDIX B**

- Azevedo I, Regalo M, Mena C, Almeida G, Carneiro L, Teixeira P, Hogg T and Gibbs PA, 2005. Incidence of *Listeria* spp. in domestic refrigerators in Portugal. *Food Control*, 16, 121-124.
- Bakalis S, Giannakourou MC and Taoukis PS, 2003. Effect of domestic storage and cooking conditions on the risk distribution in ready to cook meat products. In the 9th international congress on engineering and food (ICEF9), Montpellier, 7-11 March 2003.
- Breen A, Brock S, Crawford K, Docherty M, Drummond G, Gill L, Lawton S, Mankarious V, Oustayiannis A, Rushworth G and Kerr KG, 2006. The refrigerator safari - An educational tool for undergraduate students learning about the microbiological safety of food. *British Food Journal*, 108, 487-494.
- Devriese S, Huybrechts I, Moreau M and Van Oyen H, 2006. De Belgische Voedselconsumptiepeiling 1-2004. *Epidemiologie*, maart 2006; Brussel (België). Wetenschappelijk Instituut Volksgezondheid, WIV/EPI REPORTS N 2006 – 016. Available at: [www.wiv-isp.be/epidemio/epinl/foodnl/food04nl/food9nl.pdf](http://www.wiv-isp.be/epidemio/epinl/foodnl/food04nl/food9nl.pdf)
- Evans JA, Stanton JI, Russell SL and James SJ, 1991. Consumer handling of chilled foods: A survey of time and temperature conditions. Editor. MAFF Publications, PB 0682, London.
- Flynn OMJ, Blair I and McDowell D, 1992. The efficiency and consumer operation of domestic refrigerators. *International Journal of Refrigeration-Revue Internationale Du Froid*, 15, 307-312.
- Ghebrehewet S and Stevenson L, 2003. Effectiveness of home-based food storage training: a community development approach. *International Journal of Environmental Health Research*, 13, S169-S174.
- James SJ, Evans J and James C, 2008. A review of the performance of domestic refrigerators. *Journal of Food Engineering*, 87, 2-10.
- Johnson AE, Donkin AJM, Morgan K, Lilley JM, Neale RJ, Page RM and Silburn R, 1998. Food safety knowledge and practice among elderly people living at home. *Journal of Epidemiology and Community Health*, 52, 745-748.
- Kennedy J, Jackson V, Blair IS, McDowell DA, Cowan C and Bolton DJ, 2005. Food safety knowledge of consumers and the microbiological and temperature status of their refrigerators. *Journal of Food Protection*, 68, 1421-1430.
- Koutsoumanis K, Pavlis A, Nychas G-JE and Xanthiakos K, 2010. Probabilistic model for *Listeria monocytogenes* growth during distribution, retail storage, and domestic storage of Pasteurized Milk. *Applied and Environmental Microbiology*, 76, 2181-2191.
- Laguerre O, Derens E and Palagos B, 2002. Study of domestic refrigerator temperature and analysis of factors affecting temperature: a French survey. *International Journal of Refrigeration-Revue Internationale Du Froid*, 25, 653-659.
- Lezenne Coulander de PA, 1994. Koelkast temperature thuis. Report of the Regional Inspectorate for Health Protection. Leeuwarden, The Netherlands.
- Likar K and Jevsnik M, 1996. Cold chain maintaining in food trade. *Food Control* 17:10-113.
- Pierre O, 1996. Température de conservation de certaines denrées alimentaires très périssables dans les rayons "libre service" des grandes et moyenne surfaces. *Option Qualité*, 138, 12-18.
- Rose SA, Steadman S and Brunskill R, 1990. A temperature survey of domestic refrigerators. CCFRA Technical Memorandum No. 577.
- Sergelidis D, Abraham A, Sarimvei A, Panoulis C, Karaioannoglou P and Genigeorgis C, 1997. Temperature distribution and prevalence of *Listeria* spp. in domestic, retail and industrial refrigerators in Greece. *International Journal of Food Microbiology*, 34, 171-177.

- Taoukis PS, Giannakourou MC, Koutsoumanis K and Bakalis S, 2005. Modelling the effect of household chilled storage conditions on the risk distribution of meat products. In: Acta Horticulturae. MLATMNBM Hertog. 435-439.
- Terpstra MJ, Steenbekkers LPA, de Maertelaere NCM and Nijhuis S, 2005. Food storage and disposal: consumer practices and knowledge. *British Food Journal*, 107, 526-533.
- Victoria R, 1993. Ne joues pas avec le froid. 50 millions de consommateurs, 267.
- Worsfold D and Griffith C, 1997. Food safety behaviour in the home. *British Food Journal*, 99, 97-104.
- Xanthiakos K, Simos D, Angelidis AS, Nychas GJE and Koutsoumanis K., 2006. Dynamic modeling of *Listeria monocytogenes* growth in pasteurized milk. *J. App. Microbiol.* 100:1289–1298.

**C. MEAN *D*-VALUES AND *Z*-VALUES FOR VEGETATIVE BACTERIA AND BACTERIAL SPORES AT GIVEN TEMPERATURES**

**Table 17:** Mean *D*- (min) and *z*-values (°C) at different temperatures (*T*, in °C) for various vegetative and sporulated microorganisms in foods (confidence intervals at 95% are expressed between brackets).

		<b>Food matrix</b>	<b>Replicates</b>	<b><i>D</i> (min)</b>	<b><i>T</i> (°C)</b>	<b><i>z</i> (°C)</b>	<b>Reference</b>
<b>Food-borne pathogen (vegetative)</b>	<i>Salmonella</i> Typhimurium*	Chocolate syrup	4	2.4 (1.1, 3.8)	65.6	7.5 (6.1, 8.9)	Sumner et al., 1991
	<i>Salmonella</i> Typhimurium*	Egg yolk	4	9.2 (7.4, 11)	55.0	4.2 (2.7, 5.6)	Jung and Beuchat, 2000
	<i>Listeria monocytogenes</i> *	Chicken gravy	10	154 (95, 214)	50.0	4.6 (4.0, 5.3)	Huang et al., 1992
	<i>Clostridium perfringens</i> *	Minced beef	8	6.1 (2.9, 9.4)	59.0	3.9 (3.4, 4.5)	Juneja and Marmer, 1998
	<i>Escherichia coli</i> O157:H7*	Ground/minced beef	7	84 (63, 104)	50.0	4.5 (4.3, 4.7)	Ahmed et al., 1995
	<i>Escherichia coli</i> O157:H7*	Pork sausage	3	64 (26, 103)	50.0	4.7 (4.5, 4.8)	Ahmed et al., 1995
	<i>Salmonella</i> spp.*	Chicken broth	9	4.6 (4.0, 5.2)	55.0	6.2 (5.9, 6.4)	Juneja et al., 2001
	<i>Listeria monocytogenes</i> *	Ham	3	17 (9.3, 24)	55.0	5.4 (2.4, 8.4)	Carlier et al., 1996
<b>Food-borne pathogen (spore-forming; refers to spores unless otherwise mentioned)</b>	<i>Bacillus</i> spp.* (vegetative)	Milk	4	39 (5.6, 73)	72.0	9.7 (8.1, 11.2)	Xu et al., 2006
	<i>Clostridium botulinum</i> *	Mushroom extract	3	0.042 (0.038, 0.045)	121.1	15.2 (9.3, 21.2)	Brown and Martinez, 1992
	<i>Bacillus cereus</i>	Infant formula (milk-based) (pH 6.3)	-	95	15.3	8.7	ICMSF, 1996

	Food matrix	Replicates	D (min)	T (°C)	z (°C)	Reference	
	<i>Bacillus cereus</i>	0.25M phosphate buffer (pH 7.0)	6	85	33.8	9.7	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type A)	Pea puree	-	110	2.0	8.3	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type A)	Spaguetti, tomato sauce and cheese	-	116	0.5	8.3	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type A)	Spanish rice	-	110	2.4	8.6	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type A)	Mackerel in oil	-	121	0.4	12.7	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type B)	Fresh corn puree (pH 6.9)	-	110	2.9	10.6	ICMSF, 1996
	<i>Clostridium botulinum</i> (proteolytic Type B)	Canned asparagus (pH 5.04)	-	100	11.9	9.7	ICMSF, 1996
	<i>Clostridium botulinum</i> (non proteolytic Type B)	Phosphate buffer	-	85	100.0	7.6	ICMSF, 1996
	<i>Clostridium botulinum</i> (non proteolytic Type E)	Surimi	-	82	1.2	9.8	ICMSF, 1996
<b>Food spoilage bacteria (spore-forming; refers to spores)</b>	<i>Geobacillus stearothermophilus</i> *	Mushroom extract	7	6.7 (2.6, 11)	115.0	8.9 (7.8, 10.0)	Fernandez et al., 1994
	<i>Clostridium sporogenes</i> *	Peas puree	6	22 (17, 27)	110.0	10.6 (9.5, 11.7)	Cameron et al., 1980
	<i>Clostridium sporogenes</i>	Phosphate buffer (pH 7.0)	5	121	2.4	9.0	Santos and Zarzo, 1995
	<i>Clostridium sporogenes</i>	Natural asparagus (acidified) (pH 5.0)	5	121	1.1	8.7	Santos and Zarzo, 1995

\* Values collected from Lemgo Database ([www.hs-owl.de/fb4/ldzbase/index.pl](http://www.hs-owl.de/fb4/ldzbase/index.pl))



## REFERENCES OF APPENDIX C

- Ahmed NM, Conner DE and Huffman DL, 1995. Heat resistance of *Escherichia coli* O157H7 in meat and poultry as affected by product composition. *Journal of Food Science*, 60, 606-610.
- Brown KL and Martinez A, 1992. The heat-resistance of spores of *Clostridium botulinum* 213b heated at 121-130°C in acidified mushroom extract. *Journal of Food Protection*, 55, 913-915.
- Cameron MS, Leonard SJ and Barrett EL, 1980. Effect of moderately acidic pH on heat-resistance of *Clostridium sporogenes* spores in phosphate buffer and in buffered pea puree. *Applied and Environmental Microbiology*, 39, 943-949.
- Carlier V, Augustin JC and Rozier J, 1996. Heat resistance of *Listeria monocytogenes* (Phagovar 2389/2425/3274/2671/47/108/340): D- and z-values in ham. *Journal of Food Protection*, 59, 588-591.
- Fernandez PS, Ocio MJ, Sanchez T and Martinez A, 1994. Thermal-resistance of *Bacillus stearothermophilus* spores heated in acidified mushroom extract. *Journal of Food Protection*, 57, 37-41.
- Huang IPD, Yousef AE, Marth EH and Matthews ME, 1992. Thermal inactivation of *Listeria monocytogenes* in chicken gravy. *Journal of Food Protection*, 55, 492-496.
- ICMSF (International Commission on Microbiological Specifications for Food), 1996. *Microorganisms in foods 5. Characteristics of microbial pathogens*. Blackie Academic and Professional, London.
- Juneja JK and Marmer BS, 1998. Thermal inactivation of *Clostridium perfringens* vegetative cells in ground beef and turkey as affected by sodium pyrophosphate. *Food Microbiology*, 15, 281-287.
- Juneja VK, Eblen BS and Ransom GM, 2001. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken: Determination of D- and Z-values. *Journal of Food Science*, 66, 146-152.
- Jung YS and Beuchat LR, 2000. Sensitivity of multidrug-resistant *Salmonella* Typhimurium DT104 to organic acids and thermal inactivation in liquid egg products. *Food Microbiology*, 17, 63-71.
- Santos MHS and Zarzo JT, 1995. Glucono-delta-lactone and citric-acid as acidulants for lowering the heat-resistance of *Clostridium sporogenes* PA 3679 in HTST working-conditions. *International Journal of Food Microbiology*, 25, 191-197.
- Sumner SS, Sandros TM, Harmon MC, Scott VN and Bernard DT, 1991. Heat-resistance of *Salmonella* Typhimurium and *Listeria monocytogenes* in sucrose solutions of various water activities. *Journal of Food Science*, 56, 1741-1743.
- Xu S, Labuza TP and Diez-Gonzalez F, 2006. Thermal inactivation of *Bacillus anthracis* spores in cow's milk. *Applied and Environmental Microbiology*, 72, 4479-4483.

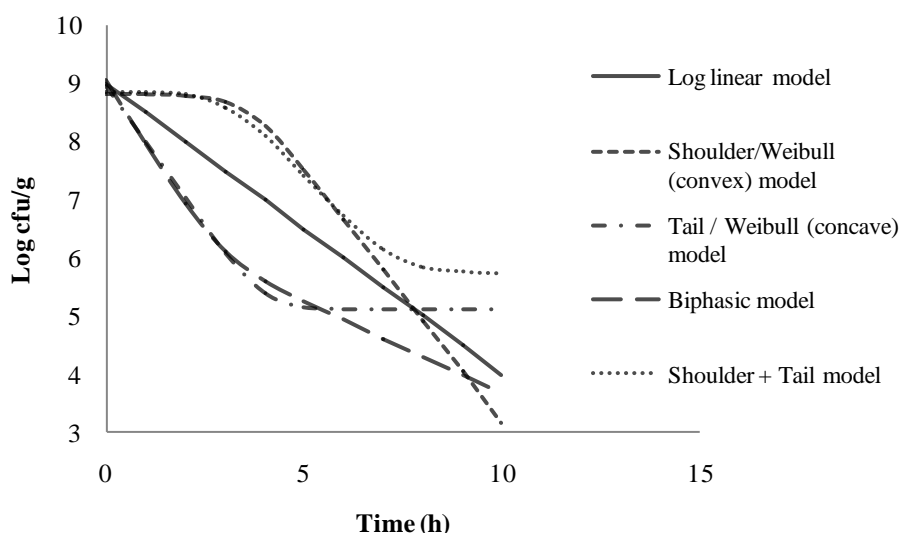
## D. MODELLING OF INACTIVATION/GROWTH OF PATHOGENIC MICROORGANISMS IN FOOD

### 1. Inactivation models of pathogenic microorganisms in food

#### 1.1 Primary inactivation models

One of the quantitative microbiology tools for microbial inactivation is a freeware Add-in for Microsoft Excel, so-called GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool), which includes a large variety of primary inactivation models by the use of linear and non linear regression approaches. The application, described by Geeraerd et al. (2005) comprises nine model types: (i) classical log-linear curves, (ii) curves displaying a so-called shoulder before a log-linear decrease is apparent, (iii) curves displaying a so-called tail after a log-linear decrease, (iv) survival curves displaying both shoulder and tailing behaviour, (v) concave curves, (vi) convex curves, (vii) convex/concave curves followed by tailing, (viii) biphasic inactivation kinetics, and (ix) biphasic inactivation kinetics preceded by a shoulder.

The most used models for describing the inactivation of pathogens in foods are presented in Figure 13, and a brief description of the various models is provided below.



**Figure 13:** Most used models for describing the inactivation of pathogens in foods.

#### **Bigelow model (linear model)**

This model was established to quantify microbial inactivation in the canning industry, assuming first-order kinetics. Apparently, cell death occurs due to an inactivation of a critical enzyme, and it is commonly stated that this inactivation follows first-order kinetics (although there might be some exceptions to this rule). The model has the following form:

$$\ln N = \ln N_0 - kt \quad \text{Eq.1}$$

with  $N$  as the number of microorganisms,  $N_0$  as the initial number of microorganisms, and  $k$  as the first order rate constant ( $s^{-1}$ ). This equation is then rearranged into:

$$\log \frac{N}{N_0} = \log S(t) = -\frac{t}{D} \quad \text{Eq.2}$$

where  $D$  is the decimal reduction time ( $D = 2.303/k$ , units in min or sec.) and  $S(t)$  is the momentary survival ratio.

A drawback of this model is that it is mainly focused on thermal treatments rather than on novel preservation techniques.

When  $\log D$ -values are plotted against temperature, the reciprocal of the slope is equal to the  $z$ -value, which is the temperature increase needed to reduce  $D$  by a factor 10, so to increase the destruction rate by a factor 10.

$$D = D_{ref} 10^{\left(\frac{T_{ref}-T}{z}\right)} \quad \text{Eq.3}$$

$$z = \frac{T_{ref} - T}{\log D - \log D_{ref}} \quad \text{Eq.4}$$

where  $D_{ref}$  is the  $D$ -value at the reference temperature,  $T_{ref}$  (the usual  $T_{ref}$  is 121.1°C).  $D$ -values could be influenced by the type of organism (strain), treatment temperature, physiological state of cells (cells in stationary phase often have higher  $D$ -values), pH (neutral pH values have higher  $D$ -values), fat and  $a_w$  content (high fat and low  $a_w$  values are generally associated to higher  $D$ -values because they exert a protective effect).

The rate constant can also be related to the temperature by the Arrhenius equation:

$$k = k_0 e^{\left(-\frac{Ea}{RT}\right)} \quad \text{Eq.5}$$

where  $Ea$  is the activation energy,  $R$  the universal gas constant and  $T$  is the temperature in degrees Kelvin. The Arrhenius model has been used for describing the kinetic behaviour of *E. coli* as a function of temperature, pH and  $a_w$  (Cerf et al., 1996).

There is one parameter, named sterilizing value ( $F$ ) that is used to designate the thermal death time, or the time to kill all pathogenic organisms at 121.1°C. Schmidt (1957), in attempting to correlate the values of  $D$  and  $F$  from existing data, showed that “to kill all” in practical terms meant to lower the population to  $10^{-12}$  organisms.

The  $F$  value can be defined as the time taken to reduce initial microbial numbers, at a specified temperature, by a particular value, normally a multiple of the  $D$ -value for the target organism. The process goal as described by the sterilizing value is given by the expression:

$$F = D (\log N_0 - \log N) \quad \text{Eq.6}$$

For a non-acid food the minimum process must assure safety by destroying any contaminating *C. botulinum*. As suggested by Stumbo et al. (1975), this is considered to be accomplished by 12D process, which is a  $10^{-12}$  reduction of the  $N_0$  value.

Another view to microbial inactivation has been discussed in a series of articles (Peleg and Cole, 1998, 2000; Peleg and Penchina, 2000), although the basic principles can already be found in older literature (Vas and Prosz, 1957). It considers lethal events as probabilities, rather than as deterministic events.

The alternative view thus looks upon survival curves as an expression of underlying statistical distributions of inactivation times.

Due to its broad applicability, the log linear model is most appropriate to obtain a first impression on the performance of an inactivation process. This is especially useful for the food industry, where elaborate knowledge and the necessary tools for complicated models and generic parameter values are not available (van Asselt and Zwietering, 2006).

### **Weibull model**

The Weibull model has been used as a primary thermal inactivation model for vegetative bacteria (Van Boekel, 2002). This model assumes non linearity of semi logarithmic survivor curves in the inactivation process through considering biological variation with respect to thermal inactivation, and is basically a statistical model of distribution of inactivation times. The model is built by two parameters, the scale parameter  $\alpha$  (time) and the dimensionless shape parameter  $\beta$ . The logarithm of the scale parameter  $\alpha$  depends linearly on temperature; however, this relationship for parameter  $\beta$  is not so well established. The shape parameter accounts for upward concavity of a survival curve ( $\beta < 1$ ), a linear survival curve ( $\beta = 1$ ), and downward concavity ( $\beta > 1$ ). Therefore, if  $\beta = 1$  no biological variation is assumed (each cell is equally susceptible to be destroyed).

In terms of a survival curve, the cumulative function is:

$$\log S(t) = -\frac{1}{2.303} \left( \frac{t}{\alpha} \right)^\beta \quad \text{Eq.7}$$

The Weibull distribution function (which is closely related to the Gamma, Extreme Value and Log normal distributions) is widely used in reliability engineering to describe time to failure in electronic and mechanical systems and is also appropriate for the analysis of survival data, i.e. time to failure after the application of stress.

Although the Weibull model is of an empirical nature, a link can be made with physiological effects.  $\beta < 1$  indicates that the remaining cells have the ability to adapt to the applied stress, whereas  $\beta > 1$  indicates that the remaining cells become increasingly damaged.

This variation in parameters allows the Weibull model to be more flexible than the linear model based on  $D$ -values. For instance, cell behaviour may be quite different when they have been adapted to certain stress conditions in foods or when they have grown in 'ideal' laboratory conditions.

Although Eq.7 has been found to be an adequate model for a variety of microorganisms, it is in no way unique and alternative models can be just as applicable (Peleg and Pechina, 2000). Modifications of the Weibull model have been proposed by Mafart et al. (2002) for the calculation of the heat treatment efficiency to describe non linear survival curves of spores. Later on, Coroller et al. (2006) applied a general model based on two mixed Weibull distributions of cell resistance of vegetative bacteria subjected to an acidic stress (*Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium) to describe the survival curves and the change in the pattern with the modifications of resistance of two assumed subpopulations.

One development in the modelling of bacterial survival is the use of distributions. This is based on the assumption that lethal events are probabilistic rather than deterministic. The survival curve for a single cell is a step function, where a cell can be either alive or death:

$$S_i(t) = \begin{cases} 1 \text{ (alive) for } t < t_c \\ 0 \text{ (dead) for } t \geq t_c \end{cases} \quad \text{Eq.8}$$

where  $t_c$  is the inactivation time. Since all cells would not be expected to die at the same time, values of  $t_c$  would follow a distribution. In many cases, the Weibull distribution seems to be appropriate for modelling bacterial inactivation.

### Shoulder/tail models

Shoulder/tail models are based upon the existence of a shoulder or lag period (prior to inactivation) and a tail region (after the inactivation treatment). The first type of deviation is produced when the curve is flat, i.e., no change in the number of microorganisms at the beginning of the inactivation treatment. The second type is the tailing of survivor curves which occurs at the end of the inactivation treatment and is characterized by the culture showing more resistance than would be expected from the previous logarithmic order of destruction.

Stringer et al. (2000) has summarized possible explanations for this deviation from linearity, like variability in heating procedure, use of mixed cultures, clumping or protective effect of the food matrix or dead cells.

A linear approach was followed by Whiting (1993) for modelling inactivation of *L. monocytogenes* and *Salmonella* as a function of temperature and in presence of NaCl, nitrites and lactic acid.

$$\log N = \begin{cases} \log N_0 & \text{when } 0 < t < t_L \\ \log N_0 - \left(\frac{1}{D}\right)(t - t_L) & \text{when } t > t_L \end{cases} \quad \text{Eq.9}$$

where  $N$  is the number of microorganisms surviving at time  $t$ ,  $N_0$  is the initial microbial load,  $t_L$  is the time prior to inactivation and  $D$  is the  $D$ -value.

The model was successfully applied to describe non thermal inactivation of *L. monocytogenes* as a function of organic acid and nitrite concentrations (Buchanan and Golden, 1994) and under reduced oxygen (Buchanan and Golden, 1995).

Regarding the shoulder region, model fitting is more difficult since a high variability is associated to this parameter. Thus, survival is often described through the time required for a 4 log<sub>10</sub> reduction, T4D (Whiting, 1993). This value is calculated as the sum of  $t_L + 4 \cdot D$ .

Nonlinear approaches normally represent a shoulder/tailing function such as:

$$\log \frac{N}{N_0} = -\frac{t^p}{D} \quad \text{Eq.10}$$

where  $p$  is the power which takes a concave curve when is lower than 1 and a convex (shoulder curve) when is higher than 1.

Other modelling approaches use the logistic function which their inactivation forms are named as Fermi equation. For the quantification of sigmoidal decay curves, the following expression is used:

$$\text{Log} \frac{N}{N_0} = \log \left[ \frac{1 + e^{-btL}}{1 + e^{b(t-t_L)}} \right] \quad \text{Eq.11}$$

where  $N$  is the number of microorganisms surviving at time  $t$ ,  $N_0$  is the initial microbial load,  $b$  is the maximum specific decay rate and  $t_L$  is the time prior to inactivation.

Geeraerd et al. (2000) developed a shoulder/tail inactivation model.

$$N = \left[ N_0 - N_{res} e^{-k_{max} t} \frac{e^{-k_{max} t_L}}{1 + e^{((-k_{max} t_L) - 1)e^{-k_{max} t}}} + N_{res} \right] \quad \text{Eq.12}$$

where  $N$  is the number of microorganisms surviving at time  $t$ ,  $N_0$  is the initial microbial load,  $k_{max}$  is the maximum specific decay rate,  $t_L$  is the time prior to inactivation, and  $N_{res}$  is the residual population density.

Other inactivation models relied upon mechanistic processes in which predictions can be achieved outside the range of the obtained experimental data. These models have been mainly developed for bacterial spores and for high-temperature treatments (UHT, HTST sterilization). However, one drawback of more complex and mechanistic models lies in the difficulty in being able to successfully develop them, and the primary drawback is the absence of generic parameters.

## 1.2 Secondary inactivation models

Evidence for an interacting effect between temperature and pH are referred in the studies of López et al. (1996) and Fernández et al. (1996) for *Bacillus stearothermophilus* and *Clostridium sporogenes*. The model, subsequently developed by Mafart and Leguérinel (1998) considers an additive effect, on log scale, as follows:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{(pH - pH^*)^2}{z_{pH}^2} \quad \text{Eq.13}$$

where  $T^*$  is the reference temperature,  $pH^*$  is the pH of the maximum heat resistance (7.0 for spores),  $z_T$  is the commonly used thermal  $z$ -value,  $z_{pH}$  is the distance from pH to  $pH^*$  which leads to a tenfold reduction of the decimal reduction time, and  $D^*$  is the  $D$ -value at  $T^*$  and  $pH^*$ .

The  $a_w$  was taken into account for the first time by Reichart (1994) who derived a semi-empirical model for the death rate of *E. coli*. A five parameter model was proposed by Cerf et al. (1996), including an extension of the Davey's model with the  $a_w$  term:

$$\ln K_{max} = C_0 + \frac{C_1}{T} + C_2 pH + C_3 pH^2 + C_4 a_w^2 \quad \text{Eq.14}$$

where  $C_0$ ,  $C_1$ ,  $C_2$ ,  $C_3$  and  $C_4$  are empirical coefficients without biological significance.

Mafart and Leguérinel (1998) modified the Bigelow model to include an extension with the  $a_w$  term. The following relationship emerged:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{(pH - pH^*)^2}{z_{pH}^2} - \frac{a_w - 1}{z_{a_w}} \quad \text{Eq.15}$$

where  $z_{a_w}$  is the distance of  $a_w$  from 1 which leads to a tenfold increase of  $D$ -value. In that work, an interaction between temperature and pH was noted for *B. cereus*. At pH 4.5 (mean  $z_T$  value = 10.48°C) the thermal resistance of spores was less sensitive to variations of temperature than at pH 6.5 (mean  $z_T$  value = 8.78°C). Interactions were also shown between  $a_w$  and temperature. At 85°C and 95°C a decrease of sensitivity of spores to pH variations was observed with decreasing  $a_w$ .

Published models for vegetative microorganisms are available, such as the study of Blackburn et al. (1997) who developed a secondary inactivation model for *Salmonella* Enteritidis and *E. coli* O157:H7. For both organisms, predicted  $z$ -values of 4.6–7.0°C were obtained depending on conditions, with larger  $z$ -values at higher levels of NaCl. Optimum survival occurred between pH 5 and pH 7 and increasing acidity or alkalinity caused a decrease in the predicted  $D$ -values. The effect of pH on

thermal inactivation of *Salmonella* in broth medium was studied by Mañas et al. (2003). They observed that the pH of the heating medium at which heat resistance was maximum was pH 6 for cells grown at 37°C, but changed with growth temperature. The alkalization of the heating medium from pH 6 to pH 7.7 decreased the heat resistance of cells grown at 37°C by a factor of 3.

Buchanan and Golden (1994, 1995) studied the effect and interactions of temperature (4 - 42°C), lactic acid concentration (0-2 %), pH (3.3-7.3), NaCl content (0.5-19.0%), and sodium nitrite concentration (0-200 µg/ml) on the survival of a three strain mixture of *L. monocytogenes* in an aerobic and oxygen-reduced environment. Results obtained were similar in both cases and time to a 4 log<sub>10</sub> reduction was inversely related to the lactic acid concentration, temperature and NaCl content. The effect of sodium nitrite was pH dependent.

Alternative secondary models for *L. monocytogenes*, taking into account other factors such as type of organic acid and type of solute to reduce a<sub>w</sub> values, were subsequently developed (Fernández et al., 2007; Miller et al., 2009).

In Figure 14, the effect, estimated with the Pathogen Modelling Program, of thermal (Figure 14.i) and non-thermal treatments (Figure 14.ii) on inactivation kinetics of various pathogens is presented.

Regarding thermal inactivation of *L. monocytogenes* and *E. coli* O157:H7, PMP predictions at 65°C and 62.5°C, gave a significant effect since a 3 log<sub>10</sub> reduction in 1.45 min was observed for both microorganisms at optimal pH and a<sub>w</sub> conditions (Figure 14.i.a and 14.i.d). The effectiveness of thermal treatments on *L. monocytogenes* and *E. coli* O157:H7 cells increased when reducing pH and a<sub>w</sub> to 4.5 and 0.963 respectively (Figure 14.i.b, 14.i.c, 14.i.d and 14.i.f). For spores of non-proteolytic *C. botulinum*, higher temperatures are needed since 3 log<sub>10</sub> reduction is achieved in 167 minutes at 70°C. As it can be seen in Figure 14.i.g, 14.i.h and 14.i.i, the effect of temperature and pH was higher than a<sub>w</sub> in inactivating non-proteolytic *C. botulinum*.

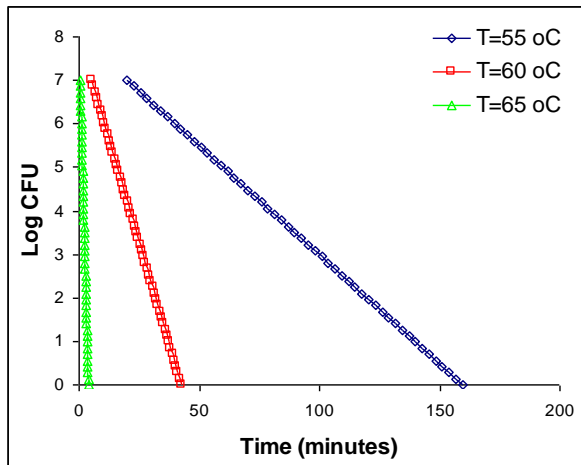
Non-thermal inactivation was described by the interaction of refrigeration temperatures (5-20°C), acidic pH (3.0-4.5) and reduced a<sub>w</sub> (0.834-0.997) for various vegetative microorganisms. Generally, *Salmonella* spp. showed a lesser resistance, according to the PMP model, to non-thermal treatments (Figure 14.ii.d, 14.ii.e and 14.ii.f). At pH 4, a temperature of 5°C produced a faster reduction of *L. monocytogenes* and *E. coli* O157:H7 populations than a temperature of 10°C, as shown in Figure 14.ii.a and 14.ii.g (3 log<sub>10</sub> reduction in 41 and 30 days respectively). However, no significant differences are obtained between 5° and 10°C for *Salmonella* spp. and *S. aureus* (Figure 14.ii.d and 14.ii.j). For *Salmonella* spp. at 10°C, 3 log<sub>10</sub> reduction was obtained substantially faster at pH 3.5 (1 h) than at pH 4.0 (5.2 h) and 4.5 (20 h) as represented in Figure 14.ii.c. When evaluating the interactive effect of temperature, pH and a<sub>w</sub>, less differences were noted for *S. aureus* because of its combined survival capacity at low a<sub>w</sub> values (Figure 14.ii.l). At 10°C, pH4, for *L. monocytogenes* reduction of a<sub>w</sub> to 0.845 produced a 3 log<sub>10</sub> reduction in 12 days, while at higher a<sub>w</sub> values (0.941), the same reduction was achieved in 42 days (Figure 14.ii.c). Combination of the three environmental factors at the studied levels in *Salmonella* spp. survival gave predictions of a 3 log<sub>10</sub> reduction in less than 1 day, as shown in Figure 14.ii.f. In relation to *E. coli* O157:H7, reduction of a<sub>w</sub> from 0.997 to 0.887 produced an increase of time to a 3 log<sub>10</sub> reduction from 18 to 44 days (Figure 14.ii.i).

**Figure 14:** Inactivation of representative pathogens at various environmental conditions predicted by the Pathogen Modeling Program (PMP).

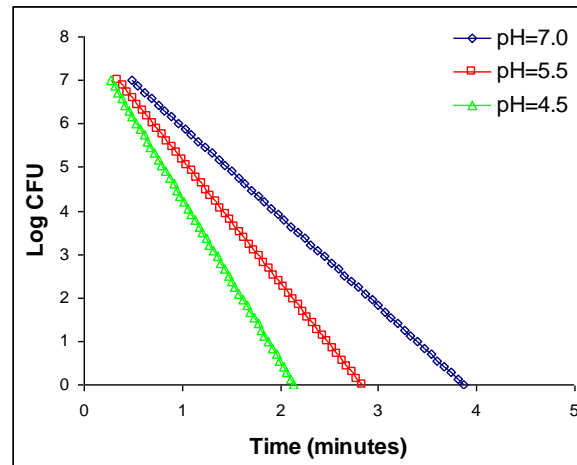
i) Thermal inactivation

*Listeria monocytogenes* (ground beef)

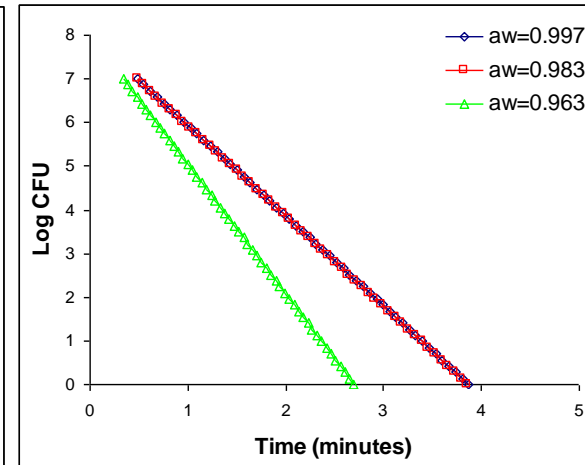
i.a) pH=7,  $a_w=0.997$



i.b) T=65 °C,  $a_w=0.997$



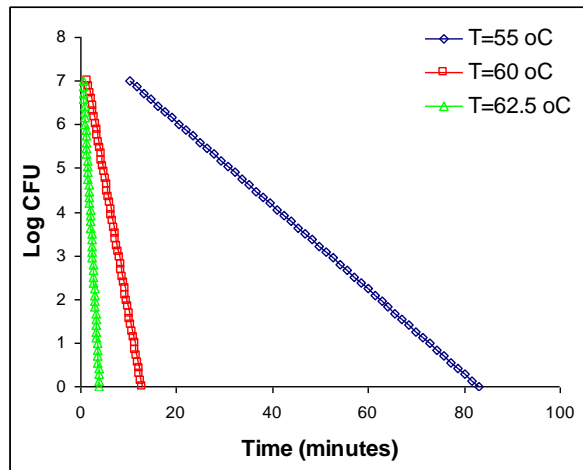
i.c) T=65 °C, pH=7.0



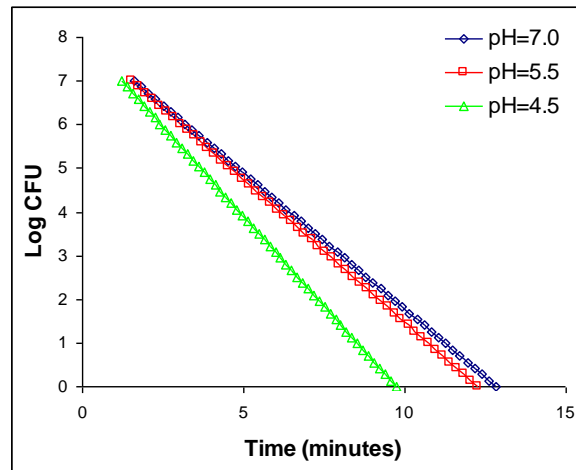


*Escherichia coli* O157:H7 (simulated beef gravy)

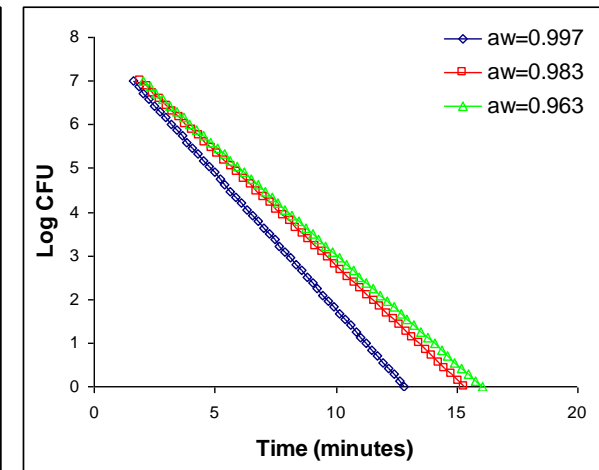
i.d) pH=7,  $a_w=0.997$



i.e) T=60 °C,  $a_w=0.997$

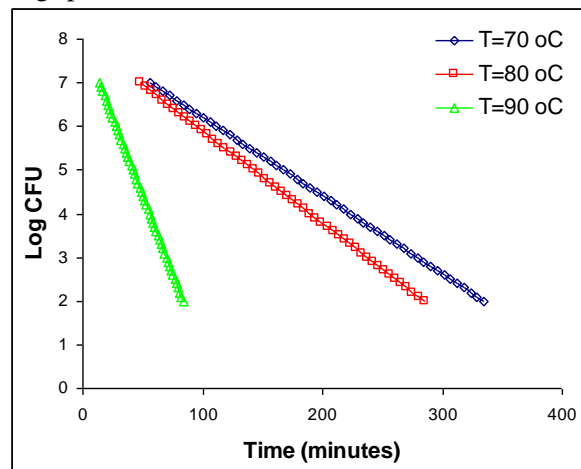


i.f) T=60 °C, pH=7.0

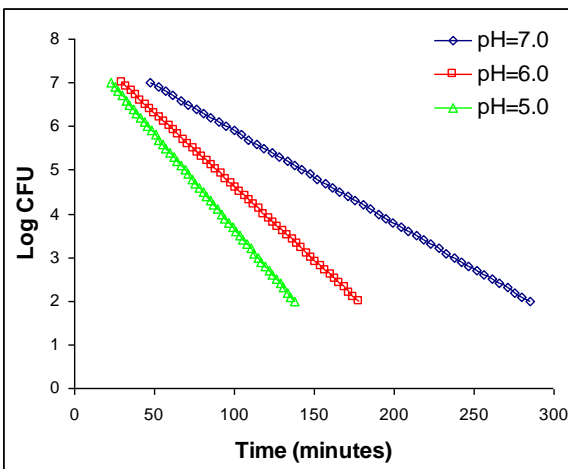


*Non-proteolytic Clostridium botulinum* (turkey slurry)

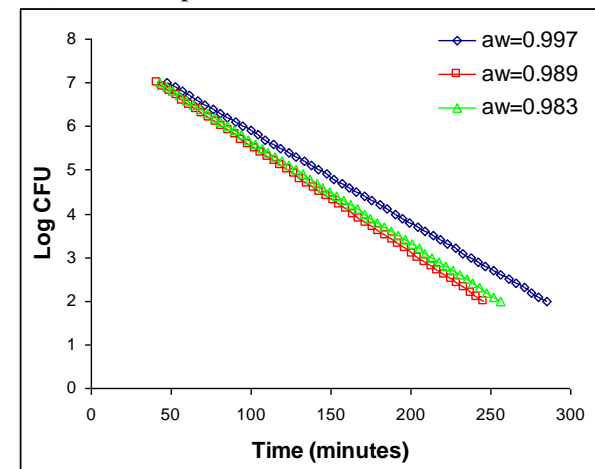
i.g) pH=7,  $a_w=0.997$



i.h) T=80 °C,  $a_w=0.997$



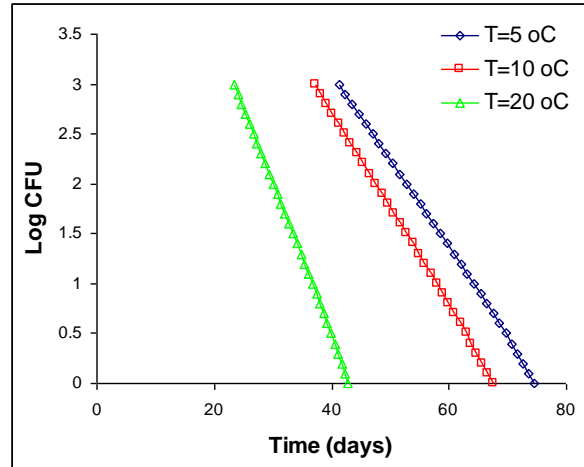
i.i) T=80 °C, pH=7.0



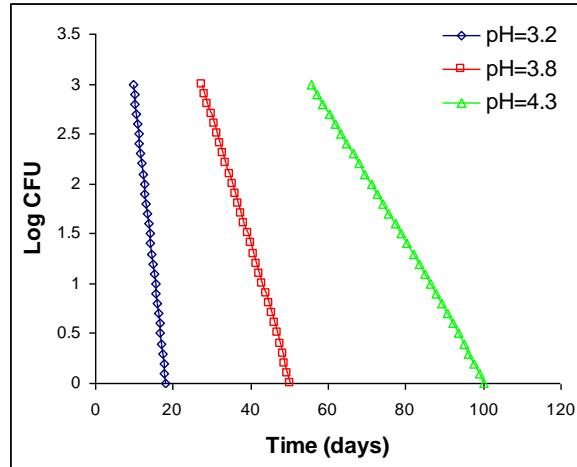
ii) Non-thermal inactivation

*Listeria monocytogenes* (broth culture)

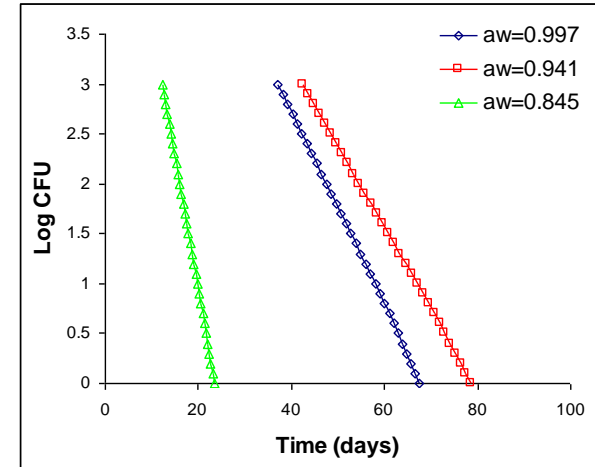
ii.a) pH=4.0,  $a_w=0.997$



ii.b) T=10 °C,  $a_w=0.997$

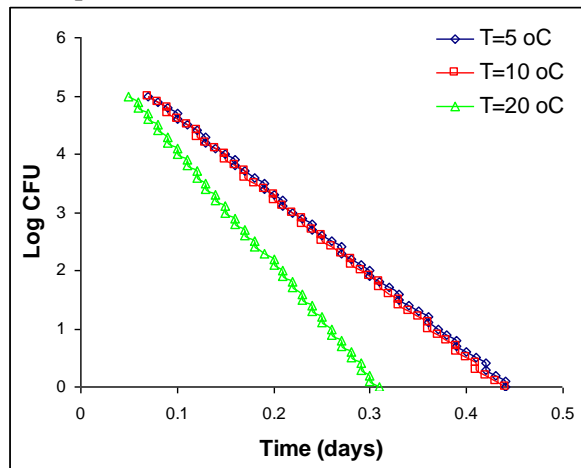


ii.c) T=10 °C, pH=4.0

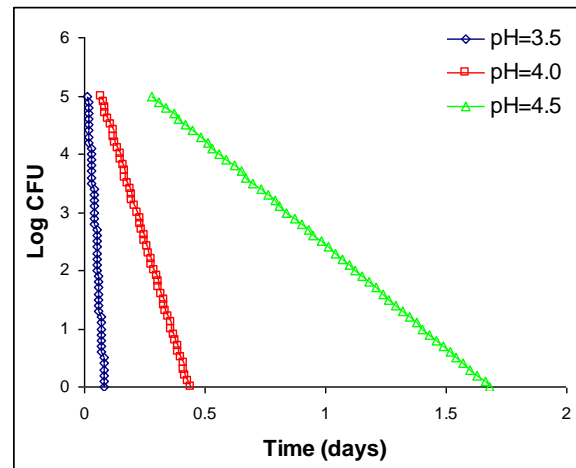


*Salmonella* spp. (broth culture)

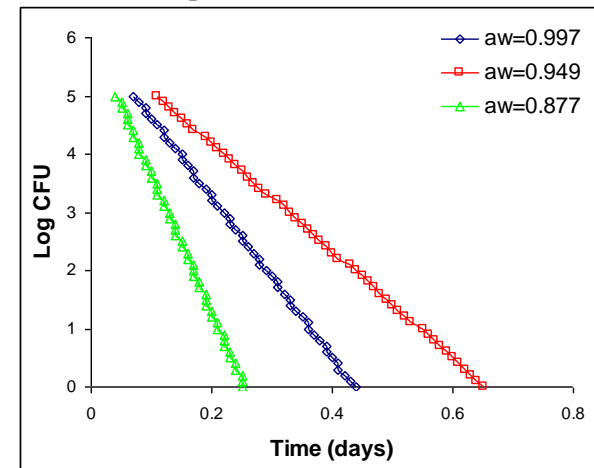
ii.d) pH=4.0,  $a_w=0.997$



ii.e) T=10 °C,  $a_w=0.997$



ii.f) T=10 °C, pH=4.0

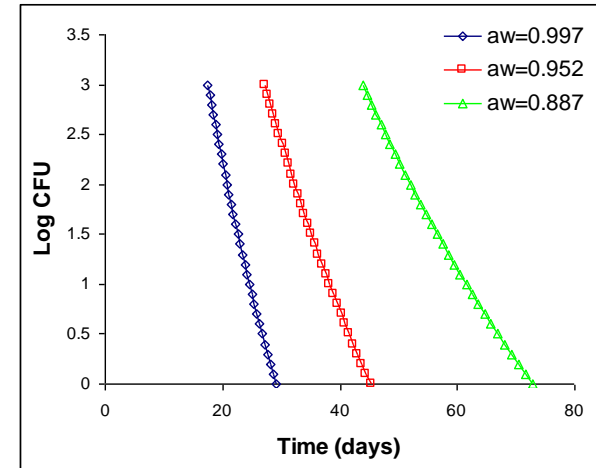
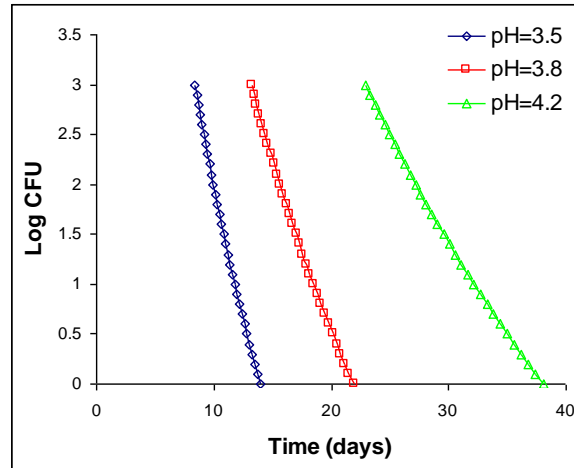
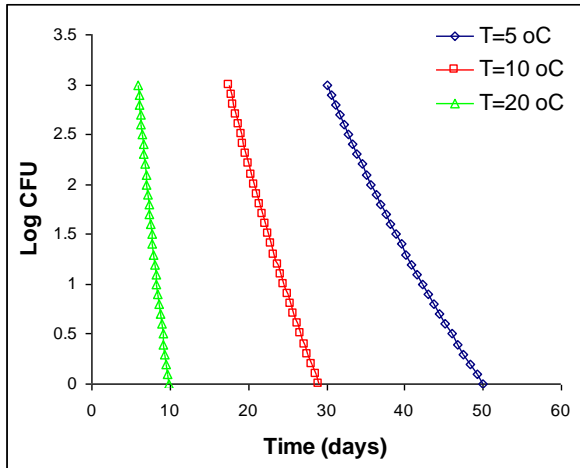


*Escherichia coli* O157:H7 (broth culture)

ii.g) pH=4.0,  $a_w=0.997$

ii.h) T=10 °C,  $a_w=0.997$

ii.i) T=10 °C, pH=4.0

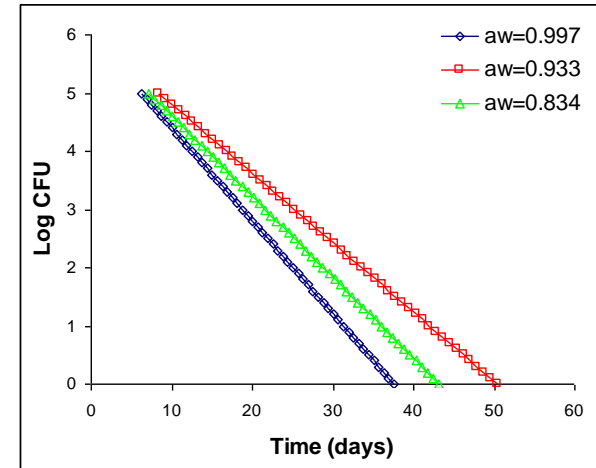
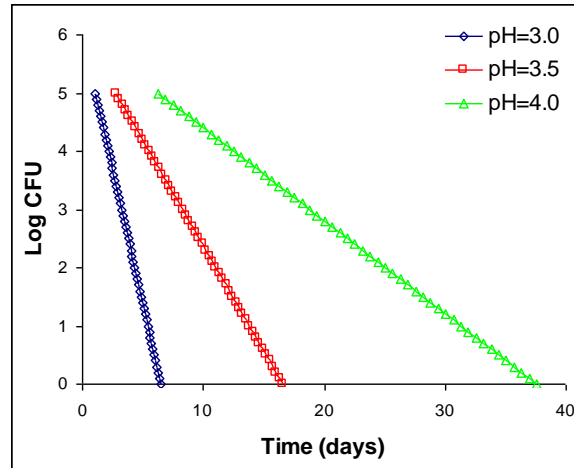
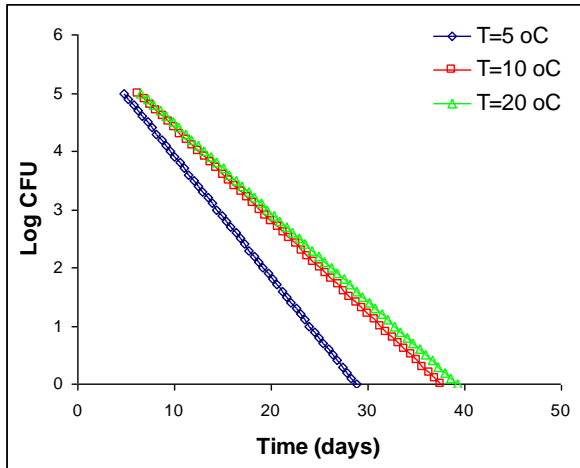


*Staphylococcus aureus* (broth culture)

ii.j) pH=4.0,  $a_w=0.997$

ii.k) T=10 °C,  $a_w=0.997$

ii.l) T=10 °C, pH=4.0



### 1.3 Example of inactivation in a real composite product

Table 18 reports the estimated inactivation parameters of the fitted linear models in relation to survival data for *Salmonella* and *Listeria monocytogenes* in fruit malt loaf (see also Chapter 3.2.3, Figure 3).

**Table 18:** Estimated inactivation parameters of the fitted linear models ( $k_{\max}$  and  $D$ -value) together with determination coefficients ( $R^2$ ) and Mean Standard Errors (MSE).

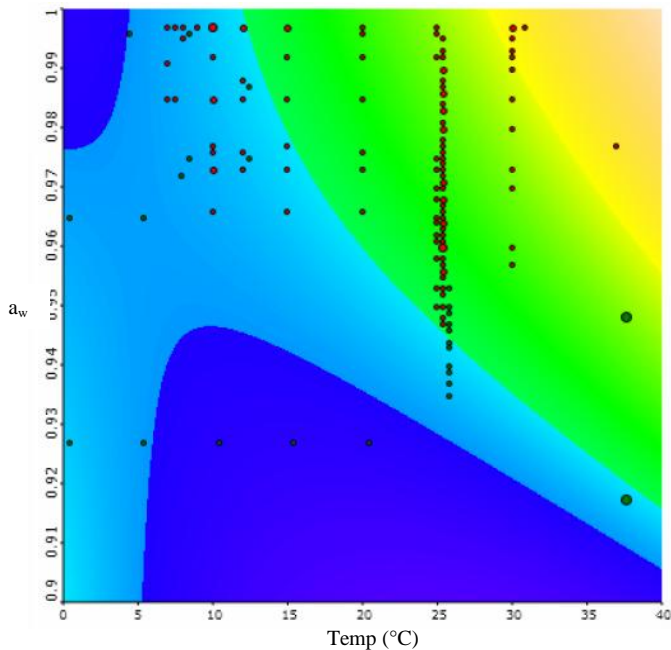
Food-borne pathogen	T, pH, $a_w$	$k_{\max}$ ( $h^{-1}$ )	$D$ -value (h)	$R^2$	MSE
<i>Salmonella</i> spp.	15°C, 5.2, 0.89	0.012	196.43	0.72	0.31
	21°C, 5.2, 0.89	0.015	145.38	0.95	0.05
	25°C, 5.2, 0.89	0.034	67.16	0.98	0.06
<i>L. monocytogenes</i>	15°C, 5.2, 0.89	0.004	560.50	0.42	0.35
	21°C, 5.2, 0.89	0.007	333.28	0.75	0.16
	25°C, 5.2, 0.89	0.017	137.17	0.97	0.03

## 2. Growth-no growth interface models of pathogenic microorganisms in food

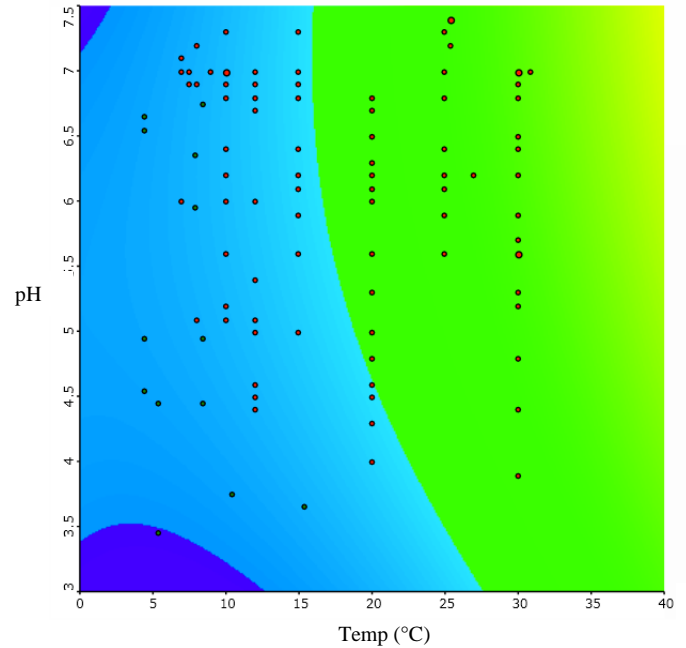
Figure 15 presents examples of the growth/no growth interface of representative pathogens at various environmental conditions predicted by the available tools, and Table 19 shows the pH limits for growth ( $P = 0.5, 0.1, 0.01$ ) of the same pathogens at various  $a_w$  and temperature conditions.

**Figure 15:** Growth/no growth response of representative pathogens at various environmental conditions predicted by the Microbial Responses Viewer (MRV). Points represent observed growth (●) and no growth (●). The size of the points indicates the number of experiments. The different colours indicate the predicted growth rate by the MRV in  $h^{-1}$ : ■:  $<-0.3$ ; ■:  $-0.1$  to  $0.1$ ; ■:  $0.1$  to  $1$  (growth); ■:  $>1$  (growth).

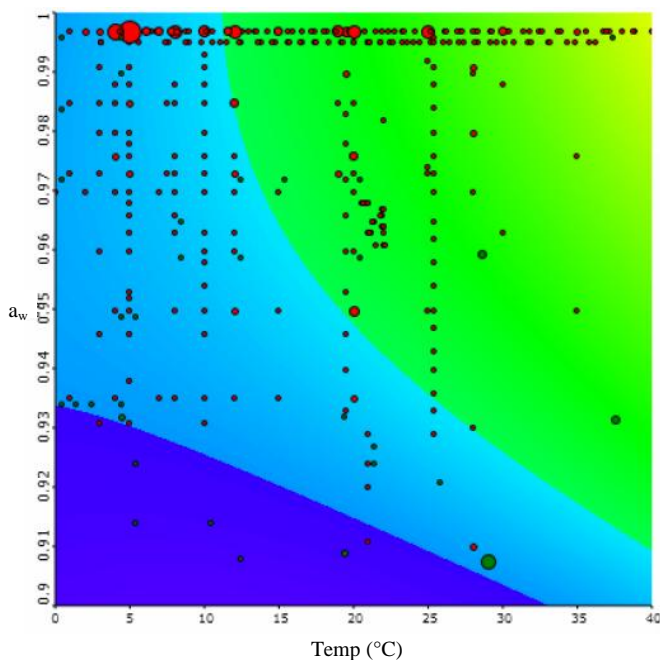
a) *Listeria monocytogenes*  
pH=7.0



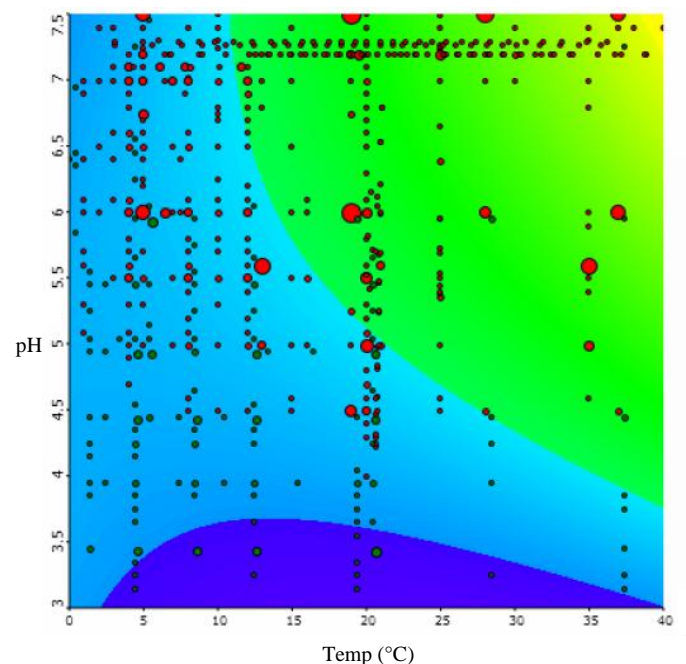
$a_w=0.997$



b) *Salmonella*  
pH=7.0

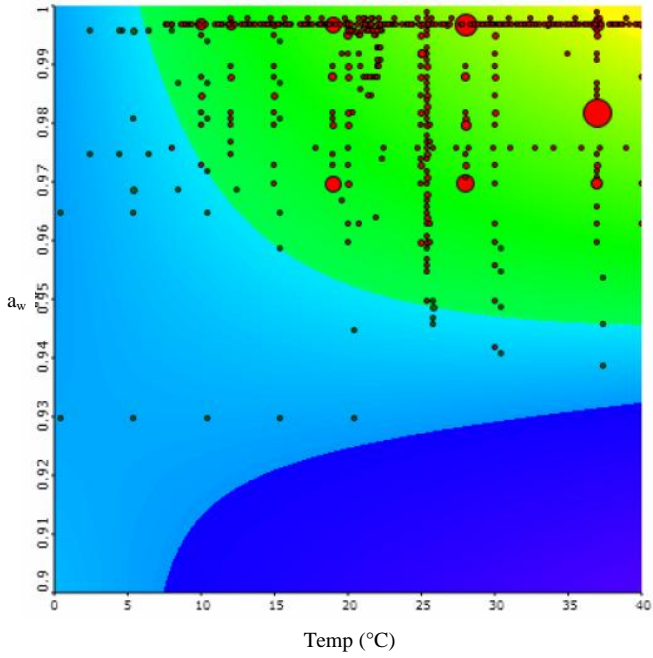


$a_w=0.997$

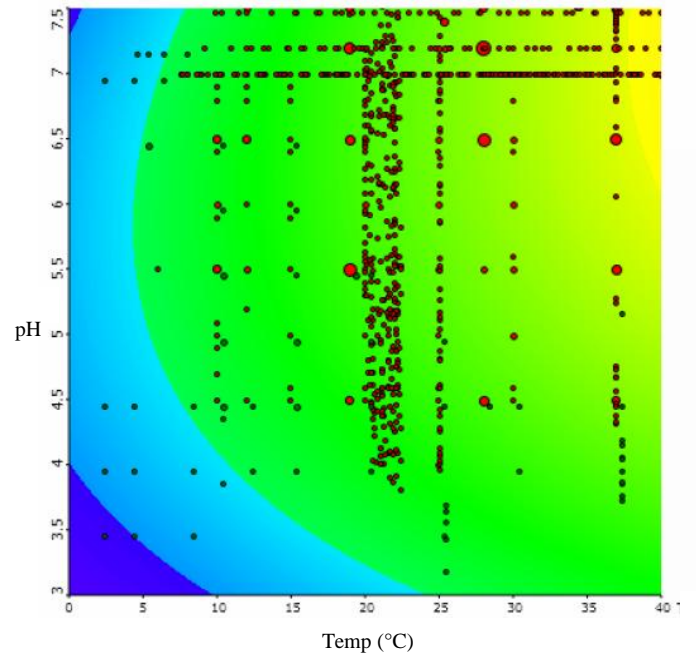


c) *Escherichia coli*

pH=7.0

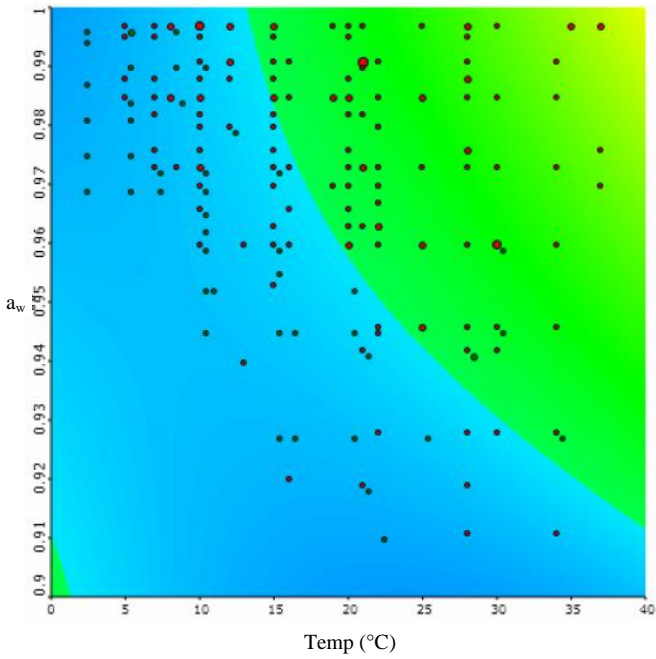


$a_w=0.997$

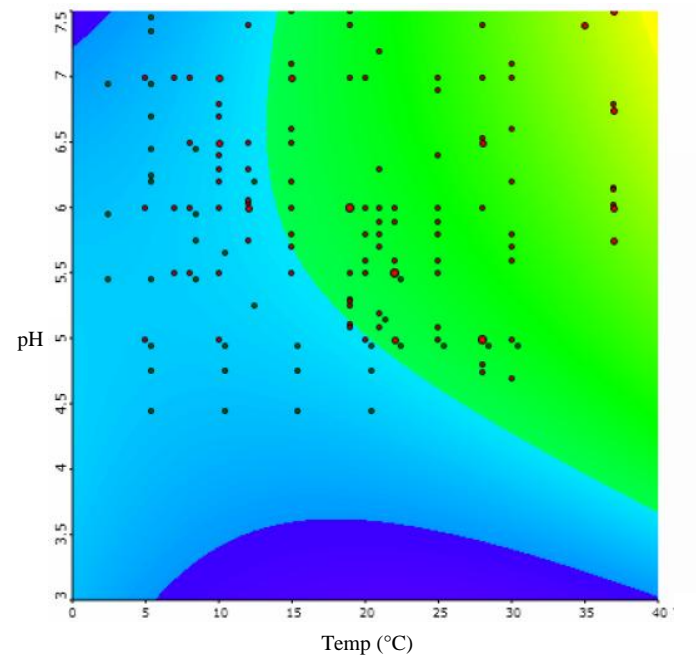


d) *Bacillus cereus*

pH=7.0

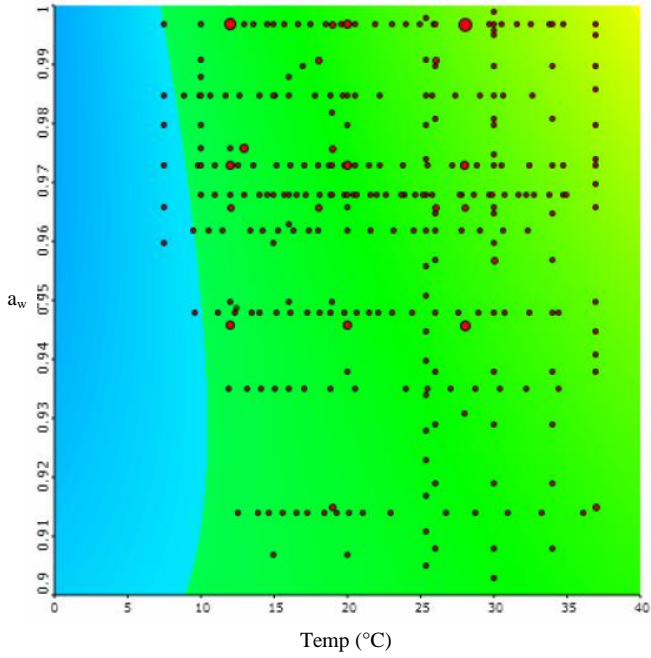


$a_w=0.997$

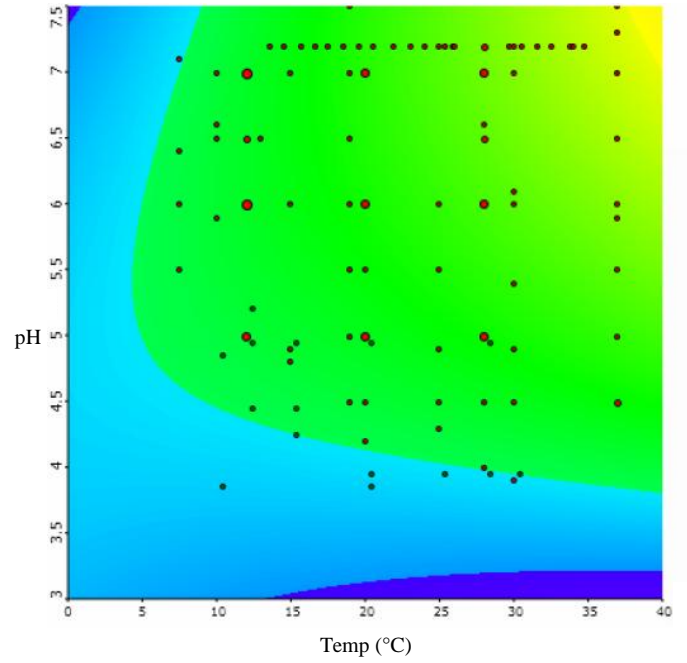


e) *Staphylococcus aureus*

pH=7.0

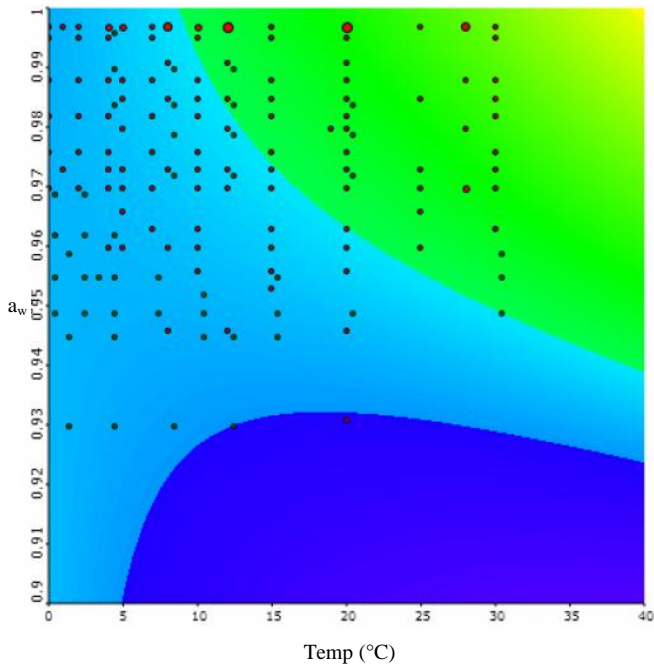


$a_w=0.997$

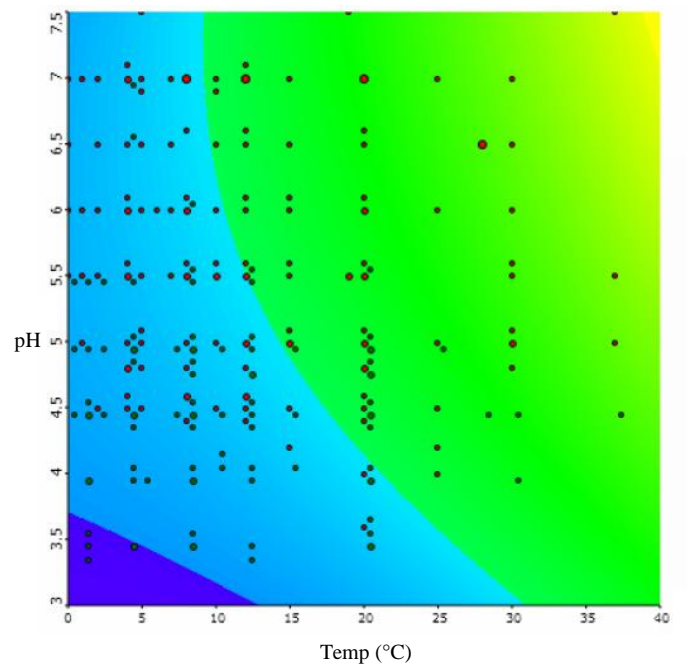


f) *Yersinia enterocolitica*

pH=7.0



$a_w=0.997$



**Table 19:** Predicted pH limits for growth (P = 0.5, 0.1, 0.01) of selected pathogens at various  $a_w$  and temperature conditions.

Pathogen	$a_w$	Predicted pH limits at various $a_w$ , temperature and P values											
		5°C			10°C			15°C			25°C		
		P 0.5	P 0.1	P 0.01	P 0.5	P 0.1	P 0.01	P 0.5	P 0.1	P 0.01	P 0.5	P 0.1	P 0.01
<i>Listeria monocytogenes</i> (Koutsoumanis et al., 2004)	0.99	4.76	4.69	4.61	4.45	4.39	4.34	4.29	4.24	4.19	4.23	4.19	4.14
	0.98	4.84	4.77	4.69	4.53	4.47	4.41	4.37	4.32	4.26	4.32	4.28	4.23
	0.97	4.96	4.87	4.79	4.62	4.56	4.49	4.46	4.41	4.35	4.43	4.38	4.33
	0.96	5.10	5.00	4.91	4.73	4.66	4.60	4.56	4.51	4.44	4.54	4.48	4.43
	0.95	5.28	5.16	5.05	4.86	4.78	4.71	4.68	4.61	4.55	4.66	4.60	4.54
<i>Salmonella</i> (Koutsoumanis et al., 2004)	0.99	-	-	-	4.66	4.41	4.37	4.40	4.18	4.14	3.95	3.91	3.87
	0.98	-	-	-	4.85	4.61	4.56	4.56	4.35	4.31	4.23	4.06	4.01
	0.97	-	-	-	5.04	4.80	4.74	4.71	4.51	4.45	4.45	4.18	4.13
	0.96	-	-	-	5.24	4.98	4.92	4.86	4.65	4.59	4.66	4.29	4.23
	0.95	-	-	-	5.47	5.17	5.10	5.02	4.80	4.73	4.88	4.39	4.33
<i>Escherichia coli</i> O157:H7 (Skandamis et al., 2007)	0.99	-	-	-	5.31	5.10	4.89	4.46	4.33	4.20	3.94	3.83	3.72
	0.98	-	-	-	5.16	5.02	4.88	4.63	4.51	4.38	4.03	3.92	3.80
	0.97	-	-	-	6.20	5.95	5.69	5.28	5.09	4.89	4.38	4.22	4.05
	0.96	-	-	-	-	-	-	-	6.69	6.06	5.01	4.74	4.48
	0.95	-	-	-	-	-	-	-	-	-	-	-	5.80
<i>Bacillus cereus</i> (Lanciotti et al., 2001)	0.99	-	-	-	-	-	-	-	-	-	4.85	-	-
	0.98	-	-	-	-	-	-	-	-	-	4.92	-	-
	0.97	-	-	-	-	-	-	-	-	-	5.10	-	-
	0.96	-	-	-	-	-	-	-	-	-	6.02	-	-
	0.95	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i> (Lanciotti et al., 2001)	0.99	-	-	-	-	-	-	5.44	5.22	5.03	4.79	4.68	4.59
	0.98	-	-	-	-	-	-	5.64	5.39	5.16	4.89	4.77	4.65
	0.97	-	-	-	-	-	-	5.97	5.66	5.38	5.06	4.90	4.77
	0.96	-	-	-	-	-	-	6.66	6.24	5.85	5.40	5.19	5.00
	0.95	-	-	-	-	-	-	-	-	-	7.39	6.85	6.36



### 3. Growth models of pathogenic microorganisms in food

Secondary models used for describing the effect of the environmental conditions on microbial growth include (McKellar and Lu, 2004):

- Arrhenius-type models;
- Belehradek-type models;
- models based on the gamma concept;
- cardinal parameter models;
- polynomial models.

A brief description of the various growth models is provided below.

#### Arrhenius-type models

The Arrhenius relation, developed theoretically for reversible molecular chemical reactions, has been experimentally shown to hold empirically for a number of more complex phenomena including microbial growth. The simplest form of Arrhenius-type model in use in predictive microbiology is:

$$k = k_A \exp\left(-\frac{E_A}{RT}\right) \quad \text{Eq.16}$$

where,  $k$  is the rate of growth,  $k_A$  is the Arrhenius equation constant,  $T$  is the absolute temperature (K),  $R$  is the universal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $E_A$  ( $\text{J mol}^{-1}$ ) is a thermodynamically defined quantity generally referred to as activation energy. For applications of the Arrhenius-type models in predictive microbiology, activation energy could be defined as the sensitivity of the reaction(s) responsible for cell division. This sensitivity can be obtained from the slope of the straight line obtained by plotting  $\ln(k)$  against the reciprocal absolute temperature ( $1/T$ ).

In order to overcome the observed deviations of the simple Arrhenius model when applied to microbial growth data at low and high temperatures, a number of modified models have been proposed. All these models are based on the assumption that microbial growth depends on a single, enzyme-catalyzed, rate limiting reaction that shows an Arrhenius type temperature dependency. The Arrhenius-type models that have received attention in the predictive microbiology literature are the model of Hinshelwood (Zwietering et al., 1991) and the model of Schoolfield et al. (1981) which is a reparameterization of the Sharpe et al. (1977) model.

#### Belehradek-type models

Ratkowsky et al. (1982) introduced a simple 2-parameter empirical model (Equation 17) to describe the effect of sub-optimal temperatures on microbial growth. This model and its numerous expansions are called Belehradek-type, or square-root-type or Ratkowsky-type models (McMeekin et al., 1993). The model is based upon the observation that the square root of the growth is linearly related to temperature. The form of the model is

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \quad \text{Eq.17}$$

where,  $\mu_{\max}$  is the rate of growth,  $T$  is the temperature,  $b$  is a coefficient to be estimated and  $T_{\min}$  is the theoretical minimum temperature for growth.  $T_{\min}$  is a model parameter and its value is usually lower than the observed lowest temperature for growth.

Ratkowsky et al. (1983) expanded Equation 17 to describe the effect of the entire biokinetic range of growth temperatures. The form of the expanded model is:

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \{1 - \exp[c(T - T_{\max})]\} \quad \text{Eq.18}$$

where  $b$  and  $c$  are constants and  $T_{\max}$  is the theoretical maximum temperature for growth.

Belehradel-type models have been also expanded to include the combined effect of temperature with other environmental factors such as  $a_w$  (Equation 19), pH (Equation 20) and both  $a_w$  and pH (Equation 21) (McMeekin et al., 1987; Adams et al., 1991; Wijtzes et al., 1995, 2001).

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) (\sqrt{a_w - a_{w\min}}) \quad \text{Eq.19}$$

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) (\sqrt{pH - pH_{\min}}) \quad \text{Eq.20}$$

$$\mu_{\max} = b(T - T_{\min})^2 (a_w - a_{w\min}) (pH - pH_{\min}) \quad \text{Eq.21}$$

### **Models based on the gamma concept**

Gamma concept models were introduced in predictive microbiology by Zwietering et al. (1992). The gamma concept is based on the observation that the effect of each environmental factors on microbial growth is independent (Adams et al., 1991; McMeekin et al., 1987) and can be described by a discrete term that is multiplied by terms for the effect of all others growth affecting factors. Another assumption of gamma concept is that the effect of any environmental factor on growth can be expressed as a function of the rate observed when this factor is at the optimal for growth level. The form of the model is

$$\mu_{\max} = \mu_{\max\text{opt}} \gamma(T) \gamma(a_w) \gamma(pH) \quad \text{Eq.22}$$

where,  $\mu_{\max}$  is the growth rate,  $\mu_{\max\text{opt}}$  is the growth rate at optimum conditions and  $\gamma(T)$ ,  $\gamma(a_w)$  and  $\gamma(pH)$  are gamma factors that describe the relative inhibitory effect of temperature,  $a_w$  and pH, respectively. The growth factors of the gamma model are dimensionless measures that have a value between 0 and 1. The forms of gamma factors for temperature,  $a_w$  and pH are for example:

$$\gamma(T) = \left( \frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right)^2 \quad \text{Eq.23}$$

$$\gamma(a_w) = \frac{a_w - a_{w\min}}{1 - a_{w\min}} \quad \text{Eq.24}$$

$$\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{\text{opt}} - pH_{\min})(pH_{\max} - pH_{\text{opt}})} \quad \text{Eq.25}$$

### **Cardinal Parameter Models (CPM)**

Cardinal parameter models are an important group of empirical models (Augustin and Carrier, 2000ab; Rosso et al., 1993, 1995). The main characteristic of this type of models is that their parameters have a biological or graphical interpretation. This has the obvious advantage that, during nonlinear regression, appropriate starting values for the parameters are easy to determine. As in the case of the gamma concept, cardinal parameter models are based on the assumption that the inhibitory effect of

environmental factors is multiplicative. Equation 19 show a cardinal parameter model for the combined effect of temperature, pH and  $a_w$ . As it is shown, the model consists of a discrete term for each environmental factor expressed as the growth rate relative to that when that factor is optimal. Each term has a numerical value between 0 and 1, while at optimal growth conditions all terms are equal to 1 and thus the growth rate  $\mu_{\max}$  is equal to optimum growth rate  $\mu_{\text{opt}}$ .

$$\mu_{\max} = \mu_{\text{opt}} CM_2(T) CM_2(a_w) CM_1(pH) \quad \text{Eq.26}$$

$$CM_n = \begin{cases} 0, X \leq X_{\min} \\ \frac{(X - X_{\max})(X - X_{\min})^n}{(X_{\text{opt}} - X_{\min})^{n-1} [(X_{\text{opt}} - X_{\min})(X - X_{\text{opt}}) - (X_{\text{opt}} - X_{\max})(n-1)X_{\text{opt}} + X_{\min} - nX]}], X_{\min} < X < X_{\max} \\ 0, X \geq X_{\max} \end{cases} \quad \text{Eq.27}$$

where, X is any of temperature,  $a_w$  or pH,  $X_{\min}$  and  $X_{\max}$  are, respectively, the minimum and maximum for growth values of X,  $X_{\text{opt}}$  is the value at which growth rate  $\mu_{\max}$  is equal to optimum growth rate  $\mu_{\text{opt}}$  and n (subscript of CM in Equation 27) is a shape parameter.

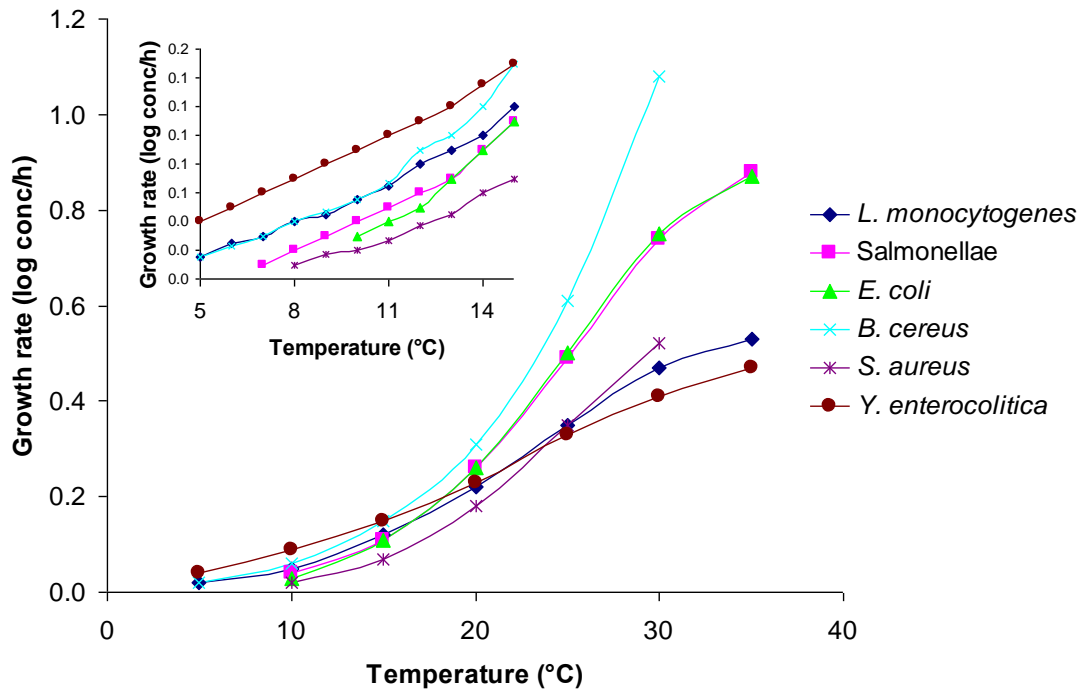
### **Polynomial models**

Polynomial models are probably the most widely used models in predictive microbiology. They present a purely empirical approach on describing the effect of environmental factors on microbial growth. Their main advantage is that they are easy to fit to experimental data by multiple linear regression. Furthermore, polynomial models allow virtually any of the environmental factors and their interaction to be taken into account. The second order form of polynomial models for the effect of three environmental factors is:

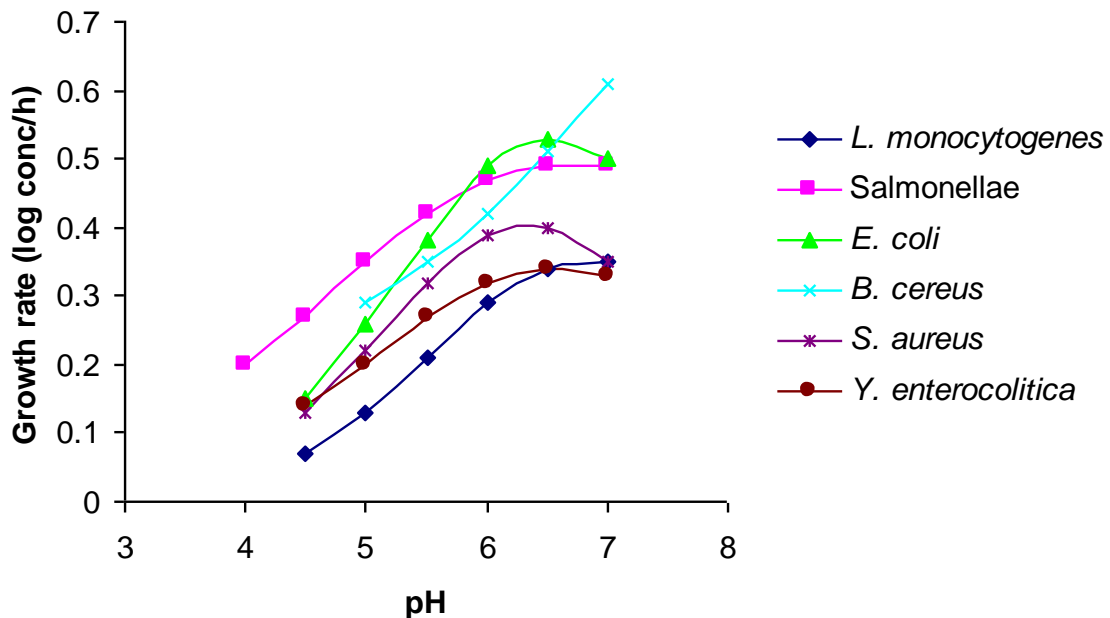
$$f(y) = p_1 + p_2 X_1 + p_3 X_2 + p_4 X_3 + p_5 X_1 X_2 + p_6 X_1 X_3 + p_7 X_2 X_3 + p_8 X_1^2 + p_9 X_2^2 + p_{10} X_3^2 + e \quad \text{Eq.28}$$

where y is the modelled growth response, f is a transformation function (e.g. natural logarithm or square root) used to reduce the variance of the response,  $p_i$  are the coefficients to be estimated,  $X_i$  are the environmental factors, and e is a random error. The main disadvantage of polynomial models is that parameters have no biological meaning and thus comparison with other models is difficult. In addition they do not provide useful information such as the  $T_{\min}$  and the activation energy provided by the Belehradek and Arrhenius models.

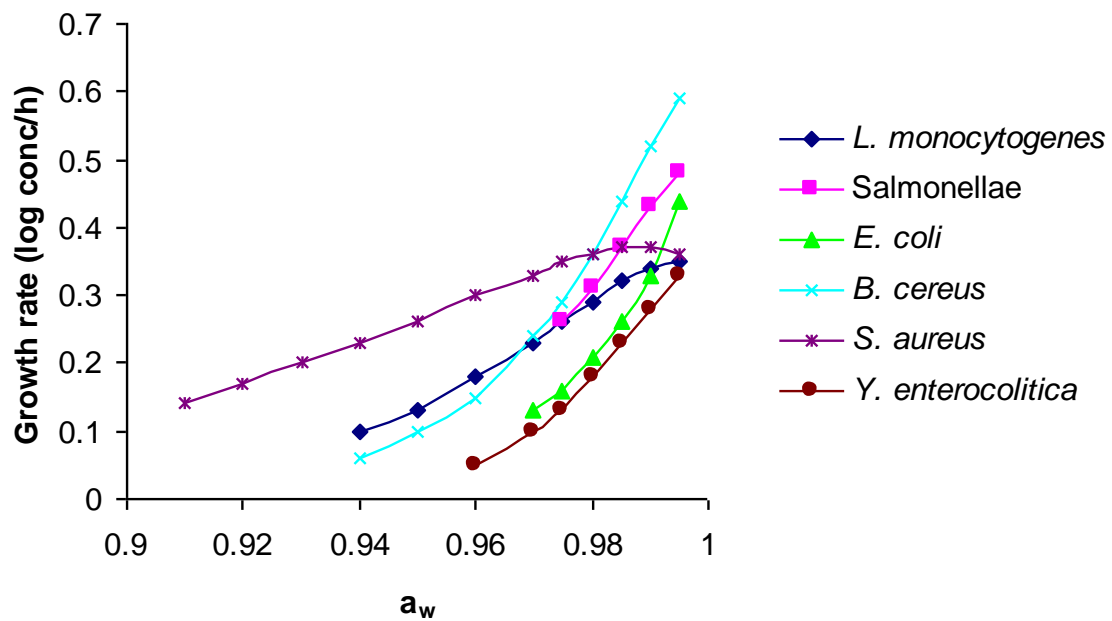
Figures 16, 17 and 18 show the predicted growth rate of selected pathogens at various temperature, pH and  $a_w$  conditions.



**Figure 16:** Effect of storage temperature on the growth rate of selected pathogens at pH=7 and  $a_w=0.99$  predicted by the ComBase predictor.



**Figure 17:** Effect of pH on the growth rate of selected pathogens at T=25 °C and  $a_w=0.99$  predicted by the ComBase predictor.



**Figure 18:** Effect of  $a_w$  on the growth rate of selected pathogens at  $T=25\text{ }^\circ\text{C}$  and  $\text{pH}=7$  predicted by the ComBase predictor.

#### 4. Summary tables on the available models of inactivation/growth of pathogenic microorganisms in foods

**Table 20:** Available models predicting inactivation of pathogenic microorganisms in food.

Pathogen	Reference	Temperature range	Other factors (ranges)	Type of model	
				Primary	Secondary
<i>L. monocytogenes</i>	Buchanan and Golden, 1995	4-42°C	lactic acid concentration (0-2 % ), (pH 3.3-7.3), NaCl (0.5-19%) and NaNO <sub>2</sub> (0-200 ppm), aerobic environment	Linear/non Linear	Response Surface
	Buchanan et al., 1997	4-42°C	lactic acid concentration (0-2 % ), (pH 3.0-6.0), NaCl (0.5-19%) and NaNO <sub>2</sub> (0-200 ppm)	Linear/non Linear	Response Surface
	Fernández et al., 2007	58-64°C	a <sub>w</sub> values (0.90-0.99) in sucrose solutions	Weibull	Response Surface
	Buchanan and Golden, 1995	4-42°C	lactic acid concentration (0-2 % ), (pH 3.0-6.0), NaCl (0.5-19%) and NaNO <sub>2</sub> (0-200 ppm), anaerobic environment	Linear/non Linear	Response Surface
	Miller, 1992	28°C	a <sub>w</sub> values (0.80-0.99) controlled by Glycerol and Polypropylene glycol	Biphasic logistic	-
	Buchanan and Golden, 1994	28°C	citric acid (0.1-2.0M), pH (4.0-7.0)	Linear	-
	Chen and Hoover, 2004	20-25°C	food matrix: UHT milk, high hydrostatic pressure (300-600MPa)	Linear/Weibull	-
	Hwang et al., 2009	40-55°C	food matrix: smoked salmon; phenol (0-15ppm), NaCl (0-6%)	Linear	Response Surface
<i>L. monocytogenes</i> and <i>Salmonella</i> spp.	Whiting, 1993	4-42°C	NaCl (0-19%), NaNO <sub>2</sub> 0-200 ppm.), and Lactic acid (0-1.5%)	Linear and Log logistic	Response Surface
<i>L. innocua</i> and <i>Salmonella</i> spp.	Murphy et al., 2000	55-70°C	food matrix: ground chicken breast meat	Linear and Arrhenius	-

Pathogen	Reference	Temperature range	Other factors (ranges)	Type of model	
				Primary	Secondary
<i>S. Enteritidis</i> and <i>S. Oranienburg</i>	Jordan et al., 2011	58-66°C	food matrix: egg yolk	Weibull/non Linear/ Spline equations	Linear
<i>Salmonella</i> Typhimurium	Erkmen, 2009	15-45°C	High Hydrostatic Pressure (200-350MPa)	Gompertz	-
<i>Salmonella</i> spp.	Juneja and Marks, 2003	10-58°C	food matrix: sous vide cooked beef; heating rate 1-3h	Logistic	-
<i>Salmonella</i> Enteritidis	Corradini et al., 2007	49-60°C	data from Humpheson et al. (1998)	Biphasic	Empiric logistic term expressing the dependence of temperature on the inactivation rate
	Humpheson et al., 1998	49-60°C	protein synthesis inhibition, regrowth of survivors and subsequent heat challenge	Linear	-
<i>S. Enteritidis</i> and <i>E. coli</i> O157:H7	Blackburn et al., 1997	54.5-64.5°C	pH (4.2-9.6), NaCl (0.5-8.5%), aerobic and anaerobic environments	Log logistic	Response Surface
<i>E. coli</i>	Cerf et al., 1996	52-63.1°C	a <sub>w</sub> values (0.928-0.995), pH (3-9)	Arrhenius	Davey
<i>E. coli</i> O157:H7	Juneja et al., 1999	55-62.5°C	food matrix: beef gravy; pH (4-8), NaCl (0-6%) and sodium pyrophosphate (0-0.3%)	Linear	Response Surface
	Riordan et al., 1998	-	food matrix: pepperoni; NaCl (2.5-4.8%) and NaNO <sub>2</sub> (100-400 ppm) and pH (4.4-5.6).	-	Response Surface
<i>Staphylococcus aureus</i>	Whiting et al., 1996	4-42°C	pH 3-7, Lactate (0-1%), NaCl (0.5-20%) and NaNO <sub>2</sub> 0-200 ppm.)	Logistic	Response Surface
<i>Bacillus sporothermodurans</i>	Periago et al., 2004	117-125°C	food matrices: chicken, mushrooms and pea soups	Log logistic	-

Pathogen	Reference	Temperature range	Other factors (ranges)	Type of model	
				Primary	Secondary
<i>B. cereus</i> and <i>C. perfringens</i> (vegetative cells and spores)	Byrne et al., 2006	50-60°C: <i>B. cereus</i> cells, 85-95°C: <i>B. cereus</i> spores, 55-65°C: <i>C. perfringens</i> cells, 90-100°C: <i>C. perfringens</i> spores	food matrix: pork luncheon roll	Linear	-
<i>B. cereus</i>	Fernández et al., 2002	80-95°C	food matrix: vegetable substrate; pH (4.7-6.2),	Weibull	Arrhenius/Weibull
	Léguerinel and Mafart, 2001	95°C	pH (4.0-6.5), organic acids	Non Linear Bigelow model	-
	Léguerinel et al., 2005	95-102°C	food matrix: cream chocolate (model validation); pH (4.5-7.0), $a_w$ values (0.92-1.00) adjusted with sucrose	Bigelow model	Nested Bigelow model
<i>B. subtilis</i>	Jagannath et al., 2005	89-98°C	food matrix: milk, soy sauce and diluted <i>kayu</i> ; pH (6.0-7.0), NaCl (0-5%),	Weibull	Modified Bigelow model
<i>B. stearothermophilus</i>	Ananta et al., 2001	60-120°C	food matrix: mashed broccoli and cocoa mass, high hydrostatic pressure (50-600MPa)	Regression model based on $n^{\text{th}}$ order kinetics	-
<i>B. stearothermophilus</i> and <i>Clostridium sporogenes</i>	Fernández et al., 1996	110-130°C	food matrix: mushroom acidified extract (citric acid and glucono- $\delta$ -lactone).	Linear	Response Surface
<i>Clostridium botulinum</i>	Juneja et al., 1995	70-90°C	food matrix: turkey; pH (5.0-6.5), NaCl (0-3%) and sodium pyrophosphate (0-0.3%)	Logistic	Response Surface
	Juneja et al., 1995	75-90°C	food matrix: turkey slurry; NaCl (1-3%),	Logistic	-
	Gao and Ju, 2008	30-70°C	nisin (0-333 IU/mL), high hydrostatic pressure (300-700MPa)	Linear	Response Surface



Pathogen	Reference	Temperature range	Other factors (ranges)	Type of model	
				Primary	Secondary
	Bowles et al., 1997	90°C	aromatic flavor carbonils: phenylacetaldehyde, benzaldehyde, cinnamaldehyde and piperonal (50- 100ppm)	Linear	-
<i>Clostridium perfringens</i>	Juneja and Marmer, 1998	65°C	food matrix: ground beef and turkey; sodium pyrophosphate (0.15-0.3%)	Linear	-
<i>S. Enteritidis</i> , <i>S.</i> <i>Typhimurium</i> , <i>E. coli</i> <i>O157:H7</i> and <i>S. aureus</i>	Buzrul and Alpas, 2007	60°C	-	Weibull	
<i>S. Enteritidis</i> , <i>S.</i> <i>Typhimurium</i> , <i>E. coli</i> <i>O157:H7</i> , <i>S. aureus</i> , <i>L.</i> <i>monocytogenes</i> and <i>Vibrio</i> <i>parahaemolyticus</i>	Chen, 2007	21.5°C	food matrix: milk; high hydrostatic pressure (300-600MPa)	Weibull, Linear, Log logistic	-

**Table 21:** Overview of the available growth/no growth interface models of pathogenic microorganisms.

Pathogen	Reference	Temperature range °C	Other environmental factors (ranges)	Growth medium
<i>Listeria monocytogenes</i>	Bolton and Frank, 1999	10	NaCl (2.0-8.0%), pH (5.0-6.5), moisture (42-60%)	Mexican-style cheese
	Tienungoon et al., 2000	3.1-36.2	$a_w$ (0.928-0.995), pH (3.7-7.8), lactic acid (0-500 mM)	TSB-YE
	Koutsoumanis et al., 2004	4-30	$a_w$ (0.900-0.993), pH (4.24-6.58)	TSB, TSA
	Augustin et al., 2005		Normal distribution means: $T_{min}$ (-1.72°C), $pH_{min}$ (4.26 for HCl, 4.71 for lactic acid), $a_{wmin}$ (0.913), $MIC_{sodium\ nitrite}$ (25.0 $\mu\text{mol l}^{-1}$ ), $MIC_{phenol}$ (31.9 ppm), $MIC_{CO_2}$ (proportion of $CO_2$ in modified atmosphere, 3.04)	Microbiological media, liquid dairy products, cheeses, meat and seafood products
	Boziaris and Nychas, 2006	5-35	$a_w$ (0.937-0.998), pH (4.05-6.70), nisin (0-100 IU $\text{ml}^{-1}$ )	TSB
	Boziaris et al., 2007	5	NaCl or KCl (0.0-1.4 $\text{mol l}^{-1}$ ), pH (4.0-7.3), nisin (0-100 IU $\text{ml}^{-1}$ )	TSB-YE
	Yoon et al., 2009	4-10	Lactic acid (0-4%); dipping time: 0-4 min	Bologna
	Yoon et al., 2009	4-12	Lactic acid (0-4%); dipping time: 0-4 min	Frankfurters
	Schvartzman et al., 2010	30	$a_w$ (0.950-0.990), pH (5.6-6.5)	TSB and pasteurized milk
	Boziaris et al., 2011	5-35	$a_w$ (0.937-0.998), pH (4.05-6.70), <i>Satureja thymbra</i> essential oil (0.0-0.1% v/v)	TSB
Zuliani et al., 2006	20	$a_w$ (0.930-0.970), pH (5.0-6.2)	Ground pork	
<i>Bacillus cereus</i>	Lanciotti et al., 2001	10-45	$a_w$ (0.89-0.99), pH (4.0-8.0), ethanol (0-3% v/v)	BHI broth
<i>Salmonella</i>	Lanciotti et al., 2001	10-45	$a_w$ (0.89-0.99), pH (4.0-8.0), ethanol (0-3% v/v)	BHI broth
	Koutsoumanis et al., 2004	10-35	$a_w$ (0.913-0.990), pH (3.76-6.44)	TSB
	Boziaris et al., 2011	10-35	$a_w$ (0.937-0.998), pH (3.78-6.70), <i>Satureja thymbra</i> essential oil (0.0-0.1% v/v)	TSB
<i>Staphylococcus aureus</i>	Lanciotti et al., 2001	10-45	$a_w$ (0.84-0.99), pH (4.0-8.0), ethanol (0-3% v/v)	BHI broth
	Stewart et al., 2001	37	RH (84-95%), pH (4.5-7.0), K-sorbate (0-1000 ppm), Ca-propionate (0-1000 ppm)	TSB
<i>Escherichia coli</i>	Presser et al., 1998	10-37	$a_w$ (0.955-0.995), pH (2.8-6.9), lactic acid (0-500 mM)	Nutrient broth
	Salter et al., 2000	7.7-37	$a_w$ (0.943-0.987), pH (7.4)	Nutrient broth
	McKellar and Lu, 2001	10-30	NaCl (0.5-16.5 %), sucrose (0-8%), pH (3.5-6.0), acetic acid (0-4 %)	TSB
	Skandamis et al., 2007	10-35	NaCl (0-10%), pH (3.52-7.32)	TSB

**Table 22:** Overview of the available growth models for pathogenic microorganisms.

Pathogen	Reference	Temperature range °C	Other environmental factors (ranges)	Type of model
<i>Yersinia enterocolitica</i>	Sutherland and Bayliss, 1994	5-30	NaCl (0.5-6.5%), pH (4.0-7.0), atmosphere (aerobic)	Polynomial
	Bhaduri et al., 1995	5-42	NaCl (0.5-5.0%), pH (4.5-8.5), Na-nitrite (0-200 ppm), atmosphere (aerobic)	Polynomial
	Pin et al., 2000	1-8	atmosphere (CO <sub>2</sub> , 0-83 %)	Polynomial
	Wei et al., 2001	4-34	atmosphere (air, vacuum, CO <sub>2</sub> , 100%)	Belehradek
<i>Listeria monocytogenes</i>	Wijtzes et al., 1993	5-35	pH (4.6-7.4), a <sub>w</sub> (0.95-0.997)	Belehradek
	George et al., 1996	1-20	pH (4.5-7.2), lactic acid (0-20.000 mg/l), acetic acid (0-10.000 mg/l)	Polynomial
	Farber et al., 1996	4-10	pH (5.5-6.5), atmosphere (CO <sub>2</sub> : 10-90%)	Polynomial
	Fernandez et al., 1997	4-20	NaCl (0.5-8.0%), pH (4.0-7.2), atmosphere (CO <sub>2</sub> : 0-100%)	Polynomial
	McClure et al., 1997	1-35	NaCl (0.5-11.5%), pH (4.5-7.0), Na-nitrite (0-200 ppm)	Polynomial
	Dalgaard and Jorgensen, 1998	5-37	NaCl (0.5-4.5%), pH (4.5-7.5), atmosphere (vacuum, air)	Pathogen Modeling Program
	Dalgaard and Jorgensen, 1998	1.0-35	NaCl (0-11.5%), pH (4.4-7.4), atmosphere (air)	Food MicroModel
	Dalgaard and Jorgensen, 1998	3-35	NaCl (0-8%), pH (4.5-7.5), atmosphere (air)	Murphy-model
	Dalgaard and Jorgensen, 1998	≤37	a <sub>w</sub> (≤0.997), pH (5.6-7.0), atmosphere (air)	Ross-model
	Lebert et al., 1998	4-30	a <sub>w</sub> (0.96-1.0), pH (5.4-7.0)	Polynomial
	Nerbrink et al., 1999	9	NaCl (1.0-4.0%), pH (5.5-6.5), Na-lactate (0-0.5%), Na-acetate (0-0.6%)	Polynomial
	Buchanan and Phillips, 2000	4-37	NaCl (0.5-10.5%), pH (4.5-7.5), Na-nitrite (0-1000 ppm), atmosphere (aerobic, anaerobic)	Polynomial
	Augustin and Carlier, 2000a, 2000b	-2.7-45.5	a <sub>w</sub> (0.910-0.997), pH (4.55-9.61), lactic acid (0-5.4 mM), acetic acid (0-20.1 mM), citric acid (0-1.6 mM), Na-benzoate (0-0.7 mM), K-sorbate (0-5.1 mM), Na-nitrite (0-11.4 μM)	Cardinal parameter
	Rodriguez et al., 2000	4-20	-	Arrhenius
	Devlieghere et al., 2001	4-12	a <sub>w</sub> (0.962-0.988), pH (6.2), Na-lactate (0-3%), Na-nitrite (20 ppm)	Belehradek, Polynomial
	Le Marc et al., 2002	0.5-43	pH (4.5-9.4), lactic acid (40-138 mM), acetic acid (16-64 mM), propionic acid (18-55 mM)	Cardinal parameter
	Seman et al., 2002	4	NaCl (0.8-3.6%), pH (4.55-9.61), K-lactate (0.15-5.6%), Na-diacetate (0.0-0.2%), Na-erythorbate (317 ppm), Na-nitrite (97ppm), Na-tripolyphosphate (0.276%)	Polynomial
	Boziaris et al., 2007	30	NaCl or KCl (0.0 and 1.0 mol l <sup>-1</sup> ), pH (4.5-7.3), nisin (0 and 50 IU ml <sup>-1</sup> )	Baranyi-model

Pathogen	Reference	Temperature range °C	Other environmental factors (ranges)	Type of model
	Zuliani et al., 2007	20	$a_w$ (0.950-0.970), pH (5.6-6.2), Na-lactate (134-402 mmol/l), Na-acetate (22-66 mmol/l), K-sorbate (20-60 mmol/l)	Cardinal parameter
	Mejlholm and Dalgaard et al., 2009	5-7°C	pH (5.6-6.6), water phase salt (1.5-2.4%), CO <sub>2</sub> (28.0-33.6%), organic acids (acetic, benzoic, citric, lactic, sorbic)	Cardinal parameter
<i>Aeromonas hydrophila</i>	Palumbo et al., 1992	5-42	NaCl (0.5-4.5%), pH (5.0-7.3), Na-nitrite (0-200ppm), atmosphere (anaerobic)	Polynomial
	McClure et al., 1994	3-20	NaCl (0.5-4.5%), pH (4.6-7.0), atmosphere (aerobic)	Polynomial
	Palumbo et al., 1996	5-42	NaCl (0.5-4.5%), pH (5.0-7.3), Na-nitrite (0-200ppm), atmosphere (aerobic)	Polynomial
	Devlieghere et al., 2000	1-12	$a_w$ (0.974-0.992), pH (6.12), Na-nitrite (22 ppm), atmosphere (CO <sub>2</sub> 0-2403 ppm)	Belerhaddek, Polynomial
<i>Bacillus cereus</i>	Benedict et al., 1993	5-42	NaCl (0.5-5.0%), pH (4.5-7.5), Na-nitrite (0-200 ppm), atmosphere (aerobic)	Polynomial
	Sutherland et al., 1996	10-30	NaCl (0.5-10.5%), pH (4.5-7.0), atmosphere (CO <sub>2</sub> 10-80 %)	Polynomial
	Zwietering et al., 1996	10-30	$a_w$ (0.95-1.00), pH (4.9-6.6)	Gamma concept
	Chorin et al., 1997	7-30	$a_w$ (0.95-0.991), pH (4.5-6.5)	Polynomial
<i>Salmonella</i>	Gibson et al., 1988	10-30	NaCl (0.5-4.5 %), pH (5.6-6.8), atmosphere (aerobic)	Polynomial
	Koutsoumanis et al., 1998	22-42	pH (5.5-7.0), oleuropein (0-0.8%)	Polynomial
	Oscar, 1999	15-40	pH (5.2-7.4), atmosphere (aerobic)	Polynomial
	Oscar, 2002	8-48	atmosphere (aerobic)	Belerhaddek, Cardinal parameter
	Basti and Razavilar, 2004	15-35	NaCl (0.5-3.0 %), pH (5.0-7.4), K-sorbate (0-0.3%)	Polynomial
<i>Shigella</i>	Zaika et al., 1994, 1998	10-37	NaCl (0.5-5.0 %), pH (5.0-7.5), Na-nitrite (0-1000 ppm), atmosphere (aerobic, anaerobic)	Polynomial
<i>Staphylococcus aureus</i>	Ross and McMeekin, 1991	5-35	$a_w$ (0.848-0.997)	Belerhaddek
	Buchanan et al., 1993	12-45	NaCl (0.5-16.5 %), pH (4.5-9.0), Na-nitrite (1-200 ppm), atmosphere (aerobic, anaerobic)	Polynomial
	Sutherland et al., 1994	10-30	NaCl (0.5-13.5 %), pH (4.0-7.0)	Polynomial
	Dengremont and Membre, 1995	10-37	NaCl (0-10 %), pH (5.0-8.0)	Belerhaddek
	Eifert et al., 1997	12-28	NaCl (0.5-8.5 %), pH (5.0-7.0), acidulants HCl, acetic acid, lactic acid, atmosphere (aerobic)	Polynomial
<i>Escherichia coli</i>	Buchanan and Bagi, 1994	5-42	NaCl (0.5-5.0 %), pH (4.0-7.0), Na-nitrite (0-200 ppm), atmosphere (aerobic)	Polynomial
	Sutherland et al., 1995, 1997	10-30	NaCl (0.5-6.5 %), pH (4.0-7.0), Na-nitrite (0-200 ppm), atmosphere (aerobic, anaerobic)	Polynomial

Pathogen	Reference	Temperature range °C	Other environmental factors (ranges)	Type of model
	Ross et al., 2003	7.6-47.4	a <sub>w</sub> (0.951-0.999), pH (4.02-8.28), lactic acid (0-500 mM)	Belerhadek
	Fujikawa et al., 2004	27.6-36	-	Arrhenius
<i>Clostridium botulinum</i>	Baker et al., 1990	4-30	Initial concentration (spores: -2-4 log cfu/g; aerobic plate count: -2-3 log cfu/g)	Polynomial
	Graham et al., 1996	4-30	NaCl (1.0-5.0 %), pH (5.0-7.3)	Polynomial
	Whiting and Oriente, 1997; Whiting and Strobaugh, 1998	4-28	NaCl (0-4.0 %), pH (5.0-7.0), initial spore concentration (1-5 log cfu/g)	Polynomial
	Fernandez et al., 2001	5-12	NaCl (0.5-2.5 %), pH (5.5-6.5), atmosphere (CO <sub>2</sub> : 0-90%)	Polynomial
<i>Clostridium perfringens</i>	Juneja et al., 1996	12-42	NaCl (0-3.0 %), pH (5.5-7.0), Na-pyrophosphate (0-3 %)	Polynomial

## REFERENCES OF APPENDIX D

- Adams MR, Little CL and Easter MC, 1991. Modelling the effect of pH, acidulant and temperature on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol., 71, 65-71.
- Ananta E, Heinz V, Schlüter O and Knorr D, 2001. Kinetic studies on high-pressure inactivation of *Bacillus stearothermophilus* spores suspended in food matrices. Innovative Food Science and Emerging Technologies, 2, 261-272.
- Augustin JC and Carlier V, 2000a. Mathematical modelling of the growth rate and lag time for *Listeria monocytogenes*. International Journal of Food Microbiology, 56, 29-51.
- Augustin JC and Carlier V, 2000b. Modelling the growth rate of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. International Journal of Food Microbiology, 56, 53-70.
- Augustin JC, Zuliani V, Cornu M and Guillier L, 2005. Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. Journal of Applied Microbiology, 99, 1019-1042.
- Baker DA, Genigeorgis C, Glover J and Razavilar V, 1990. Growth and toxigenesis of *C. botulinum* type E in fishes packaged under modified atmospheres. International Journal of Food Microbiology, 10, 269-289.
- Basti AA and Razavilar V, 2004. Growth response and modeling of the effects of selected factors on the time-to-detection and probability of growth initiation of *Salmonella* Typhimurium. Food Microbiology, 21, 431-438.
- Benedict RC, Partridge T, Wells D and Buchanan RL, 1993. *Bacillus cereus* aerobic growth kinetics. Journal of Food Protection, 56, 211-214.
- Bhaduri S, Buchanan RL and Phillips JG, 1995. Expanded response-surface model for predicting the effects of temperatures, pH, sodium-chloride contents and sodium-nitrite concentrations on the growth-rate of *Yersinia enterocolitica*. Journal of Applied Bacteriology, 79, 163-170.
- Blackburn CW, Curtis LM, Humpheson L, Billon C and McClure PJ, 1997. Development of thermal inactivation models for *Salmonella* Enteritidis and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. International Journal of Food Microbiology, 38, 31-44.
- Bolton LF and Frank JF, 1999. Defining the growth/no-growth interface for *Listeria monocytogenes* in Mexican-style cheese based on salt, pH, and moisture content. Journal of Food Protection, 62, 601-609.
- Bowles BL, Williams AC, Marmer BS and Juneja VK, 1997. Modulation of thermal resistance of proteolytic *Clostridium botulinum* spores by aromatic flavor carbonyls. Food Microbiology, 14, 3-9.
- Boziaris IS, Chorianopoulos NG, Haroutounian SA and Nychas GJE, 2011. Effect of Satureja thymbra essential oil on growth-no growth interfaces of *Listeria monocytogenes* Scott A and *Salmonella* Enteritidis PT4, at various temperatures, pH, and water activities. Journal of Food Protection, 74, 45-54.
- Boziaris IS and Nychas GJE, 2006. Effect of nisin on growth boundaries of *Listeria monocytogenes* Scott A, at various temperatures, pH and water activities. Food Microbiology, 23, 779-784.
- Boziaris IS, Skandamis PN, Anastasiadi M and Nychas GJE, 2007. Effect of NaCl and KCl on fate and growth/no growth interfaces of *Listeria monocytogenes* Scott A at different pH and nisin concentrations. Journal of Applied Microbiology, 102, 796-805.
- Buchanan RL and Bagi LK, 1994. Expansion of response-surface models for the growth of *Escherichia coli* O157-H7 to include sodium-nitrite as a variable. International Journal of Food Microbiology, 23, 317-332.

- Buchanan RL and Golden MH, 1994. Interaction of citric acid concentration and pH on the kinetics of *Listeria monocytogenes* inactivation. *Journal of Food Protection*, 57, 567-570.
- Buchanan RL and Golden MH, 1995. Model for the non-thermal inactivation of *Listeria monocytogenes* in a reduced oxygen environment. *Food Microbiology*, 12, 203-212.
- Buchanan RL, Golden MH and Phillips JG, 1997. Expanded models for the non-thermal inactivation of *Listeria monocytogenes*. *Journal of Applied Microbiology*, 82, 567-577.
- Buchanan RL and Phillips JG, 2000. Updated models for the effects of temperature, initial pH, NaCl, and NaNO<sub>2</sub> on aerobic and anaerobic growth of *Listeria monocytogenes*. *Quant Microbiol*, 2.
- Buchanan RL, Smith JL, McColgan C, Marmer BS, Golden M and Dell B, 1993. Response-surface models for the effects of temperature, pH, sodium-chloride, and sodium-nitrite on the aerobic and anaerobic growth of *Staphylococcus aureus* 196E. *Journal of Food Safety*, 13, 159-175.
- Buzrul S and Alpas H, 2007. Modeling inactivation kinetics of food borne pathogens at a constant temperature. *LWT Food Science and Technology*, 40, 632-637.
- Byrne B, Dunne G and Bolton DJ, 2006. Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll. *Food Microbiology*, 23, 803-808.
- Cerf O, Davey KR and Sadoudi AK, 1996. Thermal inactivation of bacteria-a new predictive model for the combined effect of three environmental factors: temperature, pH and water activity. *Food Research International*, 29, 219-226.
- Chen H, 2007. Use of linear, Weibull, and log-logistic functions to model pressure inactivation of seven foodborne pathogens in milk. *Food Microbiology*, 24, 197-204.
- Chen H and Hoover DG, 2004. Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk. *Innovative Food Science and Emerging Technologies*, 5, 269-276.
- Chorin E, Thuault D, Cleret JJ and Bourgeois CM, 1997. Modelling *Bacillus cereus* growth. *International Journal of Food Microbiology*, 38, 229-234.
- Coroller L, Leguerinel I, Mettler E, Savy N and Mafart P, 2006. General model, based on two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. *Applied and Environmental Microbiology*, 72, 6493-6502.
- Corradini MG, Normand MD and Peleg M, 2007. Modeling non-isothermal heat inactivation of microorganisms having biphasic isothermal survival curves. *International Journal of Food Microbiology*, 116, 391-399.
- Dalgaard P and Jorgensen LV, 1998. Predicted and observed growth of *Listeria monocytogenes* in seafood challenge tests and in naturally contaminated cold-smoked salmon. *International Journal of Food Microbiology*, 40, 105-115.
- Dengremont E and Membre JM, 1995. Statistical approach for comparison of the growth rates of 5 strains of *Staphylococcus aureus*. *Applied and Environmental Microbiology*, 61, 4389-4395.
- Devlieghere F, Geeraerd AH, Versyck KJ, Vandewaetere B, Van Impe J and Debevere J, 2001. Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. *Food Microbiology*, 18, 53-66.
- Devlieghere F, Lefevre I, Magnin A and Debevere J, 2000. Growth of *Aeromonas hydrophila* in modified-atmosphere-packed cooked meat products. *Food Microbiology*, 17, 185-196.
- Eifert JD, Hackney CR, Pierson MD, Duncan SE and Eigel WN, 1997. Acetic, lactic, and hydrochloric acid effects on *Staphylococcus aureus* 196E growth based on a predictive model. *Journal of Food Science*, 62, 174-178.
- Erkmen O, 2009. Mathematical modeling of *Salmonella* Typhimurium inactivation under high hydrostatic pressure at different temperatures. *Food and Bioprocess Processing*, 87, 68-73.

- Farber JM, Ross WH and Harwig J, 1996. Health risk assessment of *Listeria monocytogenes* in Canada. *International Journal of Food Microbiology*, 30, 145-156.
- Fernández A, Collado J, Cunha LM, Ocio MJ and Martínez A, 2002. Empirical model building based on Weibull distribution to describe the joint effect of pH and temperature on the thermal resistance of *Bacillus cereus* in vegetable substrate. *International Journal of Food Microbiology*, 77, 147-153.
- Fernández A, López M, Bernardo A, Condón S and Raso J, 2007. Modelling thermal inactivation of *Listeria monocytogenes* in sucrose solutions of various water activities. *Food Microbiology*, 24, 372-379.
- Fernandez PS, Baranyi J and Peck MW, 2001. A predictive model of growth from spores of non-proteolytic *Clostridium botulinum* in the presence of different CO<sub>2</sub> concentrations as influenced by chill temperature, pH and NaCl. *Food Microbiology*, 18, 453-461.
- Fernandez PS, George SM, Sills CC and Peck MW, 1997. Predictive model of the effect of CO<sub>2</sub>, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 37, 37-45.
- Fernández PS, Ocio MJ, Rodrigo F, Rodrigo M and Martínez A, 1996. Mathematical model for the combined effect of temperature and pH on the thermal resistance of *Bacillus stearothermophilus* and *Clostridium sporogenes* spores. *International Journal of Food Microbiology*, 32, 225-233.
- Fujikawa H, Kai A and Morozumi S, 2004. A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures. *Food Microbiology*, 21, 501-509.
- Gao YL and Ju XR, 2008. Exploiting the combined effects of high pressure and moderate heat with nisin on inactivation of *Clostridium botulinum* spores. *Journal of Microbiological Methods*, 72, 20-28.
- Geraerd AH, Herremans CH and Van Impe JF, 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, 59, 185-209.
- Geraerd AH, Valdramidis V and Van Impe JF, 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, 102, 95-105.
- George SM, Richardson LCC and Peck MW, 1996. Predictive models of the effect of temperature, pH and acetic and lactic acids on the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 32, 73-90.
- Gibson AM, Bratchell N and Roberts TA, 1988. Predicting microbial-growth - Growth-responses of *salmonellae* in a laboratory medium as affected by pH, sodium-chloride and storage-temperature. *International Journal of Food Microbiology*, 6, 155-178.
- Graham AF, Mason DR and Peck MW, 1996. Predictive model of the effect of temperature, pH and sodium chloride on growth from spores of non-proteolytic *Clostridium botulinum*. *International Journal of Food Microbiology*, 31, 69-85.
- Humpheson L, Adams MR, Anderson WA and Cole MB, 1998. Biphasic thermal inactivation kinetics in *Salmonella* Enteritidis PT4. *Applied and Environmental Microbiology*, 64, 459-464.
- Hwang CA, Sheen S and Juneja VK, 2009. Effect of salt, smoke compound, and temperature on the survival of *Listeria monocytogenes* in salmon during simulated smoking processes. *Journal of Food Science*, 74, M522-M529.
- Jagannath A, Tsuchido T and Membré JM, 2005. Comparison of the thermal inactivation of *Bacillus subtilis* spores in foods using the modified Weibull and Bigelow equations. *Food Microbiology*, 22, 233-239.
- Jordan JS, Gurtler JB, Marks HM, Jones DR and Shaw WK, 2011. A mathematical model of inactivation kinetics for a four-strain composite of *Salmonella* Enteritidis and Oranienburg in commercial liquid egg yolk. *Food Microbiology*, 28, 67-75.



- Juneja JK and Marmer BS, 1998. Thermal inactivation of *Clostridium perfringens* vegetative cells in Ground beef and turkey as affected by sodium pyrophosphate. *Food Microbiology*, 15, 281-287.
- Juneja JK, Marmer BS, Phillips JG and Miller AJ, 1995. Influence of the intrinsic properties of food on thermal inactivation of spores of nonproteolytic *Clostridium botulinum*: Development of a predictive model. *Journal of Food Safety*, 15, 349-364.
- Juneja VK and Marks HM, 2003. Characterizing asymptotic *D*-values for *Salmonella* spp. subjected to different heating rates in sous-vide cooked beef. *Innovative Food Science and Emerging Technologies*, 4, 395-402, 4, 395-402.
- Juneja VK, Marmer BS and Eblen BS, 1999. Predictive model for the combined effect of temperature, pH, sodium chloride and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. *Journal of Food Safety*, 19, 147-160.
- Juneja VK, Marmer BS, Phillips JG and Palumbo SA, 1996. Interactive effects of temperature, initial pH, sodium chloride, and sodium pyrophosphate on the growth kinetics of *Clostridium perfringens*. *Journal of Food Protection*, 59, 963-968.
- Koutsoumanis K, Tassou CC, Taoukis PS and Nychas GJE, 1998. Modelling the effectiveness of a natural antimicrobial on *Salmonella* Enteritidis as a function of concentration, temperature and pH, using conductance measurements. *Journal of Applied Microbiology*, 84, 981-987.
- Koutsoumanis KP, Kendall PA and Sofos JN, 2004. Modeling the boundaries of growth of *Salmonella* Typhimurium in broth as a function of temperature, water activity, and pH. *Journal of Food Protection*, 67, 53-59.
- Lanciotti R, Sinigaglia M, Gardini F, Vannini L and Guerzoni ME, 2001. Growth/no growth interfaces of *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella* Enteritidis in model systems based on water activity, pH, temperature and ethanol concentration. *Food Microbiology*, 18, 659-668.
- Le Marc Y, Huchet V, Bourgeois CM, Guyonnet JP, Mafart P and Thuault D, 2002. Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *International Journal of Food Microbiology*, 73, 219-237.
- Lebert I, Begot C and Lebert A, 1998. Development of two *Listeria monocytogenes* growth models in a meat broth and their application to beef meal. *Food Microbiology*, 15, 499-509.
- Leguérinel I and Mafart P, 2001. Modelling the influence of pH and organic acid types on thermal inactivation of *Bacillus cereus* spores. *International Journal of Food Microbiology*, 63, 29-34.
- Leguérinel I, Spegagne I, Couvert O, Gaillard S and Mafart P, 2005. Validation of an overall model describing the effect of three environmental factors on the apparent *D*-value of *Bacillus cereus* spores. *International Journal of Food Microbiology*, 100, 223-229.
- López M, Gonzalez I, Condon S and Bernardo A, 1996. Effect of pH heating medium on the thermal resistance of *Bacillus stearothermophilus* spores. *International Journal of Food Microbiology*, 28, 405-410.
- Mafart P, Couvert O, Gaillard S and Leguerinel I, 2002. On calculating sterility in thermal preservation methods: application of Weibull frequency distribution model. *International Journal of Food Microbiology*, 72, 107-113.
- Mafart P and Leguérinel I, 1998. Modeling combined effects of temperature and pH on heat resistance of spores by a linear-Bigelow equation. *Journal of Food Science*, 63, 6-8.
- Mañas P, Pagan R, Raso J and Condon S, 2003. Predicting thermal inactivation in media of different pH of *Salmonella* grown at different temperatures. *International Journal of Food Microbiology*, 87, 45-53.
- McClure PJ, Beaumont AL, Sutherland JP and Roberts TA, 1997. Predictive modelling of growth of *Listeria monocytogenes* - The effects on growth of NaCl, pH, storage temperature and NaNO<sub>2</sub>. *International Journal of Food Microbiology*, 34, 221-232.

- McClure PJ, Cole MB and Davies KW, 1994. An example of the stages in the development of a predictive mathematical-model for microbial-growth - The effects of NaCl, pH and temperature on the growth of *Aeromonas hydrophila*. *International Journal of Food Microbiology*, 23, 359-375.
- McKellar RC and Lu XW, 2001. A probability of growth model for *Escherichia coli* O157 : H7 as a function of temperature, pH, acetic acid, and salt. *Journal of Food Protection*, 64, 1922-1928.
- McKellar RC and Lu X, 2004. *Modelling microbial responses in food*. CRC Press.
- McMeekin TA, Chandler RE, Doe PE, Garland CD, Olley J, Putro S and Ratkowsky DA, 1987. Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosum*. *J. Appl. Bacteriol.*, 62, 543-550.
- McMeekin TA, Olley JN, Ross T and Ratkowsky DA, 1993. *Predictive microbiology: theory and application*.
- Mejlholm O and Dalgaard P, 2009. Development and validation of an extensive growth and growth boundary model for *Listeria monocytogenes* in lightly preserved and ready-to-eat shrimp. *Journal of Food Protection*, 72, 2132-2143.
- Miller AJ, 1992. Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. *Journal of Food Protection*, 55, 414-418.
- Miller FA, Ramos B, Gil MM, Brandao TRS, Teixeira P and Silva CLM, 2009. Influence of pH, type of acid and recovery media on the thermal inactivation of *Listeria innocua*. *International Journal of Food Microbiology*, 133, 121-128.
- Murphy RY, Marks BP, Johnson ER and Johnson MG, 2000. Thermal inactivation kinetics of *Salmonella* and *Listeria* in ground chicken breast meat and liquid medium. *Journal of Food Science*, 65, 706-710.
- Nerbrink E, Borch E, Blom H and Nesbakken T, 1999. A model based on absorbance data on the growth rate of *Listeria monocytogenes* and including the effects of pH, NaCl, Na-lactate and Na-acetate. *International Journal of Food Microbiology*, 47, 99-109.
- Oscar TP, 1999. Response surface models for effects of temperature, pH, and previous growth pH on growth kinetics of *Salmonella* Typhimurium in brain heart infusion broth. *Journal of Food Protection*, 62, 106-111.
- Oscar TP, 2002. Development and validation of a tertiary simulation model for predicting the potential growth of *Salmonella* Typhimurium on cooked chicken. *International Journal of Food Microbiology*, 76, 177-190.
- Palumbo SA, Williams AC, Buchanan RL, Call JC and Phillips JG, 1996. Expanded model for the aerobic growth of *Aeromonas hydrophila*. *Journal of Food Safety*, 16, 1-13.
- Palumbo SA, Williams AC, Buchanan RL and Phillips JG, 1992. Model for the anaerobic growth of *Aeromonas hydrophila* K144. *Journal of Food Protection*, 55, 260-265.
- Peleg M and Cole MB, 1998. Reinterpretation of microbial survival curves. *Critical Reviews in Food Science and Nutrition*, 38, 353-380.
- Peleg M and Cole MB, 2000. Estimating the survival of *Clostridium botulinum* spores during heat treatments. *Journal of Food Protection*, 63, 190-195.
- Peleg M and Pechina CM, 2000. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Reviews in Food Science and Nutrition*, 40, 159-172.
- Periago PM, van Zuijlen A, Fernandez PS, Klapwijk PM, ter Steeg PF, Corradini MG and Peleg M, 2004. Estimation of the non-isothermal inactivation patterns of *Bacillus sporothermodurans* IC4 spores in soups from their isothermal survival data. *International Journal of Food Microbiology*, 95, 205-218.

- Pin C, Baranyi J and de Fernando G, 2000. Predictive model for the growth of *Yersinia enterocolitica* under modified atmospheres. *Journal of Applied Microbiology*, 88, 521-530.
- Presser KA, Ross T and Ratkowsky DA, 1998. Modelling the growth limits (growth no growth interface) of *Escherichia coli* as a function of temperature, pH, lactic acid concentration, and water activity. *Applied and Environmental Microbiology*, 64, 1773-1779.
- Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN and Chandler RE, 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriol.*, 154, 1222-1226.
- Ratkowsky DA, Olley J, McMeekin TA and Ball A, 1982. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.*, 149, 565-568.
- Reichart O, 1994. Modeling the destruction of *Escherichia coli* on the base of reaction kinetics. *International Journal of Food Microbiology*, 23, 449-465.
- Riordan DCR, Duffy G, Sheridan JJ, Eblen BS, Whiting RC, Blair S and McDowell DA, 1998. Survival of *Escherichia coli* O157:H7 during the manufacture of pepperoni. *Journal of Food Protection*, 61, 146-151.
- Rodriguez AMC, Alcalá EB, Gimeno RMG and Cosano GZ, 2000. Growth modelling of *Listeria monocytogenes* in packaged fresh green asparagus. *Food Microbiology*, 17, 421-427.
- Ross T and McMeekin TA, 1991. Predictive microbiology - applications of a square root model. *Food Australia*, 43, 202-207.
- Ross T, Ratkowsky DA, Mellefont LA and McMeekin TA, 2003. Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli*. *International Journal of Food Microbiology*, 82, 33-43.
- Rosso L, Lobry JR, Bajard S and Flandrois JP, 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Applied and Environmental Microbiology*, 61, 610-616.
- Rosso L, Lobry JR and Flandrois JP, 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology*, 162, 447-463.
- Salter MA, Ratkowsky DA, Ross T and McMeekin TA, 2000. Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using nonlinear logistic regression. *International Journal of Food Microbiology*, 61, 159-167.
- Schmidt CF, 1957. Thermal resistance of microorganisms, p. 831-884. In: *Antiseptics, disinfectants, fungicides and sterilization*. GF Reddish. Lea and Febiger, Philadelphia.
- Schoolfield RM, Sharpe PJH and Magnuson CE, 1981. Non linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. *J. Theor. Biol.*, 88, 719-731.
- Schvartzman MS, Belessi X, Butler F, Skandamis P and Jordan K, 2010. Comparison of growth limits of *Listeria monocytogenes* in milk, broth and cheese. *Journal of Applied Microbiology*, 109, 1790-1799.
- Seman DL, Borger AC, Meyer JD, Hall PA and Milkowski AL, 2002. Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. *Journal of Food Protection*, 65, 651-658.
- Sharpe PJH, Curry GL, DeMichele DW and Cole CL, 1977. Distribution model of organism development times. *J. Theor. Biol.*, 66, 21-38.
- Skandamis PN, Stopforth JD, Kendall PA, Belk KE, Scanga JA, Smith GC and Sofos JN, 2007. Modeling the effect of inoculum size and acid adaptation on growth/no growth interface of *Escherichia coli* O157 : H7. *International Journal of Food Microbiology*, 120, 237-249.

- Stewart CM, Cole MB, Legan JD, Slade L, Vandeven MH and Schaffner DW, 2001. Modeling the growth boundary of *Staphylococcus aureus* for risk assessment purposes. *Journal of Food Protection*, 64, 51-57.
- Stringer SC, George SM and Peck MW, 2000. Thermal inactivation of *Escherichia coli* O157:H7. *Journal of Applied Microbiology*, 88, 79s-89s.
- Stumbo CR, Purohit KS and Ramakrishnan TV, 1975. Thermal process lethality guide for low acid foods in metal containers. *Journal of Food Science*, 40, 1316-1323.
- Sutherland JP, Aherne A and Beaumont AL, 1996. Preparation and validation of a growth model for *Bacillus cereus*: The effects of temperature, pH, sodium chloride, and carbon dioxide. *International Journal of Food Microbiology*, 30, 359-372.
- Sutherland JP and Bayliss AJ, 1994. Predictive modeling of growth of *Yersinia enterocolitica* - the effects of temperature, pH and sodium-chloride. *International Journal of Food Microbiology*, 21, 197-215.
- Sutherland JP, Bayliss AJ and Braxton DS, 1995. Predictive modeling of growth of *Escherichia coli* O157-H7 - The effects of temperature, ph and sodium-chloride. *International Journal of Food Microbiology*, 25, 29-49.
- Sutherland JP, Bayliss AJ, Braxton DS and Beaumont AL, 1997. Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model. *International Journal of Food Microbiology*, 37, 113-120.
- Sutherland JP, Bayliss AJ and Roberts TA, 1994. Predictive modeling of growth of *Staphylococcus aureus* - The effects of temperature, pH and sodium-chloride. *International Journal of Food Microbiology*, 21, 217-236.
- Tienungoon S, Ratkowsky DA, McMeekin TA and Ross T, 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology*, 66, 4979-+.
- van Asselt ED and Zwietering MH, 2006. A systematic approach to determine global thermal inactivation parameters for various food pathogens. *International Journal of Food Microbiology*, 107, 73-82.
- Van Boekel M, 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, 74, 139-159.
- Vas K and Proszk G, 1957. Observations on the destruction of spores of *Bacillus cereus*. *Journal of Applied Bacteriology*, 21, 431-441.
- Wei QK, Fang TJ and Chen WC, 2001. Development and validation of growth model for *Yersinia enterocolitica* in cooked chicken meats packaged under various atmosphere packaging and stored at different temperatures. *Journal of Food Protection*, 64, 987-993.
- Whiting RC, 1993. Modeling bacterial survival in unfavorable environments. *Journal of Industrial Microbiology*, 12, 240-246.
- Whiting RC and Oriente JC, 1997. Time-to-turbidity model for non-proteolytic type B *Clostridium botulinum*. *International Journal of Food Microbiology*, 36, 49-60.
- Whiting RC, Sackitey S, Calderone S, Merely K and Phillips JG, 1996. Model for the survival of *Staphylococcus aureus* in non growth environments. *International Journal of Food Microbiology*, 31, 231-243.
- Whiting RC and Strobaugh TP, 1998. Expansion of the time-to-turbidity model for proteolytic *Clostridium botulinum* to include spore numbers. *Food Microbiology*, 15, 449-453.
- Wijtzes T, de Wit JC, Huis in 't Veld JHJ, van 't Riet K and Zwietering MH, 1995. Modelling bacterial growth of *Lactobacillus curvatus* as a function of acidity and temperature. *Applied and Environmental Microbiology*, 61, 2533-2539.

- Wijtzes T, McClure PJ, Zwietering MH and Roberts TA, 1993. Modeling bacterial growth of *Listeria monocytogenes* as a function of water activity, pH and temperature. *International Journal of Food Microbiology*, 18, 139-149.
- Wijtzes T, Rombouts FM, Kant-Muermans MLT, van 't Riet K and Zwietering MH, 2001. Development and validation of a combined temperature, water activity, pH model for bacterial growth rate of *Lactobacillus curvatus*. *International Journal of Food Microbiology*, 63, 57-64.
- Yoon Y, Kendall PA, Belk KE, Scanga JA, Smith GC and Sofos JN, 2009. Modeling the Growth/No-Growth Boundaries of Postprocessing *Listeria monocytogenes* Contamination on Frankfurters and Bologna Treated with Lactic Acid. *Applied and Environmental Microbiology*, 75, 353-358.
- Zaika LL, Moulden E, Weimer L, Phillips JG and Buchanan RL, 1994. Model for the combined effects of temperature, initial pH, sodium-chloride and sodium-nitrite concentrations on anaerobic growth of *Shigella flexneri*. *International Journal of Food Microbiology*, 23, 345-358.
- Zaika LL, Phillips JG, Fanelli JS and Scullen OJ, 1998. Revised model for aerobic growth of *Shigella flexneri* to extend the validity of predictions at temperatures between 10 and 19 degrees C. *International Journal of Food Microbiology*, 41, 9-19.
- Zuliani V, Lebert I, Augustin JC, Garry P, Vendevre JL and Lebert A, 2007. Modelling the behaviour of *Listeria monocytogenes* in ground and pork as a function of pH, water activity, nature and concentration of organic acid salts. *Journal of Applied Microbiology*, 103, 536-550.
- Zuliani V, Lebert I, Garry P, Vendevre J-L, Augustin J-C and Lebert A, 2006. Effects of heat-processing regime, pH, water activity and their interactions on the behaviour of *Listeria monocytogenes* in ground pork. Modelling the boundary of the growth/no-growth areas as a function of pH, water activity and temperature. *International Journal of Food Science and Technology*, 41, 1197-1206.
- Zwietering MH, de Koos JT, Hasenack BE, de Wit JC and van 't Riet K, 1991. Modeling of bacterial growth as function of temperature. *Applied and Environmental Microbiology*, 57, 1094-1101.
- Zwietering MH, deWit JC and Notermans S, 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. *International Journal of Food Microbiology*, 30, 55-70.
- Zwietering MH, Wijtzes T, de Wit JC and van 't Riet K, 1992. A decision support system for prediction of the microbial spoilage in foods. *Journal of Food Protection*, 55, 973-979.

**E. PREVALENCE DATA OF ZOO NOTIC AGENTS IN CERTAIN COMPOSITE PRODUCTS IN THE EU (2004-2009)**

**Bread**

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	169	0	0.0%	
<i>Salmonella</i> spp.	batch	25 g	95	0	0.0%	
		<i>Total Salmonella</i>	264	0	0.0%	
Pathogenic <i>E. coli</i>	single	25 g	1	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	51	1	2.0%	

**Cakes** (includes cakes and desserts)

Causative agent	Sample unit	Weight	N	Samples		Other info
				pos	% pos	
<i>Salmonella</i> spp.	single	25 g	6975	18	0.3%	<i>S. spp.</i> : 61.1% <i>S. Enteritidis</i> : 36.1% <i>S. Senftenberg</i> : 2.8%
<i>Salmonella</i> spp.	batch	25 g	1167	7	0.6%	
<i>Salmonella</i> spp.	unknown	25 g	1331	11	0.8%	
<i>Total Salmonella</i>			9473	36	0.4%	
<i>Campylobacter</i> spp.	single	25 g	12	0	0.0%	
Pathogenic <i>E. coli</i>	single	10 g	1	0	0.0%	
Pathogenic <i>E. coli</i>	single	20 g	15	0	0.0%	
Pathogenic <i>E. coli</i>	single	25 g	3	0	0.0%	
<i>Total Pathogenic E. coli</i>			19	0	0.0%	
<i>Cronobacter</i> spp.	single	25 g	1	0	0.0%	
<i>Cronobacter</i> spp.	batch	unknown	14	0	0.0%	
<i>Total Cronobacter spp.</i>			15	0	0.0%	
<i>Listeria monocytogenes</i>	single	0.01 g	188	1	0.5%	
<i>Listeria monocytogenes</i>	single	1 g	2	0	0.0%	
<i>Listeria monocytogenes</i>	single	10-25 g	30	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	2962	13	0.4%	
<i>Listeria monocytogenes</i>	single	unknown	151	0	0.0%	
<i>Listeria monocytogenes</i>	batch	1 g	103	0	0.0%	
<i>Listeria monocytogenes</i>	batch	1-25 g	236	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	483	1	0.2%	
<i>Total Listeria monocytogenes</i>			4155	15	0.4%	

**Other bakery products** (includes pastry and unspecified bakery products)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	11889	45	0.4%	
<i>Salmonella</i> spp.	single	50 g	6	0	0.0%	<i>S. Enteritidis</i> : 54.4% <i>S. spp.</i> : 40.4% <i>S. Typhimurium</i> : 5.3%
<i>Salmonella</i> spp.	single	unknown	21	0	0.0%	
<i>Salmonella</i> spp.	batch	25 g	5500	12	0.2%	
<i>Total Salmonella</i>			17416	57	0.3%	
<i>Campylobacter</i> spp.	single	25 g	10	0	0.0%	
<i>Campylobacter</i> spp.	batch	25 g	1	0	0.0%	
<i>Total Campylobacter</i>			11	0	0.0%	
Pathogenic <i>E. coli</i>	single	unknown	1	0	0.0%	
<i>Staphylococcus enterotoxins</i>	single	20 g	2	0	0.0%	
<i>Yersinia</i> spp.	batch	25 g	2	0	0.0%	
<i>Listeria monocytogenes</i>	single	0.01 g	118	0	0.0%	
<i>Listeria monocytogenes</i>	single	1 g	60	11	18.3%	
<i>Listeria monocytogenes</i>	single	25 g	710	4	0.6%	
<i>Listeria monocytogenes</i>	single	unknown	94	1	1.1%	
<i>Listeria monocytogenes</i>	batch	1 g	11	0	0.0%	
<i>Listeria monocytogenes</i>	batch	1-25 g	13	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	32	0	0.0%	
<i>Total Listeria monocytogenes</i>			1038	16	1.5%	



## Chocolate

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	706	1	0.1%	
<i>Salmonella</i> spp.	single	50 g	1	0	0.0%	S. Infantis: 100%
<i>Salmonella</i> spp.	single	unknown	8	0	0.0%	
<i>Salmonella</i> spp.	batch	25 g	405	0	0.0%	
<i>Total Salmonella</i>			1120	1	0.1%	
<i>Staphylococcus</i> enterotoxins	single	150 g	28	4	14.3%	
Histamine	single	unknown	10	10	100.0%	
<i>Listeria monocytogenes</i>	single	25 g	156	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	11	0	0.0%	
<i>Total Listeria monocytogenes</i>			167	0	0.0%	

**Confectionery** (includes confectionery and pastes)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	1961	1	0.1%	
<i>Salmonella</i> spp.	single	unknown	238	0	0.0%	<i>S. Enteritidis</i> : 77.8% <i>S. Bareilly</i> : 14.8% <i>S. Infantis</i> : 3.7%
<i>Salmonella</i> spp.	batch	25 g	2919	11	0.4%	
<i>Salmonella</i> spp.	batch	unknown	36	9	25.0%	
<i>Salmonella</i> spp.	unknown	25 g	611	6	1.0%	<i>S. spp.</i> : 3.7%
<i>Total Salmonella</i>			5765	27	0.5%	
<i>Campylobacter</i> spp.	single	25 g	33	0	0.0%	
Pathogenic <i>E. coli</i>	single	25 g	4	0	0.0%	
<i>Staphylococcus</i> enterotoxins	single	10 g	37	5	13.5%	
<i>Yersinia</i> spp.	single	25 g	22	1	4.5%	<i>Y. Enterocolitica</i>
<i>Listeria monocytogenes</i>	single	10-25 g	258	6	2.3%	
<i>Listeria monocytogenes</i>	single	25 g	1817	18	1.0%	
<i>Listeria monocytogenes</i>	single	100 g	526	4	0.8%	
<i>Listeria monocytogenes</i>	single	unknown	109	0	0.0%	
<i>Listeria monocytogenes</i>	batch	10-25 g	6	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	128	0	0.0%	
<i>Listeria monocytogenes</i>	batch	unknown	7	0	0.0%	
<i>Listeria monocytogenes</i>	unknown	25 g	36	0	0.0%	
<i>Listeria monocytogenes</i>	unknown	unknown	23	0	0.0%	
<i>Total Listeria monocytogenes</i>			2910	28	1.0%	

## Sweets

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	482	4	0.8%	
<i>Salmonella</i> spp.	single	unknown	192	0	0.0%	<i>S. Enteritidis</i> : 90% <i>S. Bareilly</i> : 6.7% <i>S. Simi</i> : 3.3%
<i>Salmonella</i> spp.	batch	25 g	3395	26	0.8%	
<i>Salmonella</i> spp.	unknown	unknown	4	0	0.0%	
<i>Total Salmonella</i>			4073	30	0.7%	
<i>Campylobacter</i> spp.	single	25	1	0	0.0%	
<i>Campylobacter</i> spp.	single	swab	1	0	0.0%	
<i>Campylobacter</i> spp.	single	unknown	8	0	0.0%	
<i>Total Campylobacter</i>			10	0	0.0%	
<i>Yersinia</i> spp.	single	unknown	158	2	1.3%	<i>Y. Enterocolitica</i>
<i>Listeria monocytogenes</i>	single	1 g	22	0	0.0%	
<i>Listeria monocytogenes</i>	single	1-25 g	68	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	477	13	2.7%	
<i>Listeria monocytogenes</i>	single	unknown	38	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	444	0	0.0%	
<i>Total Listeria monocytogenes</i>			1049	13	1.2%	

## Gelatine (includes gelatine and collagen; information may not relate to unfilled gelatine capsules)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	batch	25 g	4	0	0.0%	

**Stuffed olives** (no information is provided on the content and therefore information may not relate to olives stuffed with fish)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Yersinia</i> spp.	single	25	6	0	0.0%	

**Pasta** (includes pasta and pasta/rice salad; pasta with some meat products may be included since this is not indicated in the data provided)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	885	3	0.3%	
<i>Salmonella</i> spp.	single	unknown	183	1	0.5%	<i>S. Enteritidis</i> : 100%
<i>Salmonella</i> spp.	batch	25 g	417	0	0.0%	
		<i>Total Salmonella</i>	1485	4	0.3%	
<i>Campylobacter</i> spp.	single	25 g	16	0	0.0%	
Pathogenic <i>E. coli</i>	single	25 g	1	0	0.0%	
	batch	25 g	119	3	2.5%	VTEC (unspecified)
		<i>Total pathogenic E. coli</i>	120	3	2.5%	
<i>Staphylococcus</i> enterotoxins	single	25 g	17	0	0.0%	
<i>Cronobacter</i> spp.	batch	unknown	15	0	0.0%	
<i>Listeria monocytogenes</i>	single	1 g	1	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	224	4	1.8%	
<i>Listeria monocytogenes</i>	single	100 g	404	15	3.7%	
<i>Listeria monocytogenes</i>	single	unknown	37	0	0.0%	
<i>Listeria monocytogenes</i>	batch	25 g	81	1	1.2%	
<i>Listeria monocytogenes</i>	batch	unknown	171	0	0.0%	
		<i>Total Listeria monocytogenes</i>	918	20	2.2%	

## Noodles

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	507	12	2.4%	<i>S. Enteritidis</i> : 81.0% <i>S. Typhimurium</i> : 9.5% <i>S. Bareilly</i> : 4.8% <i>S. Egusitoo</i> : 4.8%
<i>Salmonella</i> spp.	single	unknown	538	0	0.0%	
<i>Salmonella</i> spp.	batch	25 g	1027	9	0.9%	
<i>Total Salmonella</i>			2072	21	1.0%	
<i>Campylobacter</i> spp.	single	25 g	5	0	0.0%	
<i>Staphylococcus</i> enterotoxins	single	10 g	8	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	23	0	0.0%	

## Soup stocks (includes only information related to dehydrated soups)

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Salmonella</i> spp.	single	25 g	105	0	0.0%	
<i>Salmonella</i> spp.	batch	25 g	31	0	0.0%	
<i>Total Salmonella</i>			136	0	0.0%	
<i>Listeria monocytogenes</i>	single	25 g	32	0	0.0%	

## Flavourings

Causative agent	Sample unit	Weight	Samples			Other info
			N	pos	% pos	
<i>Campylobacter</i> spp.	single	25	150	0	0.0%	

**F. CONFIRMED FOOD-BORNE OUTBREAKS IN CERTAIN COMPOSITE PRODUCTS IN THE EU (2004-2009)**

**Bakery products excluding cakes** (includes biscuits/cookies, bread and other bakery products)

Causative agent	Number of outbreaks	Countries reporting	Cases hospitalized			Other info
			ill	hospitalized	death	
<i>Bacillus cereus</i>	2	2	29	2	0	
<i>Salmonella</i> Bredeney	1	1	2	0	0	
<i>Salmonella</i> Enteritidis*	21	7	487	154	0	The number of hospitalised cases was not reported in 2 outbreaks.
<i>Salmonella</i> spp.	3	2	38	14	0	
<i>Staphylococcus aureus</i>	3	2	52	0	0	The number of hospitalised cases was not reported in 1 outbreak.
Other/unknown	4	3	39	0	0	
<i>Total</i>	<i>34</i>		<i>647</i>	<i>170</i>	<i>0</i>	

\* One additional large outbreak (not included in the table) was reported for non-specified bakery products in 2004, which was attributed to *Salmonella* Enteritidis. However, from the data reported it is not clear whether the outbreak was suspected or confirmed and the number of cases reported is also not clear, even if it is indicated that at least 720 cases of disease were described.

**Cakes without raw eggs\***

Causative agent	Number of outbreaks	Countries reporting	Cases			Other info
			ill	hospitalized	death	
<i>Bacillus cereus</i>	2	1	21	20	0	
Calicivirus (including norovirus)	19	4	850	5	0	The number of hospitalised cases and deaths was not reported in respectively 7 and 6 outbreaks.
<i>Salmonella</i> spp.	1	1	14	n.r.	0	
<i>Salmonella</i> Enteritidis	63	12	1819	463	5	<i>S. aureus</i> was also identified as causative agent in 1 outbreak. The number of ill cases, hospitalised cases and deaths was not reported in respectively 1, 6 and 5 outbreaks.
<i>Salmonella</i> Typhimurium	1	1	21	6	0	
<i>Staphylococcus aureus</i>	6	5	46	0	0	<i>B. cereus</i> was also identified as causative agent in 1 outbreak. The number of ill cases, hospitalised cases and deaths was not reported in respectively 1, 4 and 2 outbreaks.
Other/unknown	2	2	18	0	0	
<i>Total</i>	<i>94</i>		<i>2768</i>	<i>488</i>	<i>5</i>	

\* Includes several types of cakes in which the presence among ingredients of raw eggs associated with absence of subsequent processing was not explicitly indicated (e.g. fancy cakes, layer cakes, fruit pies, pancakes, puddings, cream cakes, cakes with eggs, cakes with ice-cream, cakes stuffed with fishery products, dough for cakes). In 10 cases frozen fruits included in cakes were identified as the source of the pathogen.

### Cakes and desserts with raw eggs\*

Causative agent	Number of outbreaks	Reporting countries	Cases			Other info
			ill	hosp	death	
<i>Salmonella</i> Enteritidis	123	9	1561	379	1	The number of ill cases and hospitalised cases was not reported in 1 outbreak.
<i>Salmonella</i> Typhimurium	2	1	458	31	3	In 1 of the two large outbreaks it is assumed that some sporadic cases are also included.
<i>Salmonella</i> spp.	1	1	5	0	0	
<i>Total</i>	<i>126</i>		<i>2024</i>	<i>410</i>	<i>4</i>	

\* Includes cakes and desserts for which raw eggs were explicitly indicated among ingredients and that were not subsequently processed, and cakes and desserts for which this can be assumed (e.g. tiramisu, chocolate mousse). Cakes and desserts containing raw eggs among their ingredients and that are not subsequently processed are not composite products since they contain unprocessed products of animal origin.

### Chocolate and confectionery (including sweets\*)

Causative agent	Number of outbreaks	Countries reporting	Cases			Other info
			ill	hospitalized	death	
<i>Bacillus cereus</i>	1	1	8	0	0	chocolate
<i>Clostridium perfringens</i>	1	1	43	0	0	chocolate sauce
<i>Salmonella</i> Enteritidis	6	3	150	23	0	sweets and chocolate
<i>Total</i>	<i>8</i>		<i>201</i>	<i>23</i>	<i>0</i>	

\* One additional outbreak was reported for sweets (“flimmary”) in 2005, which involved 28 cases. However, the outbreak was attributed to an unknown agent and from the data reported it is not clear whether the outbreak is suspected or confirmed.



**Pasta** (includes various types of pasta, e.g. pasta, pasta with eggs, pasta salad etc.)

Causative agent	Number of outbreaks	Countries reporting	Cases			Other info
			ill	hospitalized	death	
<i>Bacillus cereus</i>	2	2	35	0	0	The number of hospitalised cases and deaths was not reported in 1 outbreak.
<i>Salmonella</i> spp.	2	1	8	7	0	
<i>Staphylococcus aureus</i>	3	1	6	n.r.	n.r.	
<i>Staphylococcus enterotoxins</i>	1	1	120	8	1	
<i>Total</i>	8		169	15	1	

**Noodles** (includes various types of noodles, sometimes cooked with other products, e.g. boiled noodles with vegetables, boiled noodles with shrimps)

Causative agent	Number of outbreaks	Countries reporting	cases			Other info
			ill	hospitalized	death	
<i>Bacillus cereus</i>	4	1	12	n.r.	n.r.	
<i>Salmonella</i> Enteritidis	4	2	104	18	0	
<i>Staphylococcus aureus</i>	1	1	4	n.r.	n.r.	
<i>Total</i>	9		120	18	0	

## G. RASFF NOTIFICATIONS<sup>14</sup>

Composite product	Reference	Agent	Date	Reason for notification	Origin	Notified by
cakes	2009.0886	<i>Listeria monocytogenes</i>	Jul 2009	alert - company's own check	Netherlands	Netherlands
cakes	2007.0186	<i>Escherichia coli</i> <i>Salmonella</i> Enteritidis <i>Staphylococcus aureus</i> <i>Bacillus cereus</i>	Mar 2007	alert - official control on the market	Lithuania	Germany
cakes	2006.COG	<i>Salmonella</i> group D <i>Staphylococcus aureus</i>	Nov 2006	information - official control on the market	Cyprus	Cyprus
cakes	2003.CDW	<i>Staphylococcus aureus</i>	Oct 2003	information - company's own check	Germany	Germany
cakes	2003.239	<i>Salmonella</i> Infantis	Aug 2003	alert - official control on the market	Denmark	Denmark
cakes	2005.BRF	sulphite reducing anaerobes	Jun 2005	information - official control on the market	Switzerland	France
biscuits	2011.0611	<i>Salmonella</i> Enteritidis	May 2011	information - official control on the market	Poland	Poland
chocolate	2010.1418	<i>Staphylococcus</i>	Oct 2010	alert - food poisoning	France	France
chocolate	2010.0106	<i>Salmonella</i>	Jan 2010	alert - company's own check	Slovakia	Netherlands
chocolate	2010.0080	<i>Salmonella</i> Orion	Jan 2010	alert - company's own check	Slovakia	Slovakia
chocolate	2009.1429	<i>Listeria monocytogenes</i>	Oct 2009	information - company's own check	Italy	France
chocolate	2008.0586	<i>Salmonella</i> spp.	May 2008	alert - official control on the market	Netherlands	Netherlands
chocolate	2006.0399	<i>Salmonella</i> Montevideo	Jun 2006	alert - company's own check	United Kingdom	United Kingdom
chocolate	2001.291	<i>Salmonella</i> Oranienburg	Dec 2001	alert	Germany	Germany
chocolate	2010.1332	<i>Salmonella</i>	Oct 2010	information - company's own check	Greece Poland	Greece
confectionery	2010.0164	<i>Salmonella</i> spp.	Feb 2010	information - official control on the market	Serbia	Slovenia
confectionery	2010.0112	<i>Salmonella</i> Infantis	Jan 2010	information - company's own check	Serbia	Slovenia
confectionery	2010.0049	<i>Salmonella</i> Infantis	Jan 2010	information - border control - consignment released	Serbia	Slovenia
confectionery	2010.0023	<i>Salmonella</i> Infantis	Jan 2010	information - border control - consignment released	Serbia	Slovenia
confectionery	2009.0219	<i>Salmonella</i>	Feb 2009	alert - food poisoning	United States	Slovenia
confectionery	2009.0125	<i>Salmonella</i>	Feb 2009	alert - food poisoning	United States	EU Commission

<sup>14</sup> The following RASFF hazard categories were screened: biotoxins, biocontaminants, non-pathogenic micro-organism, pathogenic micro-organism, parasitic infestation. Alerts were excluded when generically indicating infestation with “moulds” or “yeast”.

Composite product	Reference	Agent	Date	Reason for notification	Origin	Notified by
confectionery	2009.0108	<i>Salmonella</i>	Feb 2009	alert - food poisoning	United States	EU Commission
confectionery	2007.0731	coagulase-positive <i>Staphylococcus</i>	Oct 2007	alert - company's own check	Spain	France
confectionery	2007.CCB	<i>Salmonella</i>	Sep 2007	information - border control - consignment detained	Serbia	Slovenia
confectionery	2007.BSD	<i>Salmonella</i>	Jul 2007	information - border control - consignment released	Serbia	Slovenia
food supplements	2010.0400	<i>Salmonella</i> Montevideo	Mar 2010	alert - food poisoning	Netherlands	Germany
food supplements	2010.0149	<i>Salmonella</i> Jerusalem	Feb 2010	alert - company's own check	Italy	Norway
food supplements	09-585	<i>Escherichia coli</i> <i>Staphylococcus epidermis</i>	Dec 2009	news - consumer complaint	France Germany	EU Commission
food supplements	09-583	<i>Bacillus cereus</i>	Dec 2009	news - official control in non-member country	United States	EU Commission
food supplements	2006.0428	bacterial contamination	Jul 2006	alert - official control on the market	United States via Canada	EU Commission
food supplements	2006.BLA	<i>Salmonella</i>	Jun 2006	information - border control - consignment detained	Croatia	Slovenia
food supplements	2005.AJF	<i>Bacillus cereus</i> aerobic mesophiles	Feb 2005	information - border control - consignment detained	Croatia	Slovenia
food supplements	2002.227	<i>Enterobacteriaceae</i>	Jun 2002	alert	Germany	Belgium
pasta	2006.0926	<i>Bacillus cereus</i> faecal coliforms <i>Escherichia coli</i>	Dec 2006	alert - official control on the market	Italy	Italy
pasta	2005.788	<i>Bacillus cereus</i>	Nov 2005	alert - company's own check	Italy via Germany	United Kingdom
noodles	2010.1692	<i>Salmonella</i> Mbandaka <i>Staphylococcus aureus</i>	Dec 2010	information - official control on the market	Poland	Germany
noodles	2007.CFE	<i>Salmonella</i>	Sep 2007	information - border control - consignment detained	Hong Kong	Italy
noodles	2004.482	<i>Salmonella</i> Enteritidis	Sep 2004	alert - official control on the market	Slovakia	Slovakia
noodles	2003.115	<i>Salmonella</i> Mbandaka	Apr 2003	alert - official control on the market	France	Germany

## GLOSSARY

Composite product	“A <i>foodstuff intended for human consumption that contains both processed products of animal origin and products of plant origin and includes those where the processing of a primary product is an integral part of the production of the final product</i> ” (Decision 2007/275/EC).
D-value	Time needed to achieve one decimal log reduction of a microorganism.
Dose-1% value	Estimated dose that caused a certain effect in 1% of the individuals exposed.
Food supplements	“ <i>Foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities</i> ” (Directive 2002/46/EC)
F <sub>ref</sub>	Time required to achieve a certain reduction (e.g. 5 log <sub>10</sub> , 8 log <sub>10</sub> , or 10 log <sub>10</sub> reduction) of a microorganism at a given reference temperature T <sub>ref</sub> (e.g. 70°C for vegetative bacteria and 120°C for spores). Calculating F <sub>ref</sub> for a series of microorganisms allows comparing the efficiency of a certain thermal treatment in their inactivation.
Hazards needing growth and toxin production in food to cause illness	The following hazards are relevant for this Opinion: <i>Clostridium botulinum</i> , <i>Staphylococcus aureus</i> , emetic <i>Bacillus cereus</i> , bacteria producing biogenic amines.
Hazards not needing growth in food to cause illness	The following hazards are relevant for this Opinion: Norovirus, parasites, <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Campylobacter</i> spp., pathogenic <i>E. coli</i> , <i>Yersinia enterocolitica</i> .
Hazards usually needing growth in food to cause illness	The following hazards are relevant for this Opinion: <i>Listeria monocytogenes</i> , <i>Vibrio parahaemolyticus</i> , <i>Clostridium perfringens</i> , diarrhoeic <i>Bacillus cereus</i> .
Low risk	The composition and processing of the food should cause inactivation of the pathogen or prevent the pathogen to reach hazardous levels at consumption.
Moderate risk	It concerns foods cooked before consumption. It is not low risk, since the hazard may be still present at the moment of its preparation by the consumer. The possibility of cross-contamination in consumer’s kitchen of other foods consumed raw, or that the food is eaten without prior cooking, must be considered. In addition, the way of cooking will influence the level of inactivation of the pathogens.
Nutrients	“ <i>The following substances: (i) vitamins, (ii) minerals</i> ” (Directive 2002/46/EC).

P1 value	Estimated probability of a certain effect when the ingested dose is represented by one cell.
Processed products	<i>“Foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics”</i> (Regulation (EC) No 852/2004).
Processing	<i>“Any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes”</i> (Regulation (EC) No 852/2004).
<i>Qualified presumption of risk</i>	For the purpose of this Opinion, it means that the pathogens have the potential to cause disease via consumption of the composite product, if present in the food or its ingredients. <i>Qualified presumption of risk</i> must also be interpreted in light of the knowledge on the prevalence of the pathogen in the food concerned and its involvement in past outbreaks. It indicates that further information is needed for this type of foods. This can be: more accurately defining food composition and shelf-life, better defining the potential of the pathogen to survive or grow (e.g. challenge test), or having more information on the level of hygiene during production and processing.
Unprocessed products	<i>“Foodstuffs that have not undergone processing, and includes products that have been divided, parted, severed, sliced, boned, minced, skinned, ground, cut, cleaned, trimmed, husked, milled, chilled, frozen, deep-frozen or thawed”</i> (Regulation (EC) No 852/2004).
Water activity ( $a_w$ )	Ratio of the water vapor pressure of food substrate to the vapor pressure of pure water at the same temperature.
$z$ -value	Increase in temperature able to produce a reduction in the $D$ -value of a factor of 10.