Return Catering Guidance

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AIRLINE CATERING ASSOCIATION

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1.0 Introduction & Background

ACA (Airline Catering Association) is a not-for-profit international association. Its aim is to provide a forum for the promotion of cooperation among airline caterers and other stakeholders in the industry and to support the activities of its members, the onboard caterers, in their contribution to human, economic and social development globally and regionally.

Return catering is a popular method of food catering in the airline industry. Its activity is explained in some internationally recognised guidance documents, e.g., the World Food Safety Guidelines (WFSG) for Airline Catering, with some generic guidance and cooperative approach proposal in the menu design phase.

In the past, the menu selection for return catering was performed by using more robust products in regards to water activity or pH level like hard cheeses or smoked ham. Today scientific tools like “predictive modelling” are available that can simulate actual growth rates of pathogenic bacteria along different time/temperature scenarios.

The document in annex is based on the latest scientific know-how and provides orientation in the menu selection considering a set of time/temperature scenarios, based on predictive modelling. ACA recognises that additional food safe processes and procedures are required throughout the food supply chain to support return catering process and ensure time/temperature controls throughout the food supply chain.

ACA hence decided to partner with Mérieux NutriSciences to conduct a detailed scientific study on simulated variable storage conditions of components and/or meals applicable to return catering. The study uses predictive microbiology models to evaluate various times and temperatures that lead to unacceptable pathogen proliferation.

You may find the scientific dossier as an appendix to this document.

2.0 Purpose

The purpose of this document is to provide broad guidance on food safe processes and procedures and to simplify interactions between airlines and other stakeholders. This guidance document and the scientific dossier in annex should be used together to support food safety risk decision making such as, for instance, the suitability of specific components and/or meals given critical times and temperatures based on storage, handling and transportation conditions.

All return catering activities should be carried out in accordance with relevant local regulatory requirements in the country of operation where applicable.

3.0 Risk Assessment

A thorough and detailed food safety risk assessment was completed. This assessment led to the identification of food safety risks and hazards associated with return catering.

For the purpose of this guidance report, it was considered that all other food safety hazards were controlled by organisational processes and procedures and/or other internationally recognised food safety standards.

The following key hazards and control measures were identified as part of the assessment process:

Hazards	Control Measures
Pathogenic growth and/or quality issues due to unsuitable components and/or meals	Processes and procedures should be put in place to review the suitability of all components and main dishes which are stored, refrigerated and heated onboard. Components and main dishes containing hazardous components should not be provided.
Pathogenic growth due to temperature abuse through outbound flight	Thermal insulated bags with ice blocks should be used for unrefrigerated cabin stowage. Refrigerated containers should be used for hold stowage. Refrigerated containers are likely to require batteries and/or dry ice to operate and maintain appropriate temperatures. Electronic temperature monitoring equipment should be used for verification and validation. Time and temperature trials should be completed to validate controls. Contingency processes and procedures should be put in place for delayed flights.
Pathogenic growth due to temperature abuse through transportation	Refrigerated catering and/or support vehicles should be used to transport catering from the aircraft to overnight storage. Temperature readings from electronic monitoring equipment should be verified before catering is provided to the return flight. Contingency processes and procedures should be put in place for interrupted transportation activities.

4.0 Scope

This guidance document applies to all processes associated with:

1. Return catering,
2. Multiple sector (multi-hop) catering, and
3. Circumstances where overnight storage of food is required using methods to mitigate and minimise food safety risks and hazards, with specific emphasis on prevention of potential pathogenic microorganisms.

The scientific dossier can also be used as guidance for decision-making in any food processes in a food establishment that involves critical storage conditions of food products that are influenced by various intrinsic and extrinsic factors as detailed in the dossier.

5.0 Guidance

Menu Selection & Product Groups

Processes and procedures should be put in place to review and agree on the suitability of components and/or meals. It is recommended that the processes and procedures include a risk assessment, quality evaluation and microbiological analysis. The scientific dossier can be used to support the decision-making process on suitability of components and/or meals based on the known storage conditions.

The manufacturer's guidelines should always be followed for ready-to-eat (RTE) components and meals. These can include specification details, ingredients and components, microbiological criteria, chemical limits and tolerance, storage, and handling conditions, etc.

The suitability of components and/or meals should be confirmed before menu selection (including prohibited foods, restricted foods, susceptible foods, hazardous meal ingredients, and high-risk food items). The process should also consider local regulatory requirements that must be followed.

The components and/or meals should be consumed by the given date code. Time until consumption and duration of flight should be considered as part of the process.

The scientific dossier includes guidance on product grouping and can be used to support the menu selection process.

Food Safety System

A safe food management system should inevitably include return catering in its scope. Some of the key factors for consideration are:

- document control
- training and development
- infrastructure
- storage/transportation practises
- pre-requisite programmes
- inventory management
- internal/external audit management
- corrective/preventive actions, and
- continual improvement

In-flight Storage

Galley Loading:

Galley storage (or stowage) locations within an aircraft are a place where food is stored and distributed to consumers onboard. Galley locations can be both refrigerated and non-refrigerated. This depends on the airline and the aircraft type used in service.

Meals are generally stowed in refrigerated compartments in the galley of the aircraft, with all cooling systems switched on.

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Loading examples include:

- Standard units (trolleys/carts) placed directly in refrigerated stowage positions;
- Catering packed in insulated carton boxes (cartons lined with expanded polystyrene) or other types of equipment such as coolers (non-refrigerated storage galleys).

Belly Loading:

Belly loading is a practice in the airline industry where items are stored in the lower deck (belly) of a passenger aircraft. This is also termed as cargo-hold area.

Meals are stowed in the lower deck (belly) of a passenger aircraft for return catering. Such loading examples are:

- Standard units placed in ULD's (Unit Load Devices);
- Catering packed in insulated carton boxes (cartons lined with expanded polystyrene) or other types of equipment such as coolers.

Airlines may decide to belly load food items for a return sector depending on space availability within an aircraft galley. If a decision is made to load the food items and containers in the belly, airlines should evaluate the risks (including product categories used for return catering).

Based on an airline's trials using data loggers, if time and temperature trials have been effective with re-producible data, trials should be carried out periodically to validate that the process can achieve the intended food safety controls.

If time and temperature trials have not been completed before the commencement of services, it is recommended to monitor time and temperatures of products by utilising data loggers for both galley and belly loading.

The food establishments should highlight the risks to the airline if a decision has been made to return cater food items in uncontrolled temperature storage.

Food establishments and airline stakeholders should work together and highlight food safety risks if any and agree upfront before commencement of service.

Airlines can use a data logger for evaluation purpose and risk assessment for return catering, which can be lag indicators to control and prevent hazards. Airlines should have a documented process to this effect.

Contingency processes and procedures should be put in place for creeping and delayed flights.

Double Catering Multiple Sectors:

Meals are generally stowed in refrigerated compartments in the galley of the aircraft, with all cooling systems switched on. It is essential for food establishments in association with the airline stakeholders to ensure that the cooling refrigeration systems are in good working condition and switched on and working at the correct temperature before loading of food and during the flight (no switching off of the cooling system).

Although the scientific dossier in annex does not cover frozen food products and their storage conditions, food establishments and airlines may require additional food safety plans to determine potential hazards, monitor and validate processes to have an effective plan established before commencement of operations.

Overnight Storage

Overnight storage is when the catering is removed from the aircraft and transported to a facility for storage on ground in a refrigerator or when the catering remains on the aircraft overnight and until the next flight departs.

In case there is a need to offload and store overnight, the airline acts as a supplier in the logistic chain. Adherence to the caterer's food safety program, local legislation and liability risks should be clarified upfront with the food establishment or caterer. As a minimum (but it should not be limited to this), the temperature of the goods should be checked prior to their offloading, monitored in between, and checked again when loaded back onto the aircraft.

Refrigerated vehicles should be used to offload and transport catering from the aircraft to the catering facility for overnight storage. Standard documented practices should be maintained and followed as per each food establishment's HACCP Plan.

> ***N.B.:*** *The Airline and/or the food establishment may require additional authorisation by the local food safety authority as the temporary food storage on ground may be treated as transit cargo, depending on local regulations in the country of operation.*

Contingency processes and procedures should be put in place for creeping and delayed flights and such processes must be documented in order to mitigate risks.

Sanitation Programme

In-flight storage equipment should be clean, free from damage and kept in accordance with the manufacturer's instructions.

Sanitation Controls:

Sanitation Controls include procedures, practices and processes to ensure that the food establishment or caterer is maintained in a sanitary manner allowing it to control hazards such as environmental pathogens.

Associated guidance for sanitation controls includes, as appropriate:

- monitoring
- corrective actions
- verification (incl. environmental monitoring for an environmental pathogen or appropriate indicator organism as necessary), and
- records

Aircraft Without Refrigerators

Thermal insulated bags with ice blocks or dry ice should be used for unrefrigerated cabin stowage. Refrigerated containers should be used for hold stowage. Refrigerated containers are likely to require batteries and/or dry ice to operate and maintain appropriate temperatures. Electronic temperature monitoring equipment should be used for verification. Other methods can be used to maintain storage temperatures like, for example, insulated canisters or carts and/or dry ice.

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Time and temperature trials should be completed to validate controls before commencement of the activity. It is recommended that at least three trials are completed. The frequency of verification and validation should be established after studying all parameters associated with the activity (including post-trial validation example, microbiological analysis, etc).

The time and temperature trial form can be used to record time and temperature trials. The results can be referred to the storage time and temperature growth simulations of the scientific study. The time and temperature trial form is an appendix to this document.

6.0 References

Title	By
Food Processing Quality (FPQ) Standards and Interpretation Guidelines	Quality & Safety Alliance In-flight Services (QSAI)
US FDA Preventive Controls	United States Food and Drug Administration Preventive Controls
US FDA Sanitary Transportation of Food	United States Food and Drug Administration – Guidance for Industry: Sanitary Transportation of Food
WFSG	World Food Safety Guidelines

7.0 Appendices

Title	By
Scientific Dossier on Critical Storage Conditions of Food Products Applicable to Return Catering	Mérieux NutriSciences – June 2021
Time and Temperature Trial Form	ACA – July 2021

8.0 Glossary

Term	Details
Audit	A systematic, independent, and documented process for obtaining evidence and evaluating it objectively to determine the extent to which the audit criteria are fulfilled.
Calibration	A procedure for ensuring that a known measured output of an instrument such as temperature or weight corresponds to a known national standard value for that property.

Certification	A procedure by which a certification body, following its own independent assessment determines whether a business complies with the requirements of a recognized standard.
Cleaning	The process of removing soil, food residues, dirt, grease, and other objectionable matter.
Creeping Delay	It is sometimes difficult for an airline to estimate how long a delay will be during its early stages. When a flight delay unexpectedly becomes longer and longer, this is called a "creeping delay."
Contamination	The introduction or occurrence of a contaminant in food or the food environment.
Control measure	Any action at a control point which can be taken or used to prevent a hazard or reduce it to an acceptable safe level.
Corrective Action	The action taken when the monitoring of a critical control point indicates a potential loss of control, or when a critical limit is not met.
Data Loggers	Data loggers are calibrated electronic temperature monitoring equipment
Food Establishment / Food Business Operators (FBO) / Caterers / Catering facility / Suppliers	Any place where food is manufactured, prepared, processed, traded or sold directly or indirectly to the consumer. The term includes any such place regardless of whether consumption is on or off the establishment. Other common terms used are food business operator (FBO), Caterer, Catering facility and Suppliers, which also means the same.
Food Transportation Vehicle	Any mode of transport, designated for food, whether self-propelled or not and whether used on land, sea or in the air.
Good Hygiene Practices (GHP)	All practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.
Good Manufacturing Practices (GMP)	The minimum quality & safety requirements aimed at ensuring that foods are prepared in a consistent manner according to agreed specifications e.g., raw & cooked food products are stored in separate refrigerators.
Ready to Eat Foods	Any food for consumption without further treatment or processing. Examples of ready-to-eat food items may include sliced cooked meats, cooked meat products and preparations, cooked/roast chickens, sandwiches and filled rolls, dairy products such as milk and cheese, fruits, prewashed/topped and tailed vegetables, prepared vegetable salads, whole salad items such as tomatoes or cucumbers, open and canned ready-to-eat fish, and fish products such as salmon,

	tuna or sardines, shellfish, preserves and jams, condiments, bread, confectionery, and biscuits
TCS Foods	Time/temperature control for safety (TCS) foods are foods that require time or temperature control to limit pathogenic microorganism growth or toxin formation.

9.0 Q&A's

1. **Why is it important to consider time and temperature during storage and handling of food products?**
A leading cause of foodborne illness is time and/or temperature abuse of TCS (food requiring time and temperature control for safety) foods. Food establishments should have a time/temperature control system for safe handling and to prevent growth of pathogenic microorganisms to harmful levels during storage and handling of food.

2. **How do I know which product group I should use during menu selection?**
It is essential to know the food components and the ingredients used in a meal. The scientific dossier in annex gathers pH and Aw values for more than 250 products from various sources. The products are combined into 46 main product families with one product considered as the most sensitive product of the family. The sensitive product characteristics (pH, Aw) are used for growth evaluation for the relevant time/temperature scenarios (4h to 24h, 5°C to 25°C) using predictive microbiology. Specific risk assessments for high-risk products due to higher probability of pathogen contamination should be applied before selecting any product or ingredient.

3. **What are the most critical factors that I should consider in order to minimise pathogen risk during storage?**
Among multiple intrinsic and extrinsic factors, pH, Aw, temperature and time are the most critical factors to determine unacceptable pathogens proliferation during storage at a specific temperature.

4. **Why should a risk assessment and hazard analysis be carried out when we already have temperature-controlled storage?**
Risk assessment is an essential part of a food safety plan or a management system. It is used to ensure that food safety controls are effective, relevant, timely and responsive to any threat irrespective of the existing methodology used to control hazards (i.e. storage in refrigerators). Food safety risk assessments can be quite complex even for well-established food establishments or caterers. A risk assessment helps prioritising risks and identifying with the management the necessary control measures in order to protect consumers.

5. **Who is responsible for time-temperature monitoring onboard an aircraft for return catering or during storage, handling, and transportation on ground for overnight storage?**
This depends on the food establishment and the operating airline. However, the two should always agree on, and document upfront, a process in order to establish monitoring activities

for time/temperature irrespective of where the monitoring takes place (whether on the ground or onboard).

In cases where aircraft aren't equipped with time/temperature monitoring devices, a thorough process should be agreed amongst the stakeholders involved. If the cold chain cannot be guaranteed or maintained throughout the operation, then an alternative to return catering should be considered.

6. **<u>What should I do if I cannot control the time/temperature of foods after food products has left the facility?</u>**
   (Cf. Q&A 5) Food safety is everyone's responsibility. All stakeholders involved at various stages in the food supply chain should be consulted and should agree on putting in place a good and thorough management system to prevent foodborne illnesses.
   A posteriori, in the case where food is subject to time/temperature variations, it should be considered as unsuitable and unsafe for consumption and should be discarded.

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ACA PROJECT

SCIENTIFIC DOSSIER
CRITICAL STORAGE CONDITIONS OF FOOD PRODUCTS
APPLICABLE TO RETURN CATERING

ACA – EXPERTISE SERVICES_02_2021_ V3 CC

Study conducted in	2021 April-June
By	**MERIEUX NUTRISCIENCES** Food Science Center 3 Route de la Chatterie 44800 SAINT-HERBLAIN, FRANCE
Technical experts	**Anne BRILLET** *(France Engineer Food Science Center)* **Joël CROCIANI** *(France Expert Microbiology)* **Christophe DUFOUR** *(European Scientific Director Microbiology)* **Steve OSWALD** *(US Consulting* Director*)*

Client	
AIRLINE CATERING ASSOCIATION (ACA) 3 Rue du Luxembourg, 1000 BRUXELLES, Belgium	**Mr Fabio GAMBA** *Director general* +32 473 901 707 E-mail: fabio.gamba@asaworld.aero

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1	2021-06-05	Creation
2	2021-06-18	Modification 1
3	2021-06-24	Modification 2
4	2021-07-08	Modification 3

CONTENTS

EXECUTIVE SUMMARY

In order to evaluate the critical limits of time/temperature of storage during specific catering situations such as return catering, the study evaluates the key factors influencing pathogens growth risk in food products. Based on the selection of critical pathogenic microorganisms, product families, and storage conditions, the study uses predictive microbiology to evaluate the time/temperature that lead to unacceptable pathogen proliferation. The study is based on bibliographical and simulation data's, not on field experiments or testing.

The microorganisms covered in this study are microorganisms with a target limit of absence in 25g with a maximum of 100 CFU/g *(Listeria monocytogenes)* for which an increase of 1 log is considered as the maximum tolerable growth. The study also considers microorganisms with maximum safety limits of 100 000 CFU/g (S*taphylococcus aureus, Clostridium perfringens* and *Bacillus cereus)* for which an increase of 2 logs is considered as a maximum tolerable growth. For microorganisms with a target limit of absence in 25g (*Salmonella* spp. and *E. coli* STEC), we consider exclusion of risk ingredients or food practice is the effective risk management measure and no acceptable growth has been considered.

Among multiple intrinsic and extrinsic factors, pH, Aw (water activity) and temperature are the most critical factors to determine unacceptable pathogens proliferation during storage at a specific temperature. It is widely accepted that products with pH < 3,9 or Aw < 0,88 will not lead to unacceptable growth in case of cold chain abuse, when food products with pH >5,4 and Aw > 0,96 are susceptible to all pathogen growth.

The study gathers pH and Aw values from more than 250 products from various sources (Combase, CRFSFS, ACA, Mérieux NutriSciences…). The products are combined in 46 main product families with one product considered as the most sensitive product of the family. The sensitive product characteristics (pH, Aw) are used for growth evaluation for the relevant time / temperature scenarios (4h00 to 24h00, and 5°C to 25°C / 41°F to 77°F) using predictive microbiology Sym'Previus software. Specific risk assessment for high risk products due to higher probability of pathogen contamination should be applied before selecting any product or ingredient.

The results of the study are presented in a graphical mode to evaluate the sensitivity to products leading to potential growth of the pathogens. Product families are located on the pH/Aw of the matrix, and specific growth and target microorganisms are specified with colour codes for 4 time/temperature scenarios: 22h00 at 5°C (41°F), 18h00 at 10°C (50°F), 10h00 at 15°C (59°F), and 14h00 at 25°C (77°F).
For temperature of 5°C during 22h00 no growth above 1 log is identified for the microorganisms studied. For the temperature below 10°C (50°F), *Listeria monocytogenes* is the exclusive microorganism to consider to evaluate storage duration limitations. For temperature of 15°C (59°F) and above *Listeria monocytogenes* and *Staphylococcus aureus* are the critical microorganisms to consider to evaluate storage duration limitations.
In case a product is not existing in the reference tables we suggest you measure pH and Aw of the product and refer to the tables to evaluate the pathogens growth. In case a specific event occurs (temperature abuse for a defined time) you can refer to specific tables defining time or temperature to achieve the unacceptable growth of each individual pathogen.

NB: The data presented are constructed on a worst case scenario with a continuous temperature of the product, an immediate growth of the bacteria (no lag phase) and no other intrinsic factors slowing or inhibiting the growth. Potential growth of 1 log or 2 log doesn't mean the product is contaminated by a pathogen. Not all existing products are present in the study, and pH and Aw value shared are examples, not covering the full variability of all food products. Always consider complementary data when you need a specific risk assessment including full time/temperature curves, multiple pH and Aw measurements on different product batches. Predictive microbiology should be completed with real historical data and/or challenge studies to evaluate pathogen growth on a specific product.

DOCUMENT AIM

The aim of the document is to provide key scientific data to support choosing relevant menus to limit microbial pathogen risks in air-catering specific storage conditions such as return catering. The document proposes critical time-temperature storage conditions depending on food products categories intrinsic characteristics.

I. CONTEXT

ACA is issuing guidance on good practices regarding Return-Catering. In this context ACA needs scientific support to determine the critical limits of storage time and temperature to avoid any issues with microbiological pathogens due to improper storage.

II. METHODOLOGY

Key steps

The specific risk link to inadequate storage conditions is the potential multiplication of bacterial pathogens to numbers that increase the immediate danger to human health. In order to consider this issue, the following elements are gathered in this document:
- Scientific justification of factors of growth/no growth and the relevant microorganisms based on bibliographical review
- Scientific justification of product categories based on major intrinsic factors (pH, Aw) using bibliographical data, ACA members' data and Mérieux NutriSciences' data
- Scientific justification of critical limits based on bibliography / Simulation with Sym'Previus predictive modelling
- Synthetic data covering the most probable scenarios of contaminations and temperature abuse.

The study is based on bibliographical and simulation data's, not on field experiments or testing.

Microbial pathogens Hazards (EFSA 2020, Ceylan E. 2021)

This document focuses on the main microbiological hazards (pathogenic microorganisms) to be taken into account when determining whether a food item is likely to constitute an immediate danger to human health and on the types of foods where these pathogenic microorganisms are more likely to be present. The document focusses on pathogens of concern in different types of food categories and the key determinants for growth are reviewed, with a focus on relevant bacterial pathogens capable of growing under reasonably foreseeable conditions:

- Duration: 4h00 to 24h00 considering time zero is when products leave the catering facility until time of consumption
- temperature from 5°C (41°F) to 25°C (77°F) extreme foreseeable temperature of the product.

The major pathogens, relevant for perishable food shelf life determination are presented in the table below (EFSA, 2020):

Guidance on date marking and food information part 1

Table 1: Non-exhaustive summary of pathogenic microorganisms of relevance for date marking for different perishable food categories (including raw and processed prepacked foods)

	Group Genera/species	Food category of concern	Examples of food type
Gram-negative (enteric) bacteria	**Mesophilic**		
	Salmonella spp., pathogenic *E. coli*	Meat and products thereof	Raw pork meat, raw beef
		Fish and seafood	Shellfish
		Fruits and vegetables	Fresh cut/RTE vegetables (sprouts, spinach, ...) and fruits
		Milk and dairy products	Fresh/cottage cheese, raw milk
		Prepared/mix food	Prepared salads, sandwiches
	Psychrotrophic		
	Yersinia enterocolitica	Meat and products thereof	Raw minced meat
Gram-positive bacteria	**Non-toxicogenic** *Listeria monocytogenes*	Prepacked raw RTE food	Salads, fruit juices, fresh cut vegetables and fruits
		RTE food exposed to contamination after a processing step causing microbial inactivation	Cooked meat products, smoked fish, soft/semi-soft and fresh/cottage cheese
	Toxicogenic Non spore forming *Staphylococcus aureus*	Meat and products thereof	Cooked meat products
		Fish and seafood	Cooked fish products
		Cheese and dairy products	Raw milk cheese, soft cheese
		Bakery products	Cream-filled pastries, pies
		Prepared meals	Fish dishes, meat dishes, cheese containing dishes
	Spore forming aerobic *Bacillus cereus* (Diarrheic and emetic)	Food of non-animal origin, particularly heat treated	Cooked dishes/meals containing pasta or rice, such as tabbouleh, rice salad, semolina, rice pudding
		RTE prepared/mix food/meals (REPFED)	Cooked vegetables and potatoes, vegetable puree Meat-based meals with non-animal components (sauce, vegetables)
		Milk and dairy products	Pasteurised milk and dairy products and desserts
	Spore-forming anaerobic psychrotrophic non-proteolytic *Clostridium botulinum* **mesophilic** proteolytic *Clostridium botulinum*	Reduced atmosphere packed food, particularly heat treated (REPFED)	Salted fish, cooked meat products (pâté, sausages), hummus
		Seafood and meat products	Canned fish (sardines, anchovies, tuna) and meat products (corned beef, pâté)

Note: Foods exempt from the requirements to indicate a 'best before' date or covered by other EU provisions imposing other type of date marking, and excluded from this opinion, are listed in Appendix A.
RTE: ready-to-eat; REPFED: refrigerated (minimally) processed foods of extended durability.

www.efsa.europa.eu/efsajournal 15 EFSA Journal 2020;18(12):6306

The document is considering critical pathogenic bacteria identified in catering hazard analysis that can be influenced by temperature abuse.
Targeted pathogens which are in this study cover a limited list. *Campylobacter* spp., *Shigella* and *Yersinia enterocolitica* do not appear here because they are typically controlled when the pathogens listed are addressed.

The microorganisms to be considered including maximum limits inducing health issues:

- *Listeria monocytogenes* 100 CFU/g, presence in 25g
- *Staphylococcus aureus* 100 000 CFU/g, toxins development
- *Clostridium perfringens* 100 000 CFU/g
- *Bacillus cereus* 100 000 CFU/g, toxins development
- *Salmonella* spp. presence in 25g
- *Escherichia coli* STEC presence in 25g

Product categories
Principle food categories covering classical air catering (breakfast, meals and snacks) are included in this document. Based on existing bibliographic data's including Combase database, a set of pH / Aw data's are compiled for each product category, completed by ACA and Mérieux NutriSciences anonymized data's.

Risk evaluation based on predictive modelling
The next step is the use of predictive microbiology to determine the growth/no growth of each microorganism depending on pH/Aw values of the different food products. The predictive modelling program used is Sym'Previus.

The models from the Sym'Previus simulation tools describe the bacterial response to temperature, pH, water activity (and in some cases, lactic acid concentration).
These models are based on the "Gamma" concept formulated by Zwietering *et al.* (1992). This concept is based on the observation that factors influencing bacterial growth (temperature, pH, Aw) have multiplicative effects on bacterial growth rate.
The growth models in Sym'Previus are defined by the cardinal values of a bacterial strain (e.g. minimum, optimal and maximum growth temperatures), using the approach developed by Rosso *et al.* (1993, 1995). Note that in the Sym'Previus software, field isolates have been prioritized over laboratory strains for data generation. For some bacteria such as *Listeria monocytogenes*, these cardinal values have been determined for up to 14 different field strains, allowing the intra-species variability to be taken into account in the model predictions. This approach does not consider other factors such as the lag phase, the microbial ecology or antimicrobial activities of specific ingredients, a more specific approach would need specific studies such as challenge studies.

Rules of judgment

The safety of 46 meal types is evaluated during 5 storage temperatures and 5 storage times when considering pH, water activity, and other relevant food safety data (such as specific process, for example "pasteurized cooked and uncooked pressed cheese" and "pasteurised soft cheese, bloomy rind and washed rind") as referenced in the scientific literature (Cf. Chapters V and VI).

For microorganisms with a target limit of absence in 25g (*Salmonella* spp. and *E. coli* STEC*)* the acceptable value doesn't include quantitative approach and no simulation will be applied considering exclusion of risk ingredients or food practice is the effective risk management measure. This approach is not developed in this study.
For microorganisms with a target limit of absence in 25g with a maximum of 100 CFU/g *(Listeria monocytogenes),* an increase of 1 log is considered as significant growth and the maximum tolerable growth assuming levels of *Listeria monocytogenes* will never exceed 10 CFU/g at production level due to supplier controls.
For microorganisms with maximum safety limits of 100 000 CFU/g (S*taphylococcus aureus, Clostridium perfringens* and *Bacillus cereus)* an increase of 2 logs is considered as a maximum tolerable growth to avoid reaching 100 000 CFU/g considering the maximum tolerable growth assuming levels of *Staphylococcus aureus, Clostridium perfringens* and *Bacillus cereus* will never exceed 1000 CFU/g at production level due to supplier controls.

Germs	***Listeria monocytogenes***	***Clostridium perfringens***	***Bacillus cereus***	***Staphylococcus aureus***
Limit of growth (log CFU/g)	< 0	< 0		
	> 0 and < or = 1	> 0 and < or = 2		
	> or = 1	> or = 2		

For each designated microorganism and the maximum tolerated growth, the interface growth /no growth will be presented including a colour code identifying critical limits with No growth (Green), limited growth (Orange), and critical growth (Red).

A final synthetic tool including product families, growth potential of relevant pathogens and time /temperature limits is included in the report taking into account 4 targeted time/temperature scenarios.

III. FACTORS INFLUENCING THE MICROORGANISMS DURING THE STORAGE

(EFSA 2020, NACMCF 2010)

Food production includes many key factors influencing the preservation of food such as heat treatments and the addition of ingredients and preservatives resulting in changes in pH (ex: addition of acids), in Aw (ex: addition of sugar, salt) or the concentration of antimicrobial substances (e.g. organic acids, curing salts).

The factors affecting the shelf-life in relation to microbiological safety of foods are those determining the growth of microorganisms in foods. The probability of growth and, in case of growth, the growth rate, will determine the time needed for the relevant microorganism to exceed the acceptable level.

The growth affecting factors may be classified into those that are intrinsic or associated with the food material and those that are extrinsic or associated with the environment surrounding the food.

This document focuses on the intrinsic (especially pH and Aw), and extrinsic (especially temperature) factors that determine which microorganisms can grow and their growth potential during subsequent storage until consumption.

A source of information for minimum limits for growth of pathogenic microorganisms is summarized in the table below and reports combinations of pH and Aw values that may allow their growth. (NACMCF 2010).

TABLE 2. *Potential pathogens*[a] *of concern for growth studies based on interaction of product pH and* a_w[b]

	pH values:					
a_w 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[c]	NG	NG	NG	NG	NG
0.88–0.90	NG	NG	NG	NG	*Staphylococcus aureus*	*S. aureus*
>0.90–0.92	NG	NG	NG	*S. aureus*	*S. aureus*	*L. monocytogenes* *S. aureus*
>0.92–0.94	NG	NG	*L. monocytogenes* *Salmonella*	*Bacillus cereus* *Clostridium botulinum* *L. monocytogenes* *Salmonella* *S. aureus*	*B. cereus* *C. botulinum* *L. monocytogenes* *Salmonella* *S. aureus*	*B. cereus* *C. botulinum* *L. monocytogenes* *Salmonella* *S. aureus*
>0.94–0.96	NG	NG	*L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus*	*B. cereus* *C. botulinum* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *Vibrio parahaemolyticus*	*B. cereus* *C. botulinum* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *V. parahaemolyticus*	*B. cereus* *C. botulinum* *C. perfringens* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *V. parahaemolyticus*
>0.96	NG	*Salmonella*	Pathogenic *E. coli* *Salmonella* *S. aureus*	*B. cereus* *C. botulinum* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *V. parahaemolyticus*	*B. cereus* *C. botulinum* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *V. parahaemolyticus* *V. vulnificus*	*B. cereus* *C. botulinum* *C. perfringens* *L. monocytogenes* Pathogenic *E. coli* *Salmonella* *S. aureus* *V. parahaemolyticus* *V. vulnificus*

[a] *Campylobacter* spp., *Shigella*, and *Yersinia enterocolitica* do not appear here because they are typically controlled when the pathogens listed are addressed.
[b] Data are based on the PMP *(106)*, ComBase predictor *(50)*, ComBase database *(49)*, or peer-reviewed publications *(11, 17, 45)*.
[c] NG, no growth; when no pathogen growth is expected, but formulation or process inactivation studies may still be needed.

Intrinsic Factors
Intrinsic factors include water activity (Aw), pH and buffering capacity, nutrients, oxidation-reduction (redox) potential (Eh) and redox buffering capacity, antimicrobial substances naturally present in foods, and preservatives that are added or are produced by biological processes such as fermentation.
pH and Aw are the most important intrinsic factors to consider in assessing whether pathogenic microorganisms will grow in foods during their shelf-life.
Generally, it is accepted that foods with a pH below 3.9 or Aw below 0.88 do not support the growth or toxin production of foodborne pathogenic microorganisms, irrespective of the storage conditions (temperature, atmosphere...). However, other microorganisms, such as yeast and molds, could grow and cause spoilage (more of a quality concern than a food safety concern).

Combinations of pH and/ or Aw of non-heat-treated food (or heat treated but exposed to re-contamination) that inhibit the growth of any pathogen (vegetative or spore former) include (NACMCF 2010):

- Aw ≤ 0.88 or
- pH ≤3.9 or
- Aw ≤ 0.96 and pH ≤ 4.2
- Aw ≤ 0.92 and pH ≤ 4.6
- Aw ≤ 0.90 and pH ≤ 5.0

In **pasteurized foods**, where vegetative pathogens have been eliminated, the growth of pathogenic spore-forming bacteria and/or the toxin production is prevented when (NACMCF 2010; US FDA, 2017):

- pH ≤ 4.6 (i.e. acid or acidified food)
- Aw ≤ 0.92
- Aw ≤ 0.95 and pH ≤ 5.6

For foods with pH and/or Aw values above those just mentioned, **time/temperature control** for safety is required unless the Food Business Operators can show that other hurdles (such as natural antimicrobial substances or added preservatives) contribute to prevent microbial growth and/or toxin production (NACMCF, 2010).

Combining materials or ingredients to form multicomponent food products also modifies the intrinsic parameters, throughout the product or at the interface of components, depending on the type of product, resulting in new intrinsic parameters that also influence microbial growth. Multicomponent food 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 the food components or their composite.

Limit targets
In order to evaluate the potential hazard linked to a temperature deviation, limit targets should be considered for major pathogens such as *Listeria*, understanding that what is considered to be significant growth can vary depending on the region or regulating authority:
- **EU, Canada and Australia** consider the threshold as 0.5 Log_{10} for a significant growth of *Listeria* and no increase above 100 CFU/g should be achieved during shelf life**.** When food operator can guaranty punctual contamination of *Listeria monocytogenes* is < 10 CFU/g at production stage, an increase **<**1 Log_{10} over the shelf life is acceptable**.**
- US, FDA-inspected foods: <1 Log_{10} increase over two or more time intervals is the threshold for considering the microbial growth of biological relevance (Control of *Listeria monocytogenes* in Ready-To-Eat Foods: Guidance for Industry Draft Guidance, 2017).
- US, USDA-inspected foods: < 2 Log_{10} increase over the shelf life of the product (USDA FSIS *Listeria* Guideline, 2014).

In this study, for *Listeria monocytogenes,* with a target limit of absence in 25g with a maximum of 100 CFU/g, an increase of 1 log is considered as significant growth and the maximum tolerable growth assuming levels of this germ will never exceed 10 CFU/g at production level due to supplier controls.

IV. RELEVANT MICROORGANISMS

A conversion table from Celsius to Fahrenheit degrees is available in the appendix VIII.2

IV.1. LISTERIA MONOCYTOGENES

General characteristics

History

Listeria monocytogenes was identified as a significant microbiological hazard in food after large food-associated outbreaks were reported between 1980 and 1996 across Europe and North America. The 1981 Canadian outbreak that was traced back to cabbages used in coleslaw was what first spiked scientific interest (ICMSF 1996).

Taxonomy

Listeria are common environmental bacteria. The genus is comprised of at least 20 species, most of which have been identified in the last decade. The main pathogenic species of the genus is *Listeria monocytogenes* (Nwaiwu s. d.). *Listeria monocytogenes* is a gram-positive coccobacilli (short rods) (ICMSF 1996 ; Giaccone V., Ottaviani F. et al. 2007). It is a non sporing bacterium. Their size usually ranges around 0,4-0,5µm by 0,5-2µm, although they occasionally form cells up to 10µm in length (BIORISK2016SA0081Fi.pdf s. d. ; Allerberger 2003).

The disease

Symptoms

Listeriosis can be developed in two forms. A non-invasive form that usually goes undetected as it only presents with mild gastroenteritis, and an invasive form that can lead to permanent debilitation or even death, as it causes, in the worst cases, meningitis or septicemia (ICMSF 1996). Symptoms can appear 1 to 70 days after ingestion of a contaminated food, usually ranging between 1 to 4 weeks (CDC 2017).

Pathogenicity and virulence

Listeria monocytogenes enters all human cells and multiplies there in, inducing enzymatic lysis. This is why this bacterium has such a high virulence and can be very invasive. It is estimated that food contaminated at a dose in between 10^2 to 10^3 CFU/g up to 10^8 CFU/g can cause an episode of disease when ingested (Giaccone V., Ottaviani F. et al. 2007). According to these criteria, the WHO has set a safety threshold of 100 CFU/g in food (World Health Organization et FAO 2004 ; Guidelines | CODEXALIMENTARIUS FAO-WHO s. d.).
The dose-response relationship for this bacterium is highly dependent on an individual's defense mechanisms, including stomach acidity, and immune response (Rahman et al. s. d.). Natural intestine flora can also be a factor in dose-response.

Epidemiology

Tableau 1 : Incidence of listeriosis by category of population

Population at risk	Death rate	Severe symptoms	Incidence rate on French population 2015 number cases /100 000 people
Pregnant women	20-30% (fetus or baby)	Neonatal infection	14.6
Immunosuppressed	30-40%	Neurological after effect	5.9 – 47
Chronically ill	20-30%		0.67-18.8
Elderly (over 65)	30-40%		1.74
Immunocompetent	<5%	Rare bacteremia	0.13

(ICMSF 1996 ; Giaccone, V., Ottaviani F. et al. 2007 ; Ricci et al. 2018)

95% of listeriosis cases in humans are caused by the ingestion of contaminated food (Giaccone V., Ottaviani F. et al. 2007). *Listeria* is the 4[th] cause of death due to foodborne diseases in the WHO European region (50607-WHO-Food-Safety-publicationV4_Web.pdf s. d.). The number of reported listeriosis cases in the UE between 2014 and 2018 has shown a slight increase of around 400 cases/year (EFSA and ECDC 2019 - The European Union One Health 2018 Zoonoses Report).

Growth characteristics

General knowledge

Table 2 : *Listeria monocytogenes* growth limits

	Minimum	Optimum	Maximum
Temperature	-1°C	30-37	45
pH	4,3	7,0	9,4
Water activity	0,92	-	-

Listeria monocytogenes is a ubiquitous soil bacterium, it is found everywhere in the environment, particularly where animal feces can be found (wild or bred) (ICMSF 1996).
One of the main characteristics of *Listeria monocytogenes*, on top of its ability to grow at low temperatures, is its resistance to a high salt content. It can live on a product that has a concentration of up to 8-10% NaCl. Furthermore, as *Listeria monocytogenes* is facultatively anaerobic, food packaged under vacuum or nitrogen modified atmosphere are not immune to the survival or growth of the bacterium (Giaccone V., Ottaviani F. et al. 2007).
The acidity of the medium also plays a significant part in the growth of *Listeria monocytogenes,* with a pH level under 5, at low temperatures, the bacterium will, in most cases, show no growth (MRV Microbial Responses Viewer s. d.) The regulatory criteria for *Listeria monocytogenes* varies according to the type of food. In Europe, most foods that have a pH of 4.4 or less and an Aw of 0,92 or less, or pH 5 Aw 0,94 need to have no more than 100 CFU/g of product (COMMISSION REGULATION (EC) No 2073/2005 s. d.). Other types of food need an absence of the bacterium in 25g of product (AFSCA, Avis11-2019_SciCom2018-17_listerialaitcrubeurre_000.pdf s. d.).

Influence of storage

Listeria monocytogenes is capable of growth at low temperatures, however it has been shown that growth rates are significantly lower in products stored at appropriate refrigeration temperature (4°C or under) than at abuse temperature (over 4°C) (Colás-Medà et al. 2017). If the cold chain breaks, it is likely that the growth rate picks up again in the following hours (Kurpas, Wieczorek, et Osek 2018).

Concerns for the food industry and critical food types

The bacterium is widespread in the environment, and therefore can impact nearly any food industry from raw ingredients (meat, milk, vegetable…). Furthermore, it is a bacterium that has the ability to grow at low, fridge-like temperatures from -1°C to 4°C (Giaccone V., Ottaviani F. et al. 2007).
However, *Listeria monocytogenes* is sensitive to heat and inactivated at 72°C for 15 seconds or equivalent time/ temperature combination (ICMSF 1996).
One main food category of concern is prepacked, raw, ready to eat food such as raw cut fruits, unpasteurized cheeses or milk, salads… because no heat treatment is applied and refrigeration of the product will not stop growth if there is an initial contamination.
The second food category of concern is ready to eat food that has been exposed to contamination after inactivation treatments. These products have been identified as cooked meat products, smoked fish, soft/semi soft and fresh cheeses…(Koutsoumanis et al. 2021).

Raw, ready to eat food

As *Listeria monocytogenes* is an environmental bacterium, contamination for these types of products can occur pre-harvest or during harvest. Because there is no inactivating treatment for these products, the bacterium can multiply until consumption and an initial contamination that would not be harmful, can lead to contamination levels above 10^3 and cause listeriosis (MARIK et al. 2019).
Some fruits and vegetables are more likely to be contaminated by the bacterium. Celery, cabbage ad melons are some of the preferred media of the bacterium. Moisture, topography, nutrient availability, and microflora are the main factors in *Listeria* growth. It also can be noted that fruits and vegetables that have been cut or damaged are more prone to contamination. It has been demonstrated that the bacterium targets these areas (Ziegler et al. 2019).

Ready to eat food contaminated after the inactivation treatments

Initial contamination for these products is often an effect of cross-contamination. For meat products for example, if the bacterium can be found in the processing environment, contamination post inactivating treatment is likely to occur (Kurpas, Wieczorek, et Osek 2018). The growth factors are similar to those on fruits and vegetables, with the addition that nitrites in cured meat can provide effective bacteriostatic activity against *Listeria* (BUCHANAN, STAHL, et WHITING 1989). Some of these products can also be re-heated until steaming hot to inactivate the bacterium (CDC 2019).

IV.2. STAPHYLOCOCCUS AUREUS

General characteristics

History

The significance of *Staphylococcus aureus* in food poisoning was first observed by Barber in 1914, from stocked, unrefrigerated, raw milk from a cow suffering mastitis (ICMSF 1996). Staphylococcal food poisoning is one of the most common foodborne diseases in the world (Hennekinne, De Buyser, et Dragacci 2012). Instances of outbreaks across Europe (Italy, 2015) (Ercoli et al. 2017) and the US (CDC 2012 - Outbreak of Staphylococcal Food Poisoning from a Military Unit Lunch Party — United States, July 2012 s. d.) have been observed in the last decade.

Taxonomy

Staphylococcus are gram-positive, cocci. The cells are 0.5 to 1 µm in size, and can be single, paired or form “grape like” clusters (Jan McClure et al. 2007). They are non-motile, facultative aerobes. Among over 40 species of *Staphylococcus* (Hennekinne, De Buyser, et Dragacci 2012), certain species possess the ability to produce enterotoxins. These enterotoxins are low-molecular weight singe chain proteins. 18 serological types have been identified (Jan McClure et al. 2007). *Staphylococcus aureus* is capable of producing these toxins and is of greatest concern for the food industry amongst its species. Amongst the more common toxins secreted by *Staphylococcus aureus* are hemolysin, leukotoxin, exfoliative toxin, enterotoxin, and toxic-shock syndrome toxin-1 (TSST-1) (Kong, Neoh, et Nathan 2016).

The disease

Symptoms

Staphylococcal food poisoning results from ingestion of the toxins produced during growth of the bacterium. Toxin ingestion causes the typical symptoms of food poisoning such as nausea, vomiting, abdominal cramps, diarrhea… Symptoms usually appear 1 to 7 hours after ingestion and rarely last more than two days (Jan McClure et al. 2007). Because of this, Staphylococcal food poisoning goes massively unreported, as only around 10% of patients with confirmed cases will seek medical advice.

Pathogenicity and virulence

The mode of action of the toxin is believed to be the stimulation of local neuroreceptors in the intestinal tract. As *Staphylococcus aureus* is not particularly invasive, it can still spread by causing lysis in a number of cell types (Jan McClure et al. 2007).The amount of toxins causing illness depends on the individual but 0.1 to 1 µg/kg will generally cause illness in a human (ICMSF 1996). This amount of toxin is generally produced when *Staphylococcus aureus* reaches around 10^5-10^8 CFU/g in the stool of patients (Pinchuk, Beswick, et Reyes 2010 ; Jan McClure et al. 2007). The critical safety limit for this bacterium in food is set at 100,000 CFU/g (EUR-Lex - 02005R2073-20140601 - EN - EUR-Lex s. d.) while process criteria range most commonly from 100 to 1000 CFU/g.

Epidemiology

Table 3 : *S. aureus* risk and virulence by population category

Population at risk	Death rate	Severe symptoms
Healthy adult	0.03%	Dehydration
Elderly, children, immunocompromised	4.4%	Dehydration

Although there is no real population at risk as the effects of Staphylococcal food poisoning depends mostly on the individual’s weight and exposure, certain categories of the population like the elderly, children or people with chronic illness are more prone to show symptoms (Jan McClure et al. 2007).

Growth characteristics

General knowledge

Staphylococcus aureus is ubiquitous, occurring in the membranes and skin of warm blooded animals, including all food animals and humans. At all times, up to 50% of humans can be carriers of the bacterium. Often in nasal or perineal passage but also; more rarely, on hands or other parts of the body. The bacterium is able to grow at low Aw and high salt content (Up to 10%) (ICMSF 1996).

Table 4 : *S. aureus* growth limits

	Minimum	Optimum	Maximum
Temperature	6°C	35-41°C	48°C
pH	4	6-7	10
Water activity	0.83	0.99	0.99

Table 3 : *S. aureus toxins production limits*

	Minimum	Optimum	Maximum
Temperature	10°C	34-40°C	45°C
pH	0	0-4	10
Aw	0.86	0.99	0.99

(Anses s. d.)

Influence of storage

Toxin formation in *Staphylococcus aureus* is unlikely at temperatures lower than 10°C, making the maintenance of the cold chain a crucial aspect of keeping food safe from this bacterium. Exposure of more than 12 hours at temperatures between 10°C and 21°C, and greater than 3 hours above 21°C can result in toxin formation (FDA. Fish and Fishery Products Hazards and Controls Guidance s. d.). A study indicated abrupt growth/no growth interfaces occurred at low levels of temperature, pH and A_w. At 8 °C, *Staphylococcus aureus* grew only at optimum levels of pH and A_w while at temperatures above 13 °C, growth of *Staphylococcus aureus* was observed at pH = 4.5 and A_w = 0.96 (13°C), 0.941 (16°C) and 0.915 (19°C). The optimal pH at which growth of *Staphylococcus aureus* was detected earlier was 6.5. However, a slight decrease of the probability of growth was noticed in the pH interval of 7.0–7.5 at more stringent conditions (Valero et al, 2009).

Concerns for the food industry and critical food types

The bacterium is carried by healthy humans; therefore, cross contamination is likely in the food industry when human manipulation is involved. It is also carried by food animals, making contamination from the raw animal's ingredients source a possibility. *Staphylococcus aureus* competes poorly with other bacteria contaminating raw products. But it can grow at very low Aw. (ICMSF 1996). The bacterium is sensitive to heat and can be inactivated by 2 minutes at 70°C or equivalent tie-temperature combination (Kennedy et al. 2005). However, the toxins are very heat resistant and will not be affected by the treatment, rendering illness likely for the consumer if heat treatment is applied too late (Anses s. d.).

According to all these criterias, the main concern for the food industry is food from animals (milk, cheese, meat) and food that has been precooked, handled, packaged and then stocked for a few hours (3 hours to a few days depending on temperature) before consumption.

Food from animal origin

As an animal can be a carrier of *Staphylococcus aureus*, contamination can happen directly from the animal at the first production stage (slaughtering…) (EFSA 2012 - Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant Staphylococcus aureus in food-producing animals and food 2012). In processed meat products, *Staphylococcus aureus* growth can be slowed or stopped with the use of modified atmosphere or nitrites (CASTILLEJO-RODRÍGUEZ et al. 2002).
In cheese products manufactured with raw milk, the majority of the growth of *Staphylococcus aureus* at abuse conditions occurs within the first 6 hours of storage after inoculation and quickly reaches its maximum potential at around 24 hours (DELBES et al. 2006).

Pre-cooked food

Staphylococcal food poisoning occurs mostly by ingestion of food that has been cooked, contaminated by a person and then kept under warm conditions (20-40°C) for several hours (ICMSF 1996). As shown by various studies, at temperatures under 8°C, even after contamination of a pre-cooked product, most cases show no growth (potato salad and tuna), as with storage temperatures above 47°C (Huang 2015 ; Wu et Su 2014).

IV.3. CLOSTRIDIUM PERFRINGENS

General characteristics

History

Clostridium perfringens was associated with diarrhea as early as 1895. The role of this bacterium in foodborne illnesses was recognized in 1943 (ICMSF 1996). From 1998 to 2010 in the US, 289 confirmed outbreaks of foodborne illnesses where attributed to *Clostridium perfringens* (Grass, Gould, et Mahon 2013).

Taxonomy

Clostridium perfringens is a gram positive, square-ended, large (1 to 1.5 µm in diameter) anaerobic bacillus. It is a member of the bacillaceae family. The *Clostridium* genus comprises at least 12 lineages (Johnson 2009).
Clostridium perfringens produces at least 14 different toxins as a species but an individual cell will only produce a defined subset of these toxins. Types A, C and D affect humans (Vernozy-Rozand et al. 2007). Food poisoning cases almost always involve Type A toxins and to a lesser extent, type C (ICMSF 1996).

The disease

Symptoms

Type A toxins causes general symptoms of food poisoning such as vomiting, diarrhea, abdominal cramps… Symptoms appear 8 to 24 hours after consumption and recovery is achieved after 24 to 48 hours. As a result of dehydration, death can occur in weaker patients.
Type C toxins requires 5-6 hours before sudden and severe symptoms such as abdominal pain and diarrhea occur. It is generally followed by necrotic inflammation of the small intestine (also known as Darmbrand or Pigbel syndrome). This illness is much more likely to cause death of the patient, and it occurs in 15-25% of cases (Vernozy-Rozand et al. 2007).

Pathogenicity and virulence

As *Clostridium perfringens* is particularly sensitive to stomach acidity, the food ingested has to be contaminated to a high level in order to allow contamination of the host. From investigated outbreaks, around 10^8 vegetative cells/serving are necessary to cause symptoms. The vegetative cells that survive stomach acidity have the ability to sporulate in the intestine. The mother cell undergoes lysis, freeing the spores and releasing the toxin. The toxin released is then converted to a more active toxin by its environment, binds to cell receptors and creates pores in their membranes, causing the disease (Vernozy-Rozand et al. 2007 ; ICMSF 1996).

Epidemiology

Clostridium perfringens is a serious hazard representing up to 17% of all foodborne diseases confirmed cases in France in 2015 (Anses, 2017 s. d.). The A strain is the second most common cause of bacterial food poisoning in the US. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4383721/ Type C is less common but far more dangerous as it has a mortality rate of 15-25% in patient that are diagnosed with the illness. No group of people is more sensitive to the bacterium (Vernozy-Rozand et al. 2007).

The commission regulation for microbiological criteria on foodstuff does not set a limit on the presence of *Clostridium perfringens* (EUR-Lex - 32005R2073 - EN - EUR-Lex s. d.). However, most countries have 10^5 CFU/g of product set as the safety criteria upper limit (Vernozy-Rozand et al. 2007).

Growth characteristics
General knowledge

Table 1 : *C. perfringens* growth limits

	Minimum	Optimum	Maximum
Temperature	10	40-45	52
pH	5	6-7	8,3
Water activity	0.95	0.99	-

(Anses, 2017 s. d.)

Clostridium perfringens is a ubiquitous bacterium, it can be found in soil, water, dust, the surface of vegetables and the environment. Healthy humans and animals can be carriers in their intestinal tract, shedding up to 10^3 CFU/g of feces. This bacterium can withstand up to 6,5% NaCl in its growth medium (Anses, 2017 s. d.).

Vegetative cells have an optimum growth temperature of 40-45°C and inactivate at 60°C for 5 minutes**.** Spores are heat resistant, but will significantly decrease at temperatures 90°C to 100°C for 10-30 minutes (Vernozy-Rozand et al. 2007 ; Talukdar et al. 2016). Sporulation is essential to *Clostridium perfringens* pathogenicity, as the production of enterotoxin type A (the most common), occurs during the sporulated state (Li et al. 2016).

Influence of storage
During storage, it is important to prevent the growth of vegetative cells and sporulation, as these are the two forms of the bacterium that can cause illness later on. In a study on pork meat, no growth of *Clostridium perfringens* was observed for a period of 21 days at 10-12°C. In Ham, no growth was observed for 21 days at 17°C, going against the assumption that growth occurs from 15°C and above. Both studies where performed after heat treatment of the products and appropriate cooling technique (Juneja, Huang, et Thippareddi 2006). This proves that product characteristics also play a role in adequate product conservation. In fact, appropriate use of a combination of sodium tripolyphosphate, sodium lactate and NaCl can effectively prevent growth even in optimum conditions of 47°C for 24 hrs (Huang, Li, et Hwang 2018).

Concerns for the food industry and critical food types
Clostridium perfringens is a bacterium that is found in soil, dust, vegetation and the intestinal tract of humans and animals. It can be found in a wide variety of food, raw, dehydrated or cooked (ICMSF 1996). However, meat and carcasses are the most common vectors of the disease. It has been shown that about 50% (30-80%) of raw, frozen meat and poultry contain *Clostridium perfringens* (Vernozy-Rozand et al. 2007). Although a few cases of outbreaks have been reported on other types of food such as spinach or bean curd, these occurrences are rarer (8% of the reported outbreaks) (Grass, Gould, et Mahon 2013) (EFSA 2013 - Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations).

Meat is often vacuum packed in order to prevent the growth of bacteria. Unfortunately, *Clostridium perfringens* shows growth in vacuumed environments (Hassanien-Faten et al. 2014). That and its ability to form spores and increase its heat resistance make the bacterium a real threat to the meat industry. Heat treatment above 70°C immediately before consumption will kill *Clostridium perfringens* vegetative cells and inactivate the pre-existing toxins (Vernozy-Rozand et al. 2007). Spores have a higher heat resistance and will only be inactivated at temperatures of 90-100°C for 10-30 minutes (Talukdar et al. 2016). The FDA Food Code dictates that potentially hazardous cooked foods such as meats should be cooled from 60 to 21 °C within 2 h, and from 60 to 5 °C within 6 h (FDA 2020). In U.K., it is recommended that uncured cooked meats be cooled from 50 to 12 °C within 6 h and from 12 to 5 °C within 1h (Juneja, Huang, et Thippareddi 2006).

Meat Based Product
As stated before, meat products (raw, cooked and cured) are the most commons source of outbreaks. Studies have shown that the better mean of conservation for these products is to keep an internal temperature of 5-10°C as to restrict growth of the bacterium. Reports of *C. perfringens* vegetative cell growth on a 3 day period at 12-15°C show the importance of cold storage (Juneja, Huang, et Thippareddi 2006).

IV.4. BACILLUS CEREUS

General characteristics

History

Bacillus cereus has been identified more than a century ago, in the early 1900s. It is well known as a foodborne pathogen and has caused significant outbreaks over the years (ICMSF 1996). Annual reports of the European Food Safety Authority (EFSA) show that “bacterial toxins other than *Clostridium botulinum*”, including *Bacillus cereus*, generally account for 16–20% of food-poisoning outbreaks. From 2011–2015, 220–291 annual outbreaks associated with *B. cereus* were reported in several member states, which accounted for approximately 3.9–5.5% of all annual food poisoning outbreaks (Jessberger et al. 2020).

Taxonomy

Bacillus cereus is a Gram positive, spore-forming and facultative anaerobic rod. The genus is divided into three groups, based on spore and sporangium morphology. Group I, which includes *Bacillus cereus*, are defined as having a sporangium that is not swollen by the spore. It is one of the larger one in the group, with a size of over 0.9 µm in diameter (ICMSF 1996).

The disease

Symptoms

There are two form of *Bacillus cereus* food poisoning. The first is characterized by diarrhea occurring 8-24 hours after ingestion of a large number of cells or toxin, which is normally not severe and subsides within 24 hours. The second form of the disease is characterized by emesis occurring within 1 to 6 hours of consumption, with recovery taking 12-24 hours. None of the two forms should be considered life threatening for a healthy individual (ICMSF 1996).

Pathogenicity and virulence

Toxins are produced during the exponential phase of growth. *Bacillus cereus* produces two main toxins that cause illness. The diarrhoeagenic toxin that causes vascular permeability and is toxic to Vero cells and the emetic (cereulide) toxin.
Bacillus cereus outbreaks are often associated with foods that have a concentration of over 10^5 CFU/g, but there have been cases of food poisoning from foods contaminated at 10^3 CFU/g (EFSA 2016 - Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs 2016). Emesis can occur at toxin concentrations of 5-10 µg/kg of body mass. Such concentrations can be reached at around 10^6 CFU/g of product ingested (ANSES, 2021 s. d.).

Epidemiology

The real number of cases per year is difficult to obtain because the outbreaks go massively unreported. Indeed, the disease is rarely life threatening and symptoms only last for a short period of time, leading few patients to seek medical help.
No category of the population is more sensitive to this bacterium, although some severe cases were found in people with Crohn’s disease or in premature babies, although no correlation can be definitively made (ANSES, 2021 s. d.).
Because the contamination levels have to be high to cause illness, the safety criteria on *Bacillus cereus* in food is at 100,000 CFU/g of product while process criteria commonly used by professionals reach from 100 to 1000 CFU/ (COMMISSION REGULATION (EC) No 2073/2005 s. d.).

Growth characteristics

General knowledge

Spores of *Bacillus cereus* are found in soil at concentrations of the order of 10^4 to 10^5 spores per gram of soil. *Bacillus cereus* spores are also present in the digestive tracts of warm-blooded animals. It is spread across the environment and therefore can be isolated from all categories of foodstuff. There is a significantly higher risk for prevalence in starchy foods (flour, rice, pastries…) and cooked, chilled foods (EFSA 2016 - Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs 2016). While vegetative *Bacillus cereus* cells can mainly be eliminated by mild heat treatment, spores are able to survive high temperatures, such as pasteurization or spray drying of milk. Due to this survival and adjacent outgrowth of the competing microflora, growth of B. cereus occurs more often in pasteurized than in raw milk.

Table 5 : *Bacillus cereus* growth limits

	Minimum	Optimum	Maximum
Temperature	4°C	30-37°C	55°C
pH	4.3	6-7	9.3
Water activity	0.92	0.99-1	-
NaCl (g/L)	-	-	50

(ANSES, 2021 s. d.)

Table 6 : Emetic toxin production limits

	Emetic toxin	
	Min	**Max**
Temperature	10°C	40°C
pH	2	9
NaCl	0%	5%
CO_2	0%	40-50%

(Finlay, Logan, et Sutherland 2000 ; ANSES, 2021 s. d.)

Table 7 : Toxins inactivation

	Emetic toxin	Diarrhoeagenic toxin
Time/ Temperature of inactivation	90 min/ 126°C	30 min/ 56°C
pH	2-11	No data

(Dietrich et al. 2021)

The destruction of the sporulated form of *Bacillus cereus* is obtained at 105°C for 1 minute in soy (Ryang et al. 2016).
Bacillus cereus is divided in seven genetic groups; each of them has a different heat tolerance and pathogenicity. Strains that have the ability to grow at low temperature (5-9°C) are not usually involved in foodborne outbreaks (ANSES, 2021 s. d.).
In summary, the foodstuffs which favor *Bacillus cereus* survival, spore germination and outgrowth are those with suitable pH value (approximately 5–7.5), Aw value (minimum approximately 0.91–0.95), little or no competing microflora, which are additionally improperly heated or stored (Jessberger et al. 2020).

Influence of storage

Although growth is unlikely to cause illness under 10^5 CFU/g, indication of growth and possible toxin production at cool temperatures (10-12°C) testify to the fact that refrigeration is necessary to stop food poisoning by this bacterium.
Studies have shown germination and growth (up to 100 CFU/g or ml food) of psychrotolerant members at low temperatures (4–10 ◦C) during transport and storage. In this context, it was shown that fatty acids from foods enhance growth of *Bacillus cereus* under cold and anaerobic conditions (Jessberger et al. 2020).

Concerns for the food industry and critical food types

As *Bacillus cereus* sporulates and creates a heat resistant toxin, its inactivation is a hard process as a long and harsh heat treatment can modify the aspect or taste of food.
Moreover, some of the strains of this bacterium can grow at a wide range of temperatures close to 6°C, and up to 50°C. The vegetative cells are sensitive to heat treatment but it will not prevent illness if the toxin is already formed or if there is presence of sporulated cells (EFSA 2016 - Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs 2016).
Bacillus cereus is wide spread in the environment, which helps cross contamination. A wide range of foods can be contaminated by this bacterium. It is mostly present in foods that have already undergone a heat treatment, as it doesn't perform well when competing with other bacteria, but can survive heat treatment and colonize afterwards. In regards to this, pasteurized milk products, dried ingredients (flours, herbs, spices) and improperly chilled pre-cooked foods are the most common sources of outbreaks (ANSES, 2021 s. d.).

The European Food safety agency states that growth to numbers representing a hazard is limited by refrigeration. Below 10°C, only a minority of the strains present in a food product will be able to grow. According to the same report, no emetic toxin producing cells have been identified to grow below 10°C, and no diarrhoeagenic toxin producing cells have been identified to grow at less than 7°C (EFSA 2005 - Opinion of the Scientific Panel on biological hazards (BIOHAZ) on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs 2005).

IV.5. SALMONELLA SPP.

General characteristics

History

Salmonella was first reported in the 1900s as a contagious agent responsible for the ulceration of human intestinal tract (ICMSF 1996). It is to this day a great threat to the public and causes outbreaks of foodborne diseases. In 2018, EU Member States reported 5146 *Salmonella* foodborne outbreaks affecting 48365 people, it is the most common cause for foodborne outbreaks (ECDC 2019 - *Salmonella* the most common cause of foodborne outbreaks in the European Union).

Taxonomy

Salmonella spp. is a generic term that currently include more than 2,541 serovars all pathogenic to humans. *Salmonella* belongs to the family *Enterobacteriaceae.* They are Gram negative, facultatively anaerobic, rod-shaped bacteria. The genus consists of two species *S. enterica* and *S. bongori* (2,519 and 22 serovars respectively) (D'Aoust et al. 2007). In the *S. enterica* species, *S.* Typhimurium is one of the most invasive and virulent along with *S.* Paratyphi (McWhorter et Chousalkar 2015 ; Swearingen et al. 2012).

The disease

Symptoms

The symptoms of *Salmonella* Typhi / Paratyphi infection are known as enteric fever, 7-28 days following exposure to the pathogenic agent, diarrhea, spiking fever, abdominal pain and headache may appear lasting up to 30 days. Infection from other strains will develop symptoms 8-72 hours after exposure and be self-limiting in a period of 5 days. These symptoms are abdominal pain, diarrhea and slight fever (D'Aoust et al. 2007). In the worst cases, bacteremia or septicemia may appear and seriously endanger the life of patients or cause long lasting damage (ICMSF 1996).

Pathogenicity and virulence

Salmonella invades the lumen of the small bowel where they multiply, then the ileum and colon, causing an inflammatory reaction. Invasive strains penetrate the intestinal mucosa, lymphatic system and are engulfed by phagocytes within which they multiply. They then re-enter the blood stream causing septicemia (ICMSF 1996).

There are wide differences in infectivity associated with survival of the bacterium during transit through the stomach, the food associated with the outbreak, water ingested by the host and so on. The infectious doses ranges from 10^7 CFU/g to 1-100 CFU/g (ICMSF 1996).

Epidemiology

Table 8 *Salmonella* incidence by category of population

Population at risk	Death rate	Severe symptoms	Incidence rate in Europe 2017 (number cases/ 100.000 people)
Children (0-4)	0.25%	Bacteremia, septicemia	94.1
Children (5-14)			37
Immunocompetent			19.6

(ECDC 2017 - Salmonellosis-annual-epidemiological-report-2017.pdf s. d.)

Because the implications of the disease can be severe and the infectious doses very low, the European commission with the scientific advice of EFSA, stated that there should be an absence of *Salmonella* in 25g n=5 of food to meet safety criteria (COMMISSION REGULATION (EU) No 209/2013 s. d.).

Growth characteristics

General knowledge

Table 9 : *Salmonella growth limits*

	Minimum	Optimum	Maximum	Survival
Temperature	5°C	35-37°C	50°C	-23°C
pH	3.8	7-7.5	9.5	-
Water activity	0.94	0.99	0.99	0.3-0.5

Salmonella is capable of growth at high temperatures and extreme values of pH, and survives in very abusive conditions of freezing and low Aw.
The bacterium can be found in the intestinal tract of most animals (cattle, pigs, poultry…) and aquatic animals such as fish and shells. Food produced from animals is the most common type of food to be contaminated. However, the environment can be contaminated by animal feces, raw vegetables can also be a vector of the bacterium (Anses, 2017 s. d.).

Influence of storage

Studies on broth medium show *Salmonella* growth at 7°C and 10°C on a period of 6 days, but very little at 4°C (Morey et Singh 2012). These results corroborate a study on eggs, in which eggs held at 4°C showed less growth of the bacterium than those stored over 4°C (C. J. Kim et al. 1989). Multiple authors recommend that food should be stored at no more than 5°C to prevent *Salmonella* growth (MATCHES et LISTON 2006).

Concerns for the food industry and critical food types

Contamination to humans mostly occurs by consumption of raw or insufficiently cooked foods. Foodborne contamination is responsible for 95% of non-typhoidal strain case and 80% of typhoidal strain cases with *S.* Typhi and *S.* Paratyphi being a human to human contamination (Anses, 2017 s. d.).

Salmonella is a very resistant bacterium (freezing, very high and low pH, resistance to nitrite salts…) (Anses, 2017 s. d.). It is sensitive to heat over 65°C, a heat treatment of 65-80°C for 30-60 minutes will inactivate and kill the bacterium (J. Kim et al. 2012).

The products that are commonly exposed to contamination are raw meat, eggs and poultry as well as dairy products, raw fish and shells and lastly, raw vegetables. The same categories of product might be contaminated after heat treatment or being cooked (D'Aoust et al. 2007).
Exclusion of risk ingredients and food preparation practices such as undercooked meat or raw eggs is a key management measure for *Salmonella*.

Meat, poultry and eggs

In ground pork meat held at 10°C for 12 days, an increase of under 2 log of *Salmonella* was detected, the bacterium was undetectable after heat treatment, proving its efficiency against *Salmonella* (Wang et al. 2015). In liquid whole eggs, *Salmonella* growth is only detectable above 10°C (Y.-J. Kim et al. 2018).

Dairy

Salmonella behaves differently in different kinds of cheese: they survived in ripening Cheddar cheese for up to 7 month at 13°C and for 10 month at 7°C (El-Gazzar et Marth 1992). In brie cheese *Salmonella* increased at 20°C but declined at a slow rate during storage at 4°C and 8°C (Little et Knøchel 1994).

Fish and shell fish

The FDA stated that *Salmonella* is the most common contaminant in fish and fishery products. The minimum growth reported for strains of *Salmonella* that contaminate fish and shell fish is around 6.2°C, authors recommend these products should be transported at temperatures below 4.4°C for safety (Mahmoud 2012).

Vegetables

Even though vegetables are not the preferred medium of *Salmonella*, the bacterium is capable of growth, as shown by a study in which 2 log growth was detectable under 48 hours at room temperature storage (Wells et Butterfield 1999). Authors recommend these products should be kept at 4°C or less.

IV.6. ESCHERICHIA COLI STEC

General characteristics

History

E. coli has been identified over a century ago and by the 1940s, its role as an enteropathogen was firmly established (ICMSF 1996). *E. coli* STEC are *E. coli* that possess stx genes coding for Shiga toxins, they are also called verocytotoxin-producing *Escherichia coli* (Vernozy-Rozand et al. 2007). Although it is a well know bacterium, the scope of pathogenicity is still under discussion and it continues to cause major foodborne outbreaks as those due to fresh sprouts in Europe in 2011 (WHO 2011) or in ready to eat leafy greens in the US in 2020 (CDC 2020).

Taxonomy

E. coli are members of the family *Enterobacteriaceae*. They are Gram negative, facultatively anaerobic short rods (ICMSF 1996). The genus *Escherichia* includes five species. However, these are not all responsible for causing diseases.

The diarrhoeagenic *E. coli* are divided into seven pathotypes based on virulence traits and mechanism of pathogenicity and include the Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely Adherent *E. coli* (DAEC) and Adherent Invasive *E. coli* (AIEC).

The diarrhoeagenic *E. coli* are divided into seven pathotypes based on virulence traits and mechanism of pathogenicity and include the Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely Adherent *E. coli* (DAEC) and Adherent Invasive *E. coli* (AIEC).

Historically, Enterohaemorrhagic *E. coli* (EHEC) were considered a subset of STEC associated with haemorrhagic colitis. However, the EHEC terminology is now obsolete (Koutsoumanis et al. 2020).

STEC bacteria harbor the stx gene (stx1 or stx2) responsible for a toxin similar to the Shiga toxin, and also carry the eae gene (attaching/effacing) and form distinctive lesions on the surfaces of intestinal epithelial cells (Koutsoumanis et al. 2020).

In 2019, the five most commonly reported serogroups in Europe were O157, O26, O146, O103, and O91 (ECDC 2019 - Epidemiological Report-STEC-2019.pdf s. d.). In the US, the top five most commonly reported serogroups in 2016 were O157, O26, O103, O111 and O121 (CDC 2016 - National Enteric Disease Surveillance 2019).

The disease

Symptoms

The most common symptoms of the disease are hemorrhagic colitis or bloody diarrhea, fever and abdominal cramps, noted in 90% of cases. In children and elderly people, the symptoms can become more complicated with the development of Hemolytic Uremic Syndrome or thrombotic thrombocytopenic purpura in which red blood cells and platelets are destroyed, causing excessive blood clots (Vernozy-Rozand et al. 2007).

Pathogenicity and virulence

To have pathogenic activity, *E. coli* cells first need to survive stomach acidity. Then, a period of colonization of the digestive tract is necessary. STEC strains are capable of producing attaching-effacing lesions to assist in colonization. Then, the Shiga toxins have to cross the intestinal epithelium, and bind with the specific receptors of target cells (intestinal, renal and cerebral level) to block their protein synthesis and induce death (Vernozy-Rozand et al. 2007).

It is worth noting that some of the virulence factors for these strains have been acquired by genetic exchange through horizontal transfer, with bacteriophages playing an important role (Vernozy-Rozand et al. 2007).

An estimated dose-response has been made for O157:H7, of 5-10 CFU/g in cheese. In an outbreak of STEC O111:H- associated with fermented sausage, the estimated exposure dose was 1 cell per 10 g. This indicates that *E. coli* STEC can be pathogenic at very low doses of 1 CFU/g (Koutsoumanis et al. 2020).

Epidemiology

Table 10 *E. coli* STEC risk and virulence by population category

Population at risk	Death rate	Severe symptoms	Incidence rate Europe 2019 (100.000 plp)
Children (under 5)	5%	HUS	10.3
Children (6-10)			3
Elderly (over 65)			2.5
Immunocompetent	3%	HUS	2.2

(Anses, 2019 s. d. ; WHO 2018 ; ECDC 2019 - Epidemiological Report-STEC-2019.pdf s. d.)

Following the 2011 outbreak, the European commission with the scientific advice of EFSA, stated that there should be an absence of *E. coli* STEC in 25g, n=5 of food to meet safety criteria (COMMISSION REGULATION (EU) No 209/2013 s. d.).

Growth characteristics

General knowledge

Table 11 : *E. coli* STEC growth limits

	Minimum	Optimum	Maximum
Temperature	6	40	45
pH	4.4	6-7	9
Water activity	0.95-0.96	0.995	-

E. coli is capable of growth in a medium with a concentration of up to 8.5% NaCl. This bacterium is common in the intestinal tract of ruminant animals such as bovines. Other animals, like game can be healthy carriers for the bacterium. Soil, water and even crops can be cross contaminated by the animal's feces (Anses, 2019 s. d.).

Influence of storage

Various studies have shown that conditions of transport and storage of foodstuff do have an impact on the development of *E. coli* STEC (Leclair et al. 2019). Storage temperature has been identified as one of the main factors affecting growth, with a tendency to show no growth under 4°C, very little growth in between 5-9°C and significant growth above 15°C (study on 14 days) (Chauret 2011). In all products, heat inactivation at 70°C remains the most effective method to inactivate the bacterium.

Concerns for the food industry and critical food types

Because the main carrier for this bacterium is bovines and ovines, dairy products and meat from them is most likely to be contaminated. Crops watered with contaminated water can also cause outbreaks if their product is eaten raw (Anses, 2019 s. d.).
Although *E. coli* STEC is particularly resistant to acidic environments and quite resistant to salt, they are sensitive to heat treatments of 70°C for 2 minutes or equivalent time-temperature combination. Raw products and products contaminated post heat treatment are the main source of outbreaks (Vernozy-Rozand et al. 2007).

As the initial contamination to cause illness is very low and regulation on this bacterium in food is very strict, it is a main concern to the industries producing these types of food. In the European region the STEC food-borne disease burden was attributed to six food categories: beef was estimated to be the major food source, followed by dairy products and vegetables (Koutsoumanis et al. 2020).
Exclusion of risk ingredients and food preparation practices, such as undercooked meat, is a key management measure for *E coli* STEC.

Beef meat

In a study on ground beef, the minimal temperature for growth of this bacterium has been identified at 7.7°C (Hwang et Huang 2018). Some have also shown differences in growth according to the cut of beef (brisket and rump) but conclude that temperatures under 5°C are best to prevent growth and 5-10°C show little growth in a period of 14 days (Chauret 2011).

Dairy products

In raw unpasteurized milk, no change in the viable population was detected during 14 days with storage at 8°C for some strains, and multiplication from days 9 to 17 for other strains (Massa et al. 1999). In cheddar cheese made from unpasteurized milk and spiked with *E. coli* O157:H7, survival was observed for at least 120 days at 7°C (Chauret 2011). Authors recommend to keep dairy products at temperatures below 5°C.

Vegetables

In a field study, irrigation water containing various levels of *E. coli* O157:H7 was used to spray lettuce and spinach leaves. Storage temperature was shown to have a significant impact on the fate of *E. coli* O157:H7 on lettuce and spinach, with very little growth below 8°C, but moderate growth above 8°C and significant growth above 12°C (Chauret 2011).

V. PRODUCT CATEGORIES

In the document, 46 families are described according to the different categories of products composing the menus. More precisely, the categories were determined according to the type of dish (snack, meals, desserts…), the food processing (raw, cut, cooked, cured, ...), the ingredients (vegetables, animal origin products, starchy food…) and their intrinsic characteristics, including pH and Aw values. Please find in appendix the composition of the different families with examples of products and other pH and Aw values. Simulations in the next part of the document were conducted with the most sensitive and representative couple of pH/Aw of each family according to the collected data's. These families are listed below.

	FAMILY	TARGET PRODUCT	pH	Aw
1	Raw vegetables sliced seasoned	vegetables aïoli	6,25	0,997
2	Raw vegetables sliced unseasoned	avocado, beans	6,50	0,996
3	Cooked cold vegetables	roasted peppers	4,34	0,985
4	Meat delicatessen cured	cured meat, coppa	6,07	0,950
5	Meat delicatessen cooked	cooked ham	6,23	0,990
6	Cooked hot foods*	quiche lorraine (eggs, smoked bacon, cream)	6,37	0,983
7	Soups	vegetables soup	6,41	0,997
8	Salad with raw and/or cooked vegetables with starchy foods	napoli pasta salad	5,08	0,994
9	Mixed salads with raw vegetables and/or cooked vegetables with PAO**	rice vegetables surimi salad	4,94	0,992
10	Cooked cold food (other)	minced roasted chicken	6,22	0,992
11	Raw fish	fresh fish	6,80	0,997
12	Processed fish	cold smoked salmon	6,27	0,980
13	Cooked egg products	hard-boiled egg	7,60	0,970
14	Cold sandwiches	cheese sandwich	5,37	0,968
15	Cold sandwiches with cured meat	sandwich with Serrano ham	5,82	0,913
16	Cold sandwiches with cooked meat and fish (pork, poultry, beef, tuna...)	minced chicken sandwich	6,19	0,990
17	Cold sandwiches with raw vegetables	ciabatta veggie	5,18	0,973
18	Cooked dishes with PAO with starchy foods without vegetables*	hash Parmentier	6,27	0,990
19	Cooked dishes with PAO with starchy foods with vegetables*	Bolognaise pasta	5,58	0,990
20	Cooked dishes with PAO without starch without vegetables*	breaded fish	6,76	0,994
21	Cooked dishes with PAO without starch with vegetables*	veal blanquette	6,08	0,995
22	Cooked dishes without PAO with starchy foods without vegetables*	cooked rice	6,40	0,997
23	Cooked dishes without PAO with starchy foods with vegetables*	vegetables gratin	6,30	0,992
24	Cooked dishes without PAO without starch with vegetables*	mashed carrot	6,20	0,996
25	Fried products (to be reheated)	codfish accras	6,76	0,978
26	Cream or cooked egg product based sauces	hollandaise sauce	5,13	0,991
27	Cream or (raw) egg product based sauces	mayonnaise mustard sauce	3,70	0,997
28	Pasteurized cooked and uncooked pressed cheese	mozzarella	6,11	0,987
29	Pasteurised soft cheese, bloomy rind and washed rind	camembert	6,63	0,978
30	Pasteurised blue-veined cheese	blue-veined cheese (Fourme d'Ambert...)	6,87	0,966
31	Raw milk soft cheese, bloomy rind and washed rind	raw milk camembert	6,63	0,978
32	Raw milk blue-veined cheese	blue cheese (Roquefort …)	5,61	0,935
33	Fresh cheese	fresh cheese with Guerande salt	4,75	0,992
34	Fermented milk products	cottage cheese	4,60	0,991
35	Dairy desserts	vanilla cream	6,78	0,994
36	Fresh fruit salads and desserts	watermelon	5,60	0,987
37	Cooked fruit salads and desserts	cooked apple	3,39	0,987
38	Dried fruits and vegetables	dried apricots	4,30	0,750
39	Dried nuts and smoked seeds	almond slices	7,10	0,770
40	Pickled and vinegar-cured vegetables	pitted olives	5,20	0,970
41	Room temperature stable baked goods	coconut cake	6,20	0,860
42	Baked pastries with positive cold storage	morello cherry clafoutis	4,76	0,970
43	Unbaked pastries with fruit	raspberry bavarian cake	5,67	0,977
44	Uncooked pastries without fruit	chou chantilly pastry	6,55	0,987
45	Cocoa- chocolate and confectionery	milk chocolate chips	6,30	0,387
46	Fatty products	butter	6,40	0,904

* to be reheated ** PAO: Product of Animal Origin

VI. STORAGE RISK EVALUATION

Sym'Previus is a complete tool for microbiological data prediction. Recognized by the scientific community, it helps manufacturers to achieve food safety and quality.
Using the pH and Aw values collected per family, simulations will be based on worst case scenarios (most critical product). The use of predictive microbiology (Sym'Previus) allows a clear definition of growth / risk / no growth interface depending on reasonably foreseeable storage conditions.

The selected storage temperatures the most relevant are 5, 10, 15, 20, 25°C (41, 50, 59, 68, 77°F) and the storage time 4, 6, 10, 14, 18 and 24 hours. The temperature assumed in planes is 22°C (71,6°F), and for this case a temperature of 25°C (77°F) was chosen to be in extra security conditions. The Time Zero corresponds to the logistic start point.

To make an easier reading, the table below show the location of each family products according to the range of pH and Aw of the most critical product of the family (target product).

Families	pH							
Aw	**<3.5**	**3.5 - <3.9**	**3.9 - <4.2**	**4.2 - <4.6**	**4.6 - <5.0**	**5.0 - <5.4**	**5.4 - <6.0**	**>6.0 (7.0)**
<0.88				**38**				**39, 41, 45**
>0.88-0.90								
>0.90-0.92							**15**	**46**
>0.92-0.94							**32**	
>0.94-0.96								**4**
>0.96 (0,99)	**37**	**27**		**3**	**33, 34, 42, 9**	**8, 14, 17, 26, 40**	**19, 36, 43**	**1, 2, 5, 6, 7, 10, 11, 12, 13, 16, 18, 20, 21, 22, 23, 24, 25, 28, 29, 30, 31, 35, 44**

VI.1. GROWTH SIMULATION ACCORDING TO THE STORAGE TIME

Methodology

The growth simulations are carried out on Sym'Previus' tool for 4 microorganisms (*Listeria monocytogenes*, *Staphylococcus aureus, Bacillus cereus,* and *Clostridium perfringens*).
Salmonella spp. and *E. coli* have been excluded because with a target limit "absence in 25g", no simulation will be applied considering the acceptable value doesn't include quantitative approach. Exclusion of risk ingredients or food preparation practice is the effective risk management measure.

The simulations are carried out and the results obtained are expressed according to the following methodology:
- The simulations are carried out during a storage time of 24 hours. For each table of results, only the storage temperatures are modified (5°C, 10°C, 15°C, 20°C and 25°C respectively / 41, 50, 59, 68, and 77°F respectively)
- The simulations are obtained with variable pH and Aw conditions defined by value intervals; the simulations are carried out with the highest bounds of each pH and Aw interval (noted in blue in the results tables)
- The results retained are the highest values indicated by the growth simulations (upper bounds of the 90% confidence band), which positions the results in the highest risk cases
- The observed values are represented by the following colour code: green for simulations for which no growth is observed, orange for growths that do not reach the previously defined risk level (1 log for *Listeria monocytogenes* and 2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens*). For "red" results, the boxes indicate the length of time observed to reach the defined risk level.

VI.1.1. LISTERIA MONOCYTOGENES

Rules of judgment	
	No growth during 24H
	Growth <1 log for *Listeria monocytogenes* and <2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H
	Growth >1 log for *Listeria monocytogenes* and >2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H, with the time observed to reach the defined risk level

Simulation for *Listeria monocytogenes* : growth at 5°C (41°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Listeria monocytogenes* : growth at 10°C (50°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								14H

Simulation for *Listeria monocytogenes* : growth at 15°C (59°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						21H	16H	13H
>0.96 (0.99)					17H	11H	9H	7H

Simulation for *Listeria monocytogenes* : growth at 20°C (68°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94							20H	17H
>0.94-0.96					17 H	13H	9H	8H
>0.96 (0.99)				20H	10H	7H	6H	5H

Simulation for *Listeria monocytogenes* : growth at 25°C (77°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94						18 H	13H	12H
>0.94-0.96				19H	11H	8H	7H	6H
>0.96 (0.99)				11H	7H	5H	4H	3H

VI.1.2. STAPHYLOCOCCUS AUREUS

Rules of judgment	
	No growth during 24H
	Growth <1 log for *Listeria monocytogenes* and <2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H
	Growth >1 log for *Listeria monocytogenes* and >2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H, with the time observed to reach the defined risk level

Simulation for *Staphylococcus aureus* : growth at 5°C (41°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Staphylococcus aureus* : growth at 10°C (50°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Staphylococcus aureus* : growth at 15°C (59°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								23H
>0.96 (0.99)							23H	19H

Simulation for *Staphylococcus aureus* : growth at 20°C (68°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								21H
>0.92-0.94							18H	15H
>0.94-0.96						19H	14H	12H
>0.96 (0.99)					23H	16H	12H	10H

Simulation for *Staphylococcus aureus* : growth at 25°C (77°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								20H
>0.9-0.92						21H	15H	12H
>0.92-0.94					21H	15H	11H	9H
>0.94-0.96					17H	12H	11H	7H
>0.96 (0.99)					14H	12H	7H	6H

VI.1.3. CLOSTRIDIUM PERFRINGENS

Rules of judgment	
	No growth during 24H
	Growth <1 log for *Listeria monocytogenes* and <2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H
	Growth >1 log for *Listeria monocytogenes* and >2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H, with the time observed to reach the defined risk level

Simulation for *Clostridium perfringens* : growth at 5°C (41°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Clostridium perfringens* : growth at 10°C (50°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Clostridium perfringens* : growth at 15°C (59°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Clostridium perfringens* : growth at 20°C (68°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								21H
>0.96 (0.99)							24H	13H

Simulation for *Clostridium perfringens* : growth at 25°C (77°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96							16H	9H
>0.96 (0.99)							10H	6H

VI.1.4. BACILLUS CEREUS

The germs selected for the growth simulation are Group III *Bacillus cereus*, which are the cytotoxic germs with the best growth characteristics among all the *Bacillus cereus* proposed by the Sym'Previus software.

Rules of judgment	
	No growth during 24H
	Growth <1 log for *Listeria monocytogenes* and <2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H
	Growth >1 log for *Listeria monocytogenes* and >2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – during 24H, with the time observed to reach the defined risk level

Simulation for *Bacillus cereus* : growth at 5°C (41°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Bacillus cereus* : growth at 10°C (50°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Bacillus cereus* : growth at 15°C (59°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)						24H	16H	15H

Simulation for *Bacillus cereus* ; growth at 20°C (68°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						22H	14H	12H
>0.96 (0.99)					18H	10H	8H	7H

Simulation for *Bacillus cereus* : growth at 25°C (77°F)

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						12H	8H	7H
>0.96 (0.99)					10H	6H	4H	4H

VI.2. GROWTH SIMULATION ACCORDING TO THE STORAGE TEMPERATURE

Methodology

The methodology is the same as in Chapter VI.I, but in this case the results are obtained at a given temperature (with a maximum temperature of 25°C / 77°F) and the storage times vary (4H, 6H, 10H, 14H, 18H and 24H respectively). In these tables, growth simulation results that are zero or below the defined risk level are shown in yellow. The red boxes always represent growth simulation results exceeding the previously defined risk levels, the temperature indicated in these red boxes, indicate the minimum temperature for which this risk level is exceeded.

Rules of judgment	
(yellow)	Growth ≤ 1 log for *Listeria monocytogenes* and ≤ 2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* at this temperature for a maximum of 25°C / 77°F
(red)	Growth >1 log for *Listeria monocytogenes* and >2 log for *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* – at 25°C / 77°F, with the minimal T°C to reach the defined risk level

A conversion table from Celsius to Fahrenheit degrees is available in the appendix VIII.2

VI.2.1. LISTERIA MONOCYTOGENES

Simulation for *Listeria monocytogenes* during storage 4 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)							25°C	25°C

Simulation for *Listeria monocytogenes* during storage 6 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								25°C
>0.96 (0.99)						25°C	20°C	20°C

Simulation for *Listeria monocytogenes* during storage 10 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						25°C	20°C	20°C
>0.96 (0.99)					20°C	20°C	15°C	15°C

Silliker SAS - Mérieux NutriSciences, 25 Boulevard de la Paix – CS 38512 95891 CERGY-PONTOISE cedex, FRANCE
Tel. 0820 56 20 30* - Fax 01 34 41 13 11 www.mérieuxnutrisciences.com
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Simulation for *Listeria monocytogenes* during storage 14 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94							25°C	25°C
>0.94-0.96					25°C	20°C	20°C	15°C
>0.96 (0.99)				25°C	20°C	15°C	15°C	10°C

Simulation for *Listeria monocytogenes* during storage 18 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94						25°C	25°C	20°C
>0.94-0.96					20°C	20°C	15°C	15°C
>0.96 (0.99)				25°C	15°C	15°C	15°C	10°C

Simulation for *Listeria monocytogenes* during storage 24 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94						25°C	20°C	20°C
>0.94-0.96				25°C	20°C	15°C	15°C	15°C
>0.96 (0.99)				20°C	15°C	15°C	15°C	10°C

VI.2.2. STAPHYLOCOCCUS AUREUS

Simulation for *Staphylococcus aureus* during storage 4 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Staphylococcus aureus* during storage 6 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								25°C

Simulation for *Staphylococcus aureus* during storage 10 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								25°C
>0.96 (0.99)							25°C	20°C

Simulation for *Staphylococcus aureus* during storage 14 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								25°C
>0.92-0.94							25°C	25°C
>0.94-0.96						25°C	20°C	20°C
>0.96 (0.99)					25°C	25°C	20°C	20°C

Simulation for *Staphylococcus aureus* during storage 18 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92							25°C	25°C
>0.92-0.94						25°C	20°C	20°C
>0.94-0.96					25°C	25°C	20°C	20°C
>0.96 (0.99)					25°C	20°C	20°C	20°C

Simulation for *Staphylococcus aureus* during storage 24 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								25°C
>0.9-0.92						25°C	25°C	20°C
>0.92-0.94					25°C	25°C	20°C	20°C
>0.94-0.96					25°C	25°C	20°C	15°C
>0.96 (0.99)					20°C	20°C	15°C	15°C

VI.2.3. CLOSTRIDIUM PERFRINGENS

Simulation for *Clostridium perfringens* during storage 4 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								

Simulation for *Clostridium perfringens* during storage 6 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)								25°C

Simulation for *Clostridium perfringens* during storage 10 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								25°C
>0.96 (0.99)							25°C	25°C

Simulation for *Clostridium perfringens* during storage 14 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								25°C
>0.96 (0.99)							25°C	20°C

Simulation for *Clostridium perfringens* during storage 18 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96							25°C	25°C
>0.96 (0.99)							25°C	20°C

Simulation for *Clostridium perfringens* during storage 24 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96							25°C	20°C
>0.96 (0.99)							20°C	20°C

VI.2.4. BACILLUS CEREUS

Simulation for *Bacillus cereus* during storage 4 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)							25°C	25°C

Simulation for *Bacillus cereus* during storage 6 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96								
>0.96 (0.99)						25°C	25°C	25°C

Simulation for *Bacillus cereus* during storage 10 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96							25°C	25°C
>0.96 (0.99)						20°C	20°C	20°C

Simulation for *Bacillus cereus* during storage 14 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						25°C	20°C	20°C
>0.96 (0.99)					25°C	20°C	20°C	20°C

Simulation for *Bacillus cereus* during storage 18 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						25°C	20°C	20°C
>0.96 (0.99)					20°C	20°C	15°C	15°C

Simulation for *Bacillus cereus* during storage 24 H

	pH							
Aw	3.5	3.5-<3.9	3.9-<4.2	4.2-<4.6	4.6-<5	5-<5.4	5.4-<6	>6 (7)
<0.88								
>0.88-0.90								
>0.9-0.92								
>0.92-0.94								
>0.94-0.96						20°C	20°C	20°C
>0.96 (0.99)					20°C	15°C	15°C	15°C

VII. RESULTS PER PRODUCTS FAMILY

A summary is presented based on the most critical combinations of pH scale (from below 3,0 to 8,0), Aw scale (from 0,36 to 1) and most critical microorganisms identified. If required, a selected area is zoomed.

Synthetic presentation includes 4 scenarios of time/temperature with product families (results linked to source data) and the most sensitive microorganisms for each time-temperature combination.
The 4 targeted time/temperature scenarios are the following:

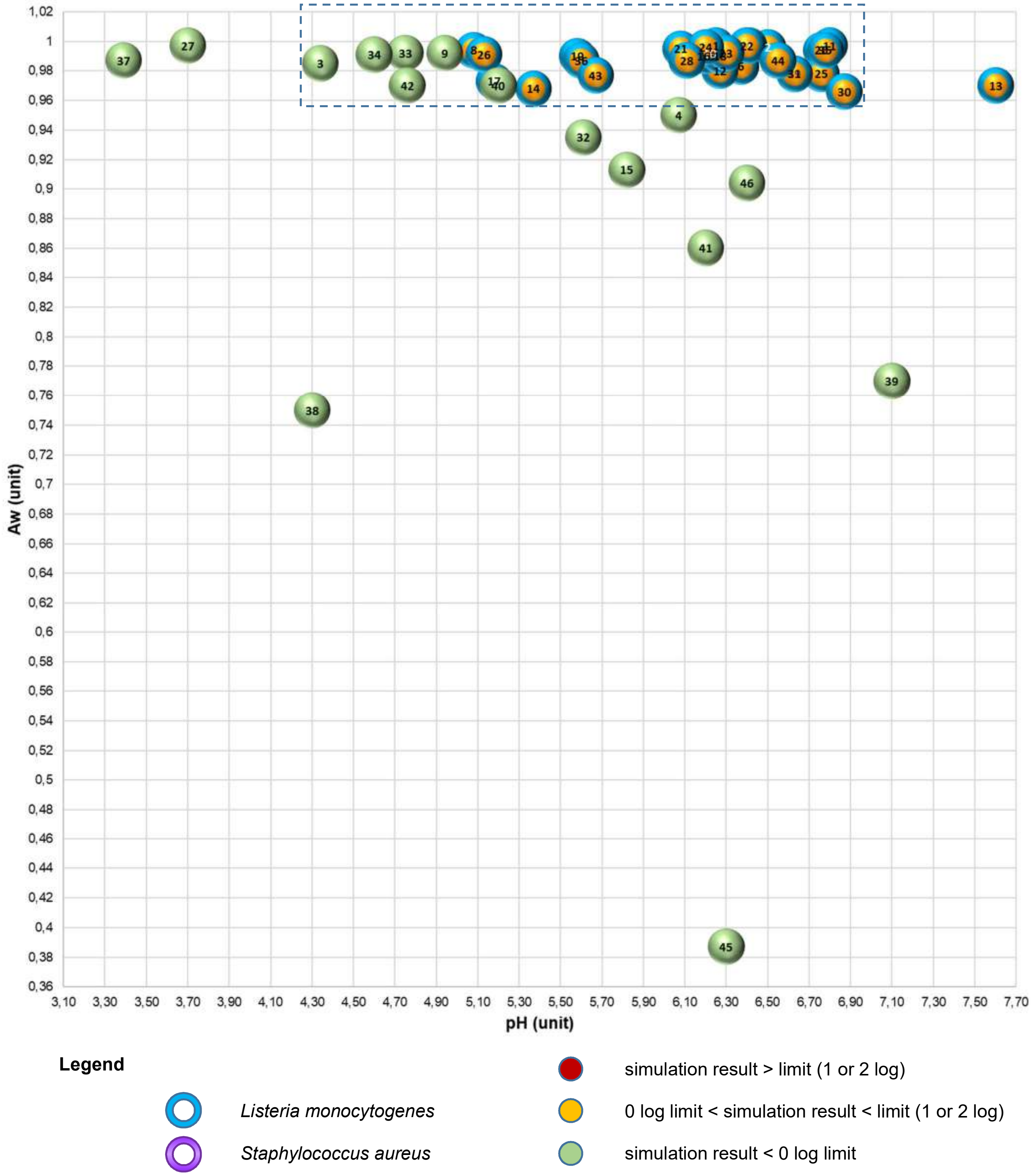
- 22H at 5°C (41°F)
- 18H at 10°C (50°F)
- 10H at 15°C (59°F)
- 14H at 25°C (77°F)

As in the previous section, the predictive microbiology study is focussed on *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens. Salmonella* spp. and *E. coli* are excluded as previously explained.

Exclusion of risk ingredients or food preparation practice is the effective risk management measure.

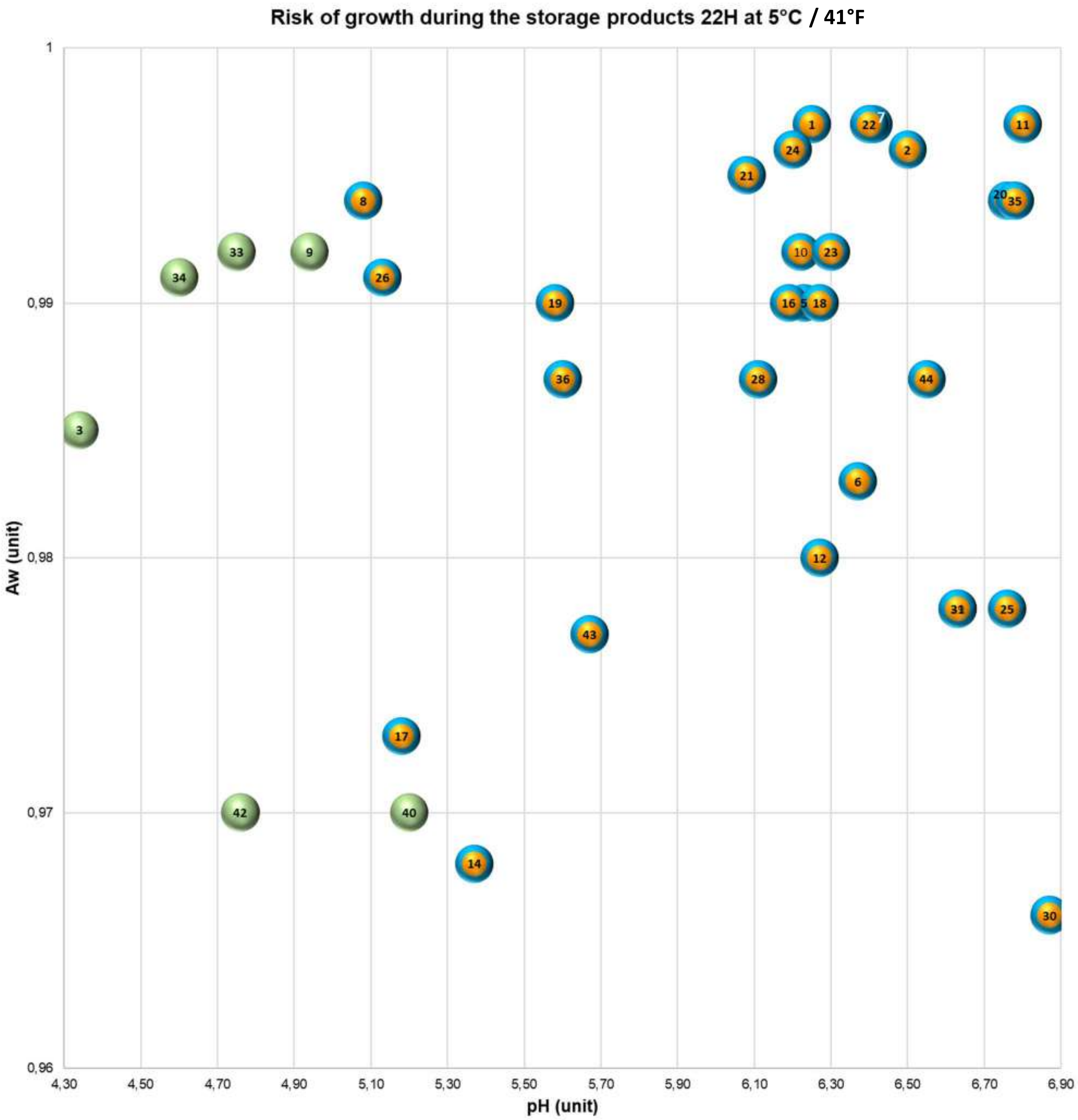
A conversion table from Celsius to Fahrenheit degrees is available in the appendix VIII.2

Risk of growth during the storage products 22H at 5°C / 41°F



1 Raw vegetables sliced seasoned
2 Raw vegetables sliced unseasoned
3 Cooked cold vegetables
4 Meat delicatessen cured
5 Meat delicatessen cooked
6 Cooked hot foods to be reheated
7 Soups
8 Salad with raw and/or cooked vegetables with starchy foods
9 Mixed salads with raw vegetables and/or cooked vegetables with PAO*
10 Cooked cold food (other)
11 Raw fish
12 Processed fish
13 Cooked egg products
14 Cold sandwiches
15 Cold sandwiches with cured meat
16 Cold sandwiches with cooked meat and fish (pork, poultry, beef, tuna...)
17 Cold sandwiches with raw vegetables
18 Cooked dishes with PAO with starchy foods without vegetables (to be reheated)
29 Cooked dishes with PAO with starchy foods with vegetables (to be reheated)
20 Cooked dishes with PAO without starch without vegetables (to be reheated)
21 Cooked dishes with PAO without starch with vegetables (to be reheated)
22 Cooked dishes without PAO with starchy foods without vegetables (to be reheated)
23 Cooked dishes without PAO with starchy foods with vegetables (to be reheated)
24 Cooked dishes without PAO without starch with vegetables (to be reheated)
25 Fried products (to be reheated)
26 Cream or cooked egg product based sauces
27 Cream or (raw) egg product based sauces
28 Pasteurized cooked and uncooked pressed cheese
29 Pasteurised soft cheese, bloomy rind and washed rind
30 Pasteurised blue-veined cheese
31 Raw milk soft cheese, bloomy rind and washed rind
32 Raw milk blue-veined cheese
33 Fresh cheese
34 Fermented milk products
35 Dairy desserts
36 Fresh fruit salads and desserts
37 Cooked fruit salads and desserts
38 Dried fruits and vegetables
39 Dried nuts and smoked seeds
40 Pickled and vinegar-cured vegetables
41 Room temperature stable baked goods
42 Baked pastries with positive cold storage
43 Unbaked pastries with fruit
44 Uncooked pastries without fruit
45 Cocoa- chocolate and confectionery
46 Fatty products

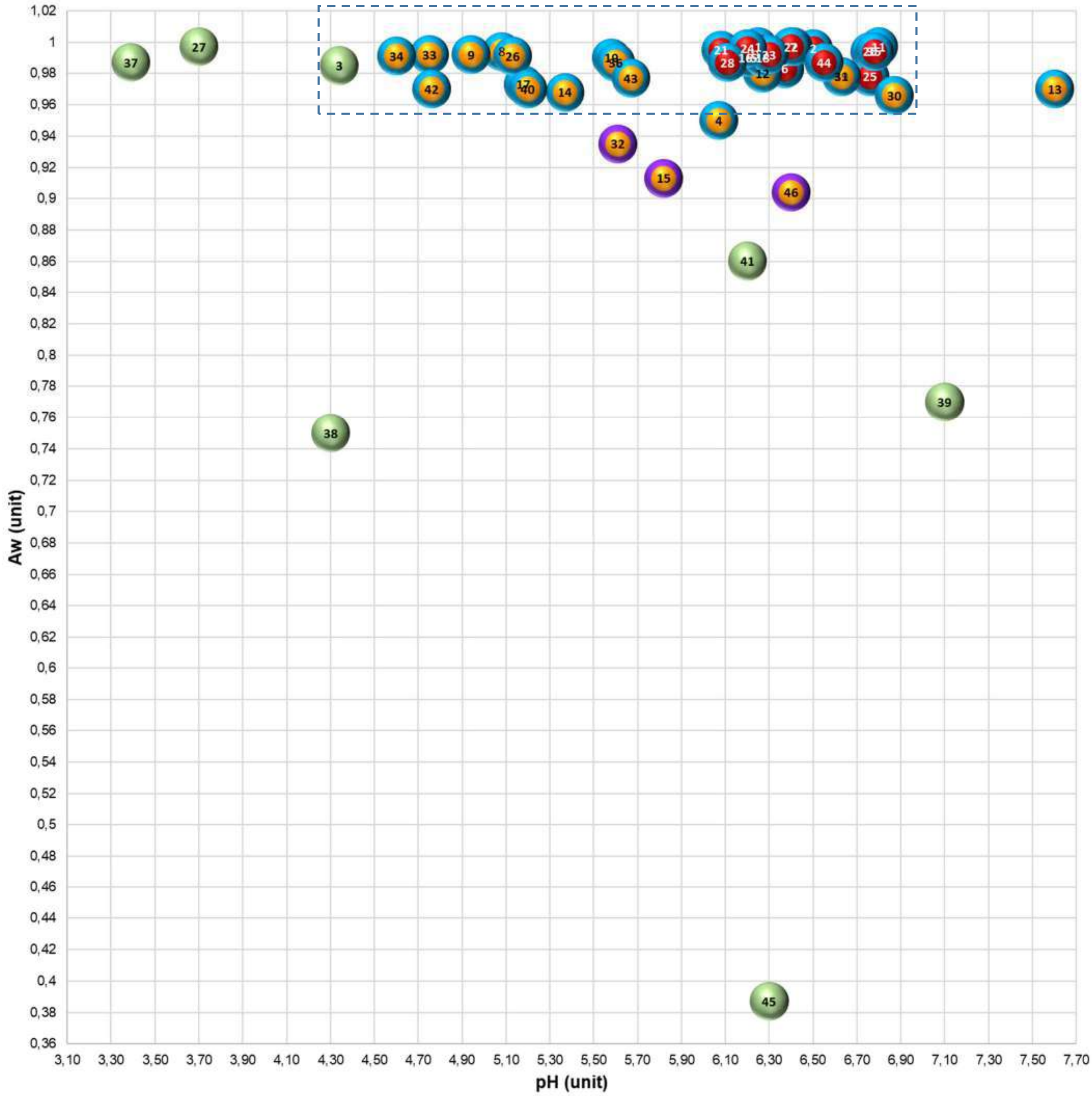
* PAO : Product of Animal Origin



Extended selection

<u>Note</u> : families 29 and 31 are superimposed (orange : risk of growth / blue circle : *Listeria monocytogenes*)

Risk of growth during the storage products 18H at 10°C / 50°F

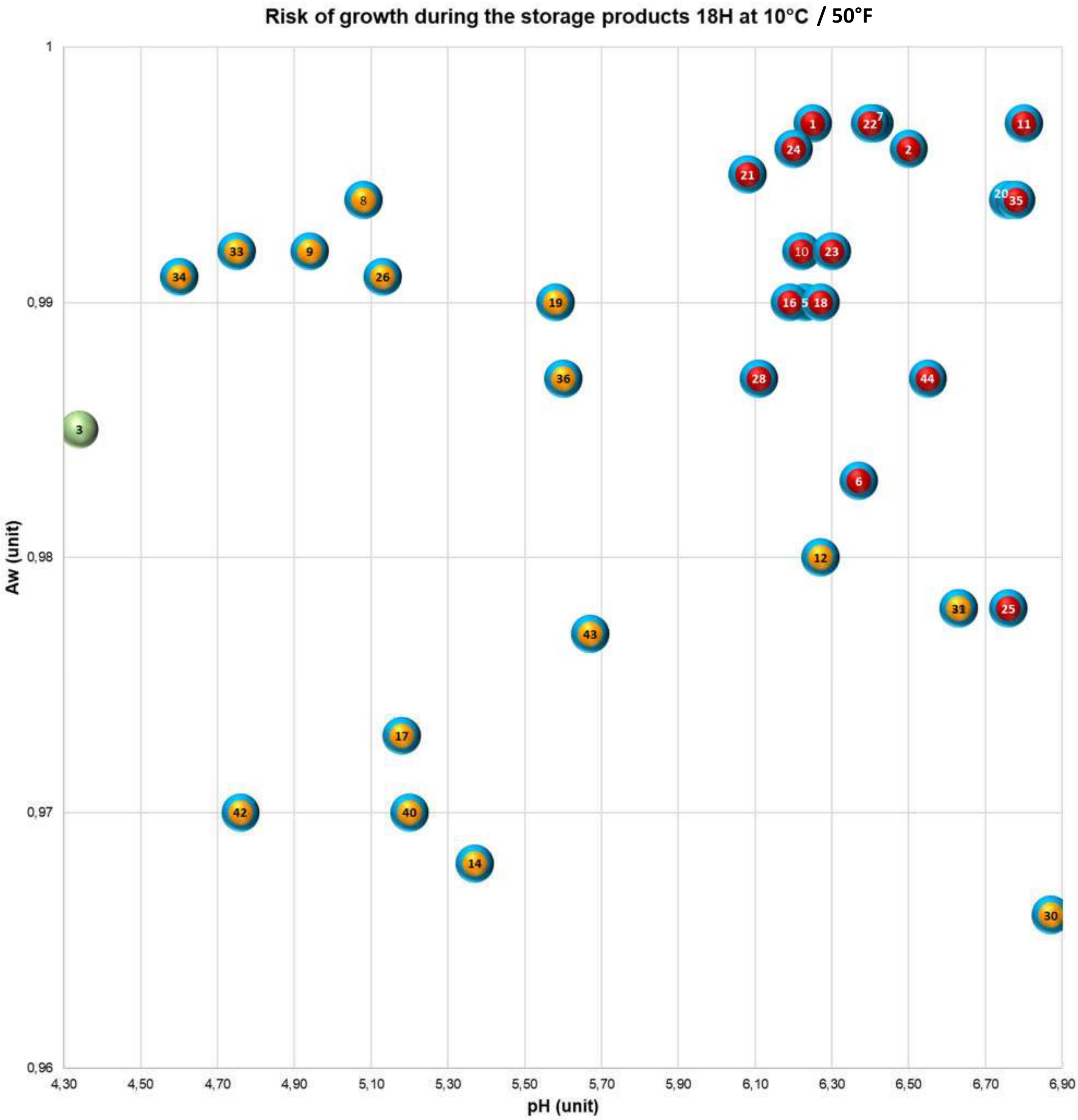


Legend

		(red)	simulation result > limit (1 or 2 log)
(blue)	*Listeria monocytogenes*	(orange)	0 log limit < simulation result < limit (1 or 2 log)
(purple)	*Staphylococcus aureus*	(green)	simulation result < 0 log limit

1 Raw vegetables sliced seasoned
2 Raw vegetables sliced unseasoned
3 Cooked cold vegetables
4 Meat delicatessen cured
5 Meat delicatessen cooked
6 Cooked hot foods to be reheated
7 Soups
8 Salad with raw and/or cooked vegetables with starchy foods
9 Mixed salads with raw vegetables and/or cooked vegetables with PAO*
10 Cooked cold food (other)
11 Raw fish
12 Processed fish
13 Cooked egg products
14 Cold sandwiches
15 Cold sandwiches with cured meat
16 Cold sandwiches with cooked meat and fish (pork, poultry, beef, tuna...)
17 Cold sandwiches with raw vegetables
18 Cooked dishes with PAO with starchy foods without vegetables (to be reheated)
29 Cooked dishes with PAO with starchy foods with vegetables (to be reheated)
20 Cooked dishes with PAO without starch without vegetables (to be reheated)
21 Cooked dishes with PAO without starch with vegetables (to be reheated)
22 Cooked dishes without PAO with starchy foods without vegetables (to be reheated)
23 Cooked dishes without PAO with starchy foods with vegetables (to be reheated)
24 Cooked dishes without PAO without starch with vegetables (to be reheated)
25 Fried products (to be reheated)
26 Cream or cooked egg product based sauces
27 Cream or (raw) egg product based sauces
28 Pasteurized cooked and uncooked pressed cheese
29 Pasteurised soft cheese, bloomy rind and washed rind
30 Pasteurised blue-veined cheese
31 Raw milk soft cheese, bloomy rind and washed rind
32 Raw milk blue-veined cheese
33 Fresh cheese
34 Fermented milk products
35 Dairy desserts
36 Fresh fruit salads and desserts
37 Cooked fruit salads and desserts
38 Dried fruits and vegetables
39 Dried nuts and smoked seeds
40 Pickled and vinegar-cured vegetables
41 Room temperature stable baked goods
42 Baked pastries with positive cold storage
43 Unbaked pastries with fruit
44 Uncooked pastries without fruit
45 Cocoa- chocolate and confectionery
46 Fatty products

* PAO : Product of Animal Origin



Extended selection

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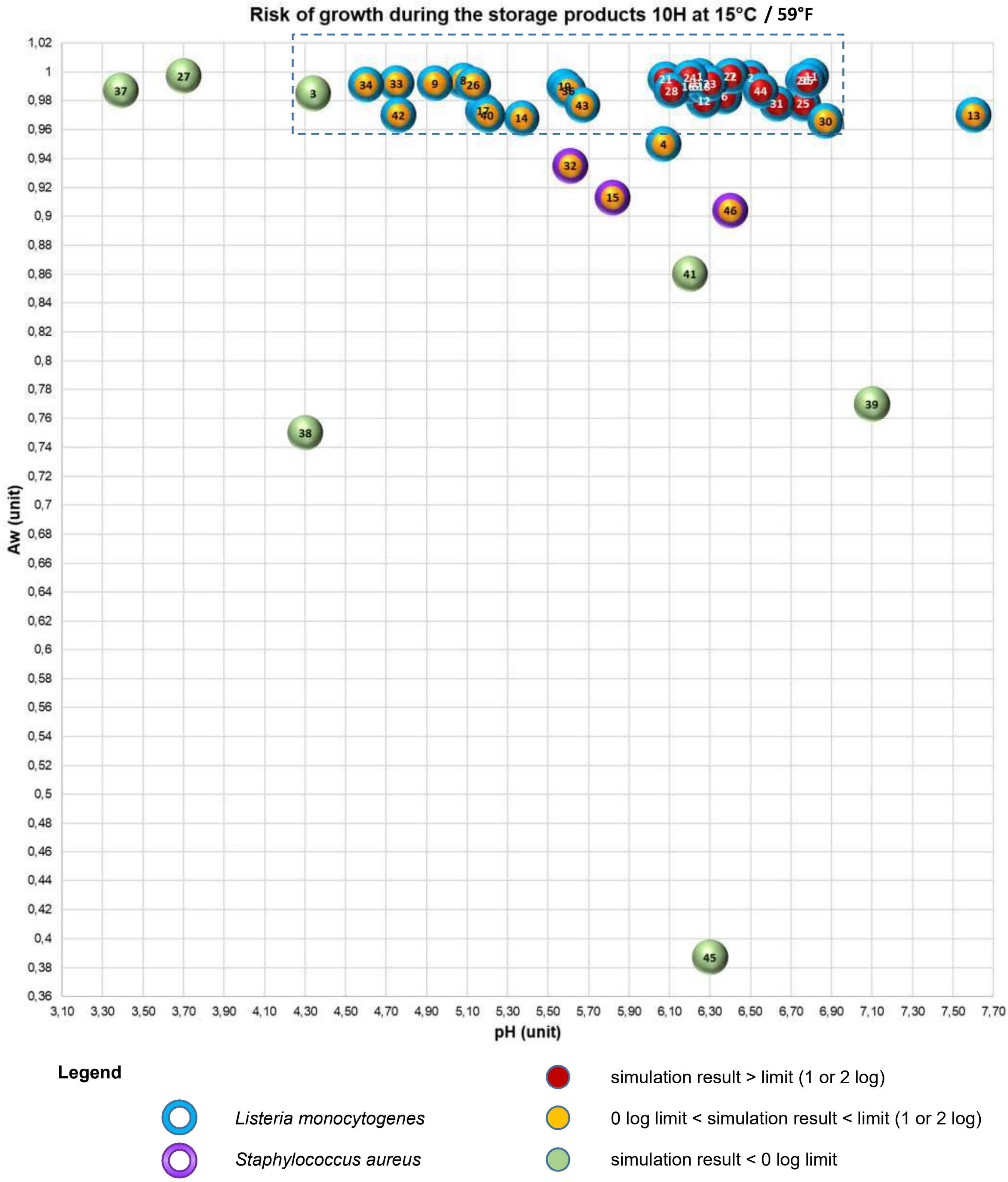

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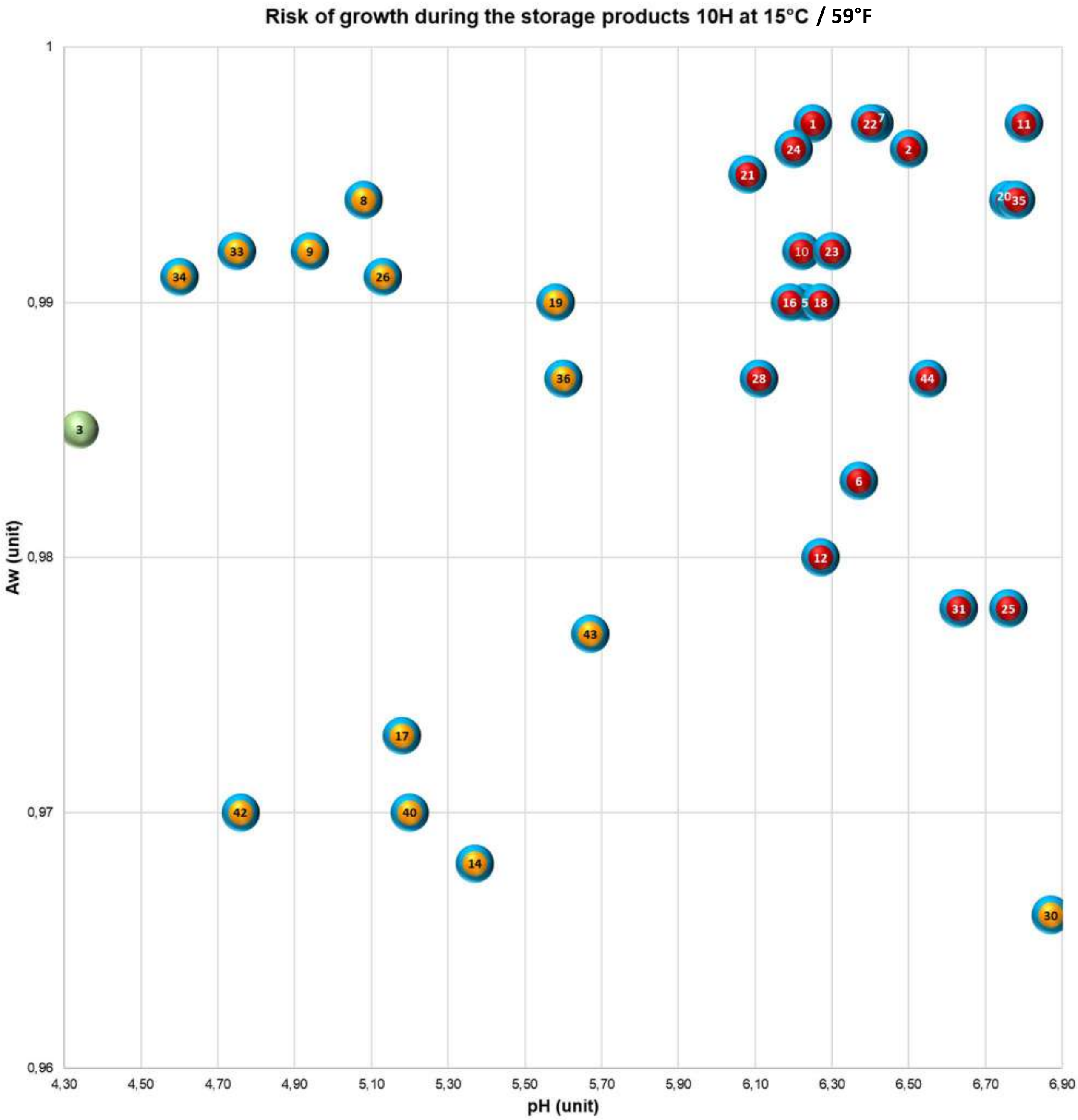
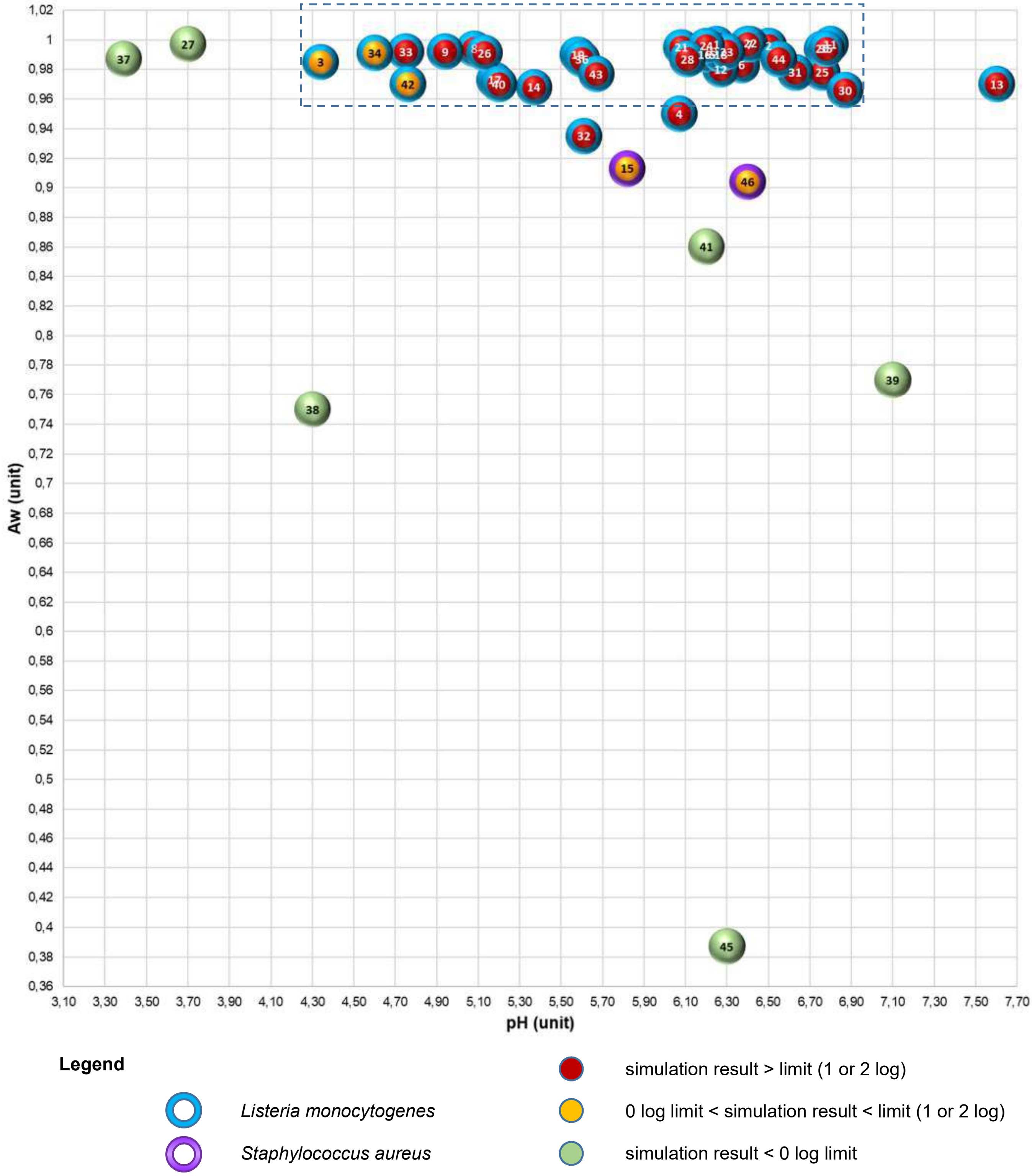


Extended selection

<u>Note</u> : families 29 and 31 are superimposed (red : growth / blue circle : *Listeria monocytogenes*)

Risk of growth during the storage products 14H at 25°C / 77°F



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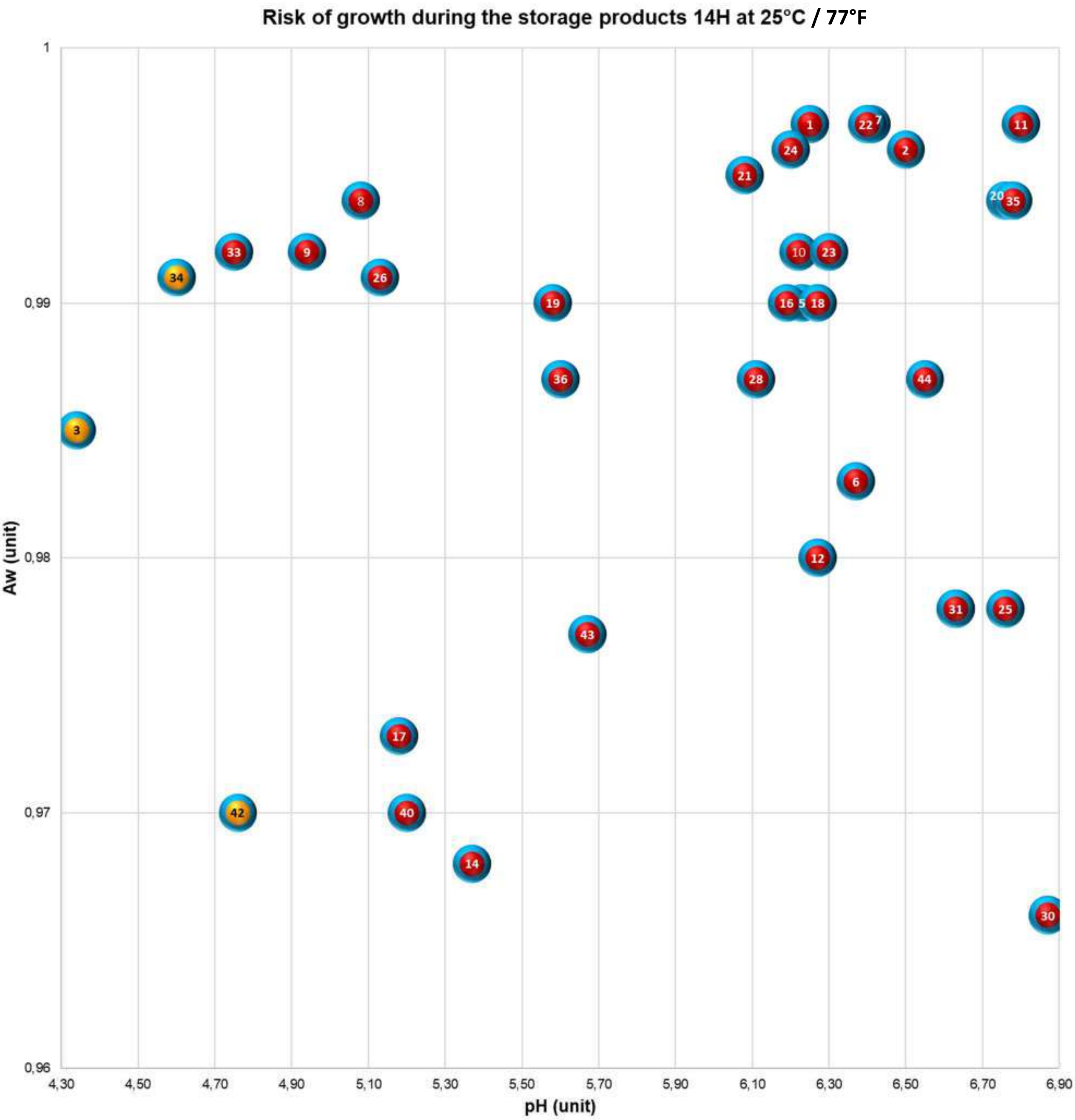

Extended selection

<u>Note</u> : families 29 and 31 are superimposed (red : growth / blue circle : *Listeria monocytogenes*)

VIII. APPENDIX

VIII.1. DETAILLED PRODUCT CATEGORIES

Some food items need specific low temperature to keep their organoleptic characteristics and their specific texture, for example ice-cream. Ice creams are a category of dessert and have the specificity to be very challenging for temperature.
If a temperature deviation goes above 0°C / 32°F (ex. 5°C / 41°F in our study), the texture of the Ice cream will be unacceptable for consumption.

	Family	Example of products	Products	Aw	pH	Reference
1	Raw vegetables sliced seasoned	Celery remoulade, coleslaw, grated carrots, gazpacho...	Celery remoulade	0.992	4.68	MXNS
			Vegetables aïoli	**0.997**	**6.25**	MXNS
			Coleslaw with cabbage and apple	0.988	4.16	MXNS
			Grated raw carrots and celery parsley	0.988	5.18	MXNS
			Carrot and celery	0.990	4.42	MXNS
2	Raw vegetables sliced unseasoned	Green salad, carrots, radishes...	Fresh vegetables	0.97-0,997	-	CRFSFS, 2003
			Asparagus (buds and stalks)	-	5.7-6.1	CRFSFS, 2003
			Avocado	**0.996**	5.54	MXNS
			Beans (string and lima)	-	4.6-**6.5**	CRFSFS, 2003
			Beets (sugar)	-	4.2-4.4	CRFSFS, 2003
			Broccoli	-	**6.5**	CRFSFS, 2003
			Brussels Sprouts	-	6.3	CRFSFS, 2003
			Cabbage (green)	-	5.4-6.0	CRFSFS, 2003
			Carrots	-	4.9-5.2 ; 6.0	CRFSFS, 2003
			Cauliflower	-	5.6	CRFSFS, 2003
			Celery	-	5.7-6.0	CRFSFS, 2003
			Corn (sweet)	-	7.3	CRFSFS, 2003
			Cucumber	-	4.5 and 5.1 - 5.7 / 3.8	ACA / CRFSFS, 2003
			Eggplant	-	4.4 / 4.5	ACA / CRFSFS, 2003
			Lettuce	-	6.0	CRFSFS, 2003
			Onions (red)	-	5.3-5.8	CRFSFS, 2003
			Parsley	-	5.7-6.0	CRFSFS, 2003
			Parsnip	-	5.3	CRFSFS, 2003
			Rhubarb	-	3.1-3.4	CRFSFS, 2003
			Spinach	-	5.5-6.0	CRFSFS, 2003
			Turnips	-	5.2-5.5	CRFSFS, 2003
			Tomatoes (small)	0.966-0.974	3.89-4.09	MXNS
			Tomatoes	-	3.8 and 4.2 - 4.9	ACA
3	Cooked cold vegetables	Beets cubes, leeks with salad dressing, cooked vegetables with mayonnaise...	**Roasted peppers**	0.982-**0.985**	4.22-**4.34**	MXNS
			Beets (seasoned)	-	4.30	MXNS
4	Meat delicatessen cured	Raw ham, sausage, chorizo…	Rosette	0.85	-	ACA
			Serrano ham	0.898-0.916	5.65-5.74	MXNS
			Aosta ham	0.91	-	ACA
			Bresaola	0.94	-	ACA
			Grisons meat	0.92	-	ACA
			Cured meat	0.87-**0.95**	-	CRFSFS, 2003
			Coppa	0.896-0.934	5.91-**6.07**	MXNS
			Dry sausage	0.882	5.24	MXNS
			Speck ham	0.902	5.75	MXNS
5	Meat delicatessen cooked	Cooked ham, pâté, rillettes, blood sausage, andouilles, poultry ham, pudding…	Pastrami	0.97	-	ACA
			Pudding (meat)	0.97-**0.99**	-	CRFSFS, 2003
			Ham	-	5.9-6.1	CRFSFS, 2003
			Cooked ham	0.972-0.983	6.00-**6.23**	MXNS
			Cooked sliced chicken ham	0.976	6.12	MXNS
			Cooked salted pork belly	0.93-0.96	5.60-5.80	MXNS
			Rillette pork	0.965-0.982	5.45-6.21	MXNS
			Saveloy salad	0.973-0.979	4.56-5.80	MXNS
			Pâté with baked pastry	0.976-0.977	6.06-6.20	MXNS
			Pâté	0.975	5.84	MXNS

6	Cooked hot food to be reheated	Puff pastry, hot dogs, buns, pizza (Margarita, 4 cheeses, mushroom, mushroom & cheese…), quiche, samosa, falafel, hot sandwiches, paninis, "cordon bleu"…	**Quiche "lorraine" (eggs, smoked bacon, cream)** Burger (beef) Cheeseburger Hot Dog Ciabatta chicken Piquillos Hot dog (beef) Muffin (eggs. bacon. cheddar) Pizza chicken cheese Hot sandwich (smoked bacon, cheese, onions, potatoes) Hot sandwich (ciabatta, ham, Emmental cheese) Pizza ham, cheese, mushroom	**0.983** 0.972 0.974 0.967 0.973 0.971 0.962 0.973 0.978 0.974 0.971	**6.37** 5.66 5.39 5.39 5.86 5.70 5.70 5.70 5.72 5.68 5.28	MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS
7	Soups	Vegetable soup with or without cream, Asian soup…	**Vegetables soup** Bean soup with noodles Sweet potato soup Asparagus soup	0.991-**0.997** 0.989 0.986 0.994	5.59-**6.41** 6.0 6.37 6.29	MXNS Da Silva et al.1993 MXNS MXNS
8	Salad with raw and/or cooked vegetables with starchy foods	Tabbouleh, Potatoes salad, pasta salad...	Piemontaise salad Pasta salad Rice salad **Napoli pasta salad**	0.99 0.987 0.995 **0.994**	4.41-4.43 4.92 4.61 **5.08**	MXNS MXNS MXNS MXNS
9	Mixed salads with raw vegetables and/or cooked vegetables with AO	Snout salad, trio of cabbage (ham, comté, cabbage), fisherman's salad (tuna...), Caesar salad, potato tuna salad...	**Rice vegetables surimi salad** Pasta and tuna salad	**0.992** 0.989	**4.94** 4.49	MXNS MXNS
10	Cooked cold food (other)	Savory cakes, hummus, eggplant caviar, tapenade, pesto sauce, cooked tuna, foie gras, fish terrine…	Chicken tomato basil cake Emmental cake Chicken curry spread Hummus Tapenade with olive and tomato Pesto sauce Pesto tomato sauce Cooked yellowfin tuna **Minced roasted chicken** Foie gras Salmon cake Fish terrine with mayonnaise	0.97 0.96 - 0.982-0.991 0.968-0.974 0.947 0.952 0.985-0.993 0.986-**0.992** 0.96-0.974 0.97-0.98 0.988	- - 3.0 4.27-4.36 4.12-4.23 4.18 4.48 5.79-5.92 6.13-**6.22** 6.09-6.16 7.44-7.80 5.92	ACA ACA ACA MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS
11	Raw fish	Sushi, fish tartar…	**Fresh fish** Tuna Fish Salmon White fish Sushi	0.99-**0.997** - - - 0.967	6.6-**6.8** 5.2-6.1 6.1-6.3 5.5 4.87	CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 MXNS
12	Processed fish	Cold smoked salmon, sprats…	**Cold smoked salmon** **Sprats** Smoked fish	0.962-**0.980** 0.967 0.95-0.97	5.94-6.17 **6.27** 5.7-6.0	Augustin et al. 2015 MXNS MXNS
13	Cooked egg products	Plain omelet with mushrooms, ham... scrambled eggs, boiled eggs …	Eggs Eggs yolks Eggs white **Hard-boiled egg (without shell)**	0.97 - - **0.97**	- 6.0-6.3 **7.6**-9.5 7.07	CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 MXNS
14	Cold sandwiches	Cheese sandwich…	**Cheese sandwich**	**0.968**	**5.37**	MXNS
15	Cold sandwiches with cured meat	Wholemeal Italian ham…	**with Serrano ham**	**0.913**	**5.82**	MXNS
16	Cold sandwiches with cooked meat and fish (pork, poultry, beef, tuna...)	Wholemeal sandwich (with ham, cooked beef, pulled beef, chicken, turkey ham, tuna…), rosette, pâté…	**Minced chicken sandwich** Eggs tuna sandwich Wrap chicken curry	**0.99** 0.982 0.979	**6.19** 5.57 5.73	MXNS MXNS MXNS
17	Cold sandwiches with raw vegetables	Vegan sandwich…	**Ciabatta veggie**	**0.973**	**5.18**	MXNS
18	Cooked dishes with PAO with starchy foods without vegetables (to be reheated)	Hash Parmentier, fish brandade, penne gratin, lentil sausage, Bolognaise pasta…	Penne gratin with two salmons Penne gratin with bacon Lentil sausage Carbonara pasta (tagliatelle) **Hash Parmentier** Gyoza chicken **Gyoza shrimp** Nikuman Chili con carne Chorizo risotto	0.99 0.98 0.99 0.99 **0.990** 0.989 0.983-0.987 0.979 0.98 0.994	5.64 5.09 5.54 5.53 6.23 5.69-6.05 5.85-**6.27** 5.80 5.29 5.86	MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS

19	Cooked dishes with PAO with starchy foods with vegetables (to be reheated)	Lasagna, paella, chicken mushroom risotto…	Chicken mushroom risotto Shrimp and coconut milk risotto Paëlla **Bolognaise pasta**	0.98 0.98 0.984-0.986 0.977-**0.99**	5.25 5.29 5.96-6.44 5.03-**5.58**	MXNS MXNS MXNS MXNS
20	Cooked dishes with PAO without starch without vegetables (to be reheated)	Roast chicken (portion), poultry supreme, cooked chicken, beef sauté, sliced cooked roast (beef, veal, pork...), paupiette, duck breast, minced steak, beef meatball, veal normandin, hake fillet with lemon sauce, cooked Neapolitan fish steak…	Cooked chicken **Breaded fish** Cooked hake fish Cooked saithe fillet with butter Cooked minced beef steak Roasted beef (slices) Slice of roasted bacon Pulled beef Roasted chicken Cooked turkey Yakitori Cooked shrimp Beef tongue with Madeira sauce Roasted guinea fowl	0.987-0.990 0.990-**0.994** 0.994 0.987 0.989-0.994 0.992 0.925-0.935 0.987-0.994 0.993 0.990 0.972 0.976-0.978 0.995 0.988	6.03-6.31 6.66-**6.76** 6.63 6.44 5.68-6.39 5.73 6.00-6.29 5.75-5.87 6.56 5.93 6.66 7.30-7.51 5.96 6.69	MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS MXNS
21	Cooked dishes with PAO without starch with vegetables (to be reheated)	Moussaka, veal blanquette…	Moussaka **Veal blanquette** Pork with vegetables	0.98-0.981 **0.995** 0.987	5.18-5.62 **6.08** 6.03	MXNS MXNS MXNS
22	Cooked dishes without PAO with starchy foods without vegetables (to be reheated)	Mashed potatoes, cooked pasta, cooked rice, cooked semolina, bulgur pancakes, noodles…	Potatoes pancake **Cooked rice** Cooked potatoes Mashed potatoes	0.983-0.987 0.994-**0.997** 0.993 0.989	5.49-5.63 6.08-**6.40** 5.69 5.8	MXNS MXNS MXNS Mahakarnchanakul et al. 1999
23	Cooked dishes without PAO with starchy foods with vegetables (to be reheated)	Mushroom risotto. asian products…	**Vegetables gratin** Gyoza with vegetables Cauliflower and potato gratin	**0.992** 0.986 0.993	**6.30** 5.94 6.24	MXNS MXNS MXNS
24	Cooked dishes without PAO without starch with vegetables (to be reheated)	Mashed zucchini, mashed pea, pan-fried vegetables, ratatouille, baked beans, tomato and pepper sauce, tofu, wok vegetables…	Cooked eggplant Ratatouille Cooked green beans Mashed zucchini **Mashed carrot** Cooked carrot	- 0.983-0.990 0.995 0.996 **0,996** 0.987	4.5-5.3 3.91-3.99 5.71 5.99 **6.20** 5.87	ACA MXNS MXNS MXNS MXNS MXNS
25	Fried products (to be reheated)	Fried potatoes, egg rolls, tempura, shrimp fritters…	Fried onions **Codfish accras**	0.348-0.371 0.975-**0.978**	5.37-5.74 **6.76**	MXNS MXNS
26	Cream or cooked egg product based sauces	Béchamel sauce, curry sauce, béarnaise sauce, hollandaise sauce…	Béarnaise sauce **Hollandaise sauce**	0.977-0.979 **0.991**	4.10-4.34 **5.13**	MXNS ADRIA Normandie, FR
27	Cream or (raw) egg product based sauces	Mayonnaise, Caesar dressing …	Light mayonnaise **Mayonnaise mustard sauce** Mayonnaise Caesar dressing	0.972-0.978 **0.997** 0,947 0.986	3.41-3.58 **3.7** - 4.66	MXNS Weagant et al. 1994 Gómez et al. 1992 MXNS
28	Pasteurized cooked and uncooked pressed cheese	Comté, Mimolette, Gouda, Parmesan, Mozzarella, Cheddar...	Jura Cheese Mimolette Gouda cumin Edam Gouda Port Salut Tomme de brebis Emmental (slices) Comté Beaufort Parmesan copeaux Parmesan cheese Cheese (American mild and cheddar) Raclette cheese Cantal (slices) Mozzarella (beads) **Mozarella**	0.96 0.96 0.97 0.96 0.96 0.96 0.96 0.970-0.982 0.955 0.94 0.93 0.68-0.76 - 0.954-0.970 0.951-0.960 0.898-0,997 **0.987**-0,997	- - - - - - - 5.48-5.61 5.77 - - - 4.9-5.9 5.44-6.03 4.88-5.03 5.52-5.62 6.03-**6.11**	ACA ACA ACA ACA ACA ACA ACA MXNS MXNS ACA ACA CRFSFS, 2003 CRFSFS, 2003 MXNS MXNS MXNS MXNS
29	Pasteurized soft cheese, bloomy rind and washed rind	Camembert, Brie...	**Camembert** St-Maure-de-Tourraine (goat cheese) Brie (slices)	0.971-**0.978** 0.972-0.985 0.970-0.982	6.30-**6.63** 4.96-5.12 5.66-6.24	MXNS MXNS MXNS
30	Pasteurized blue-veined cheese	Fourme d'Ambert…	Fourme d'Ambert **Blue-veined Cheese**	0.942 0.942-**0.966**	6.86 6.47-**6.87**	MXNS MXNS

31	Raw milk soft cheese, bloomy rind and washed rind	Raw milk Camembert, natural cheeses…	Natural cheeses **Camembert** St-Nectaire Goat cheese with raw milk Brie de Meaux	0.95-0,997 0.971-**0.978** 0.976 0.966 0.968-0.976	- 6.30-**6.63** 6.29 5.46 6.01-6.38	CRFSFS, 2003 MXNS MXNS MXNS MXNS
32	Raw milk blue-veined cheese	Roquefort…	**Blue cheese** Roquefort	**0.935** 0.926	**5.61** 5.84	MXNS MXNS
33	Fresh cheese	Fresh cheese…	Cheese with nuts Cantadou **Fresh Cheese with Guerande salt**	- - **0.992**	4.4 4.3 **4.75**	ACA ACA MXNS
34	Fermented milk products	Yoghurt, Cottage cheese…	Yoghurt **Cottage cheese** lemon "Perle de lait" (Yoplait) Actimel	- - **0.991** - -	3.8-4.2 5.1 and 4-4.5 4.4 and 4-**4.6** 4.3 4.3	CRFSFS, 2003 ACA ACA ACA ACA
35	Dairy desserts	Dessert cream (chestnut, vanilla…), ice-cream, smoothies (with milk/yoghurt)…	Bircher **Vanilla cream** Cream muslin Butter cream Ice cream	0.976-0.984 **0.994** 0.938 0.839 0.957-0.965	5.29-5.39 6.77-**6.78** 6.20 5.18 6.50-6.67	MXNS MXNS MXNS MXNS Gougouli et al. 2008
36	Fresh fruit salads and desserts	Cut-up fruits, fruits salad, fresh fruits (melon, watermelon, orange, apple, banana, grapes…), fresh fruit juice, smoothies (fruits/vegetables)…	Citrus fruit salad Grapefruit segments Grape juice Orange segments Apples Orange Kiwi Banana Figs Plums Grapes Melon Honeydew melon **Watermelon** Pumpkin Squash Cut fruits Fruit juice concentrates Limes Smoothies (with fruits)	- - - - - - - - - - - 0.989 - **0.987** - - 0.987 0.79-0.84 - 0.986-0.988	3.6 2.9 3.0 3.5 3.9 / 3.3 - 3.9 3.5 and 3.1 - 4.1 2.9 and 3.1 - 3.9 4.4 and 4.5 - 5.2 4.6 2.8-4.6 3.4-4.5 6.19 6.3-6.7 5.2-**5.6** 4.8-5.2 5.0-5.4 4.14 - 1.8-2.0 3.45-3.59	ACA ACA CRFSFS, 2003 ACA ACA / CRFSFS, 2003 ACA ACA ACA CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 Salazar et al. 2017 CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 CRFSFS, 2003 MXNS CRFSFS, 2003 CRFSFS, 2003 Moura et al. 2017
37	Cooked fruit salads and desserts	Cooked fruits, compote, redcurrant jelly pears…	4-fruit salad (in syrup) Diced pineapple (in syrup) Mini canned pear Caramel apple compote Exotic fruit compote Stewed red fruits **Cooked apple**	- - - - - - **0.987**	3.6 4.4 3.6 and 3.9-4.2 4.4 3.6 3.4 **3.39**	ACA ACA ACA ACA ACA ACA MXNS
38	Dried fruits and vegetables	Dried fruit…	Dried fruit Dried grape sultana Dried figs Dried tomatoes Dried apricots **Dried apricots**	0.55-0.80 0.575-0.634 0.484-0.695 - 0.717-0.732 **0.75**	- 4.05-4.19 4.20-4.33 <4.2 4.08-4.14 **4.3**	CRFSFS, 2003 MXNS MXNS ACA MXNS FSA
39	Dried nuts and smoked seeds	Peanuts, nuts…	**Almond slices** Peanuts, dry roasted Sunflower seeds	**0.77** 0.147 0.75	**7.1** - -	FSA Schmidt et Fontana, 2007 Schmidt et Fontana, 2007
40	Pickled and vinegar-cured vegetables	Pickles, olives…	Pickles (canned) Olives (canned) **Pitted black olives with herbs** **Pitted green olives** Olives stuffed with almonds	- - 0.87 **0.97** 0.97	5.1 and 3.2 - 3.5 4.0 and 3.6 - 3.8 **5.20** 3.62 4.09	ACA ACA / CRFSFS, 2003 MXNS MXNS MXNS
41	Room temperature stable baked goods	Dry cakes, baked cake, candied fruit cake, king's cake (galette des rois)	Candied fruit cake Baked cake Fruit cake Carrot cake (with icing cream cheese) Christmas cake Sultana and currant cake **Coconut Cake**	0.77 0.90-0.94 0.73-0.83 0.828 0.69 0.80 **0.86**	- - - 6.38 4.4 4.7 **6.2**	ACA CRFSFS, 2003 CRFSFS, 2003 MXNS FSA FSA Mattick et al. 2001

42	Baked pastries with positive cold storage	Flan, chocolate pie, apple pie, jellies, pastry with apple…	Jellies **Morello cherry clafoutis** Apple pie Blueberry pie Pears chocolate pie Foret Noire cake (chocolate cream and candied cherry) Charlotte with raspberry Plain pancakes Pancake	0.82-0.94 **0.97** 0.968-0.988 0.970 0.946 0.943 0.973 0.99 0.95	- 4.63-**4.76** 4.28-4.63 4.21 5.32 4.16 4.24 - -	CRFSFS, 2003 MXNS MXNS MXNS MXNS MXNS MXNS ACA ACA
43	Unbaked pastries with fruit	Bavarian cake…	**Raspberry bavarian cake** Pastry with buttercream and strawberry	**0.977** 0.932-0.976	**5.67** 4.97-5.53	MXNS MXNS
44	Uncooked pastries without fruit	Chocolate eclair, entremets…	Chocolate foam **Chou Chantilly pastry** Paris Brest pastry Pastry with buttercream Mille-feuille pastry Chocolate ganache	0.968-0.972 **0.987** 0.968 0.822 0.963-0.910 0.926	5.84-6.31 **6.55** 6.53 5.87 5.74-5.98 5.26	MXNS MXNS MXNS MXNS MXNS MXNS
45	Cocoa- chocolate and confectionery	Chocolate, sweetened red bean paste, milk chocolate bar…	**Milk chocolate chips**	**0.387**	**6.3**	MXNS
46	Fatty products	Butter, margarine	**Butter** Margarine	**0,904** 0,914	**6,1-6,4** -	Gómez et al. 1992 and CRFSFS

VIII.2. CONVERSION FROM CELSIUS DEGREE TO FAHRENHEIT DEGREE

Conversion from Celsius degree to Fahrenheit degree:
temperature F = (temperature C x 1,8) + 32

Conversion from Fahrenheit degree to Celsius degree:
temperature C = (temperature F – 32) / 1,8

T (°C)	T (°F)	T (°C)	T (°F)	T (°C)	T (°F)	T (°C)	T (°F)	T (°C)	T (°F)
-40,0	-40,0	0,0	32,0	40,0	104,0	80,0	176,0	120,0	248,0
-39,0	-38,2	1,0	33,8	41,0	105,8	81,0	177,8	121,0	249,8
-38,0	-36,4	2,0	35,6	42,0	107,6	82,0	179,6	122,0	251,6
-37,0	-34,6	3,0	37,4	43,0	109,4	83,0	181,4	123,0	253,4
-36,0	-32,8	4,0	39,2	44,0	111,2	84,0	183,2	124,0	255,2
-35,0	-31,0	5,0	41,0	45,0	113,0	85,0	185,0	125,0	257,0
-34,0	-29,2	6,0	42,8	46,0	114,8	86,0	186,8	126,0	258,8
-33,0	-27,4	7,0	44,6	47,0	116,6	87,0	188,6	127,0	260,6
-32,0	-25,6	8,0	46,4	48,0	118,4	88,0	190,4	128,0	262,4
-31,0	-23,8	9,0	48,2	49,0	120,2	89,0	192,2	129,0	264,2
-30,0	-22,0	10,0	50,0	50,0	122,0	90,0	194,0	130,0	266,0
-29,0	-20,2	11,0	51,8	51,0	123,8	91,0	195,8	131,0	267,8
-28,0	-18,4	12,0	53,6	52,0	125,6	92,0	197,6	132,0	269,6
-27,0	-16,6	13,0	55,4	53,0	127,4	93,0	199,4	133,0	271,4
-26,0	-14,8	14,0	57,2	54,0	129,2	94,0	201,2	134,0	273,2
-25,0	-13,0	15,0	59,0	55,0	131,0	95,0	203,0	135,0	275,0
-24,0	-11,2	16,0	60,8	56,0	132,8	96,0	204,8	136,0	276,8
-23,0	-9,4	17,0	62,6	57,0	134,6	97,0	206,6	137,0	278,6
-22,0	-7,6	18,0	64,4	58,0	136,4	98,0	208,4	138,0	280,4
-21,0	-5,8	19,0	66,2	59,0	138,2	99,0	210,2	139,0	282,2
-20,0	-4,0	20,0	68,0	60,0	140,0	100,0	212,0	140,0	284,0
-19,0	-2,2	21,0	69,8	61,0	141,8	101,0	213,8	141,0	285,8
-18,0	-0,4	22,0	71,6	62,0	143,6	102,0	215,6	142,0	287,6
-17,0	1,4	23,0	73,4	63,0	145,4	103,0	217,4	143,0	289,4
-16,0	3,2	24,0	75,2	64,0	147,2	104,0	219,2	144,0	291,2
-15,0	5,0	25,0	77,0	65,0	149,0	105,0	221,0	145,0	293,0
-14,0	6,8	26,0	78,8	66,0	150,8	106,0	222,8	146,0	294,8
-13,0	8,6	27,0	80,6	67,0	152,6	107,0	224,6	147,0	296,6
-12,0	10,4	28,0	82,4	68,0	154,4	108,0	226,4	148,0	298,4
-11,0	12,2	29,0	84,2	69,0	156,2	109,0	228,2	149,0	300,2
-10,0	14,0	30,0	86,0	70,0	158,0	110,0	230,0	150,0	302,0
-9,0	15,8	31,0	87,8	71,0	159,8	111,0	231,8	151,0	303,8
-8,0	17,6	32,0	89,6	72,0	161,6	112,0	233,6	152,0	305,6
-7,0	19,4	33,0	91,4	73,0	163,4	113,0	235,4	153,0	307,4
-6,0	21,2	34,0	93,2	74,0	165,2	114,0	237,2	154,0	309,2
-5,0	23,0	35,0	95,0	75,0	167,0	115,0	239,0	155,0	311,0
-4,0	24,8	36,0	96,8	76,0	168,8	116,0	240,8	156,0	312,8
-3,0	26,6	37,0	98,6	77,0	170,6	117,0	242,6	157,0	314,6
-2,0	28,4	38,0	100,4	78,0	172,4	118,0	244,4	158,0	316,4
-1,0	30,2	39,0	102,2	79,0	174,2	119,0	246,2	159,0	318,2

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ANNEX II

Time and Temperature Trial Form

Component/Meal Family	pH	Aw	Temperature					
			4-Hours	6-Hours	10-Hours	14-Hours	18-Hours	24-Hours
Raw Vegetables Sliced and Seasoned	6,25	0,997						
Raw Vegetables Sliced and Unseasoned	6,50	0,996						
Cooked Cold Vegetables	4,34	0,985						
Meat Delicatessen Cured	6,07	0,950						
Meat Delicatessen Cooked	6,23	0,990						
Cooked Hot Foods	6,37	0,983						
Soups	6,41	0,997						
Salad with Raw and or Cooked Vegetables with Starchy Foods	5,08	0,994						
Salads with Raw Vegetables or Cooked Vegetables with PAO	4,94	0,992						
Cooked Cold Food (Other)	6,22	0,992						
Raw Fish	6,80	0,997						
Processed Fish	6,27	0,980						
Cooked Egg Products	7,60	0,970						
Cold Sandwiches	5,37	0,968						
Cold Sandwiches with Cured Meat	5,82	0,913						
Cold Sandwiches with Cooked Meat and Fish	6,19	0,990						
Cold Sandwiches with Raw Vegetables	5,18	0,973						
Cooked Dishes with PAO with Starchy Foods w/out Vegetables	6,27	0,990						
Cooked Dishes with PAO with Starchy Foods with Vegetables	5,58	0,990						
Cooked Dishes with PAO without Starchy Food w/out Vegetables	6,76	0,994						
Cooked Dishes with PAO without Starchy Food with Vegetables	6,08	0,995						
Cooked Dishes without PAO with Starch without Vegetables	6,40	0,997						
Cooked Dishes without PAO with Starchy Foods with Vegetables	6,30	0,992						
Cooked Dishes without PAO without Starch with Vegetables	6,20	0,996						
Fried Products (to be Reheated)	6,76	0,978						
Cream or Cooked Egg Product based Sauces	5,13	0,991						
Cream or (Raw) Egg Product based Sauces	3,70	0,997						
Pasteurized Cooked and Uncooked Pressed Cheese	6,11	0,987						
Pasteurised Soft Cheese, Bloomy Rind and Washed Rind	6,63	0,978						
Pasteurised Blue-veined Cheese	6,87	0,966						

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Raw Milk Soft Cheese, Bloomy Rind and Washed Rind	6,63	0,978						
Raw Milk Blue-veined Cheese	5,61	0,935						
Fresh Cheese	4,75	0,992						
Fermented Milk Products	4,60	0,991						
Dairy Desserts	6,78	0,994						
Fresh Fruit Salads and Desserts	5,60	0,987						
Cooked Fruit Salads and Desserts	3,39	0,987						
Dried Fruits and Vegetables	4,30	0,750						
Dried Nuts and Smoked Seeds	7,10	0,770						
Pickled and Vinegar-cured Vegetables	5,20	0,970						
Room Temperature Stable Baked Goods	6,20	0,860						
Baked Pastries with Positive Cold Storage	4,76	0,970						
Unbaked Pastries with Fruit	5,67	0,977						
Uncooked Pastries without Fruit	6,55	0,987						
Cocoa-chocolate and Confectionery	6,30	0,387						
Fatty Products	6,40	0,904						