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SCIENTIFIC OPINION

Microbiological safety of ungulates meat intended to be frozen and defrosting of frozen ungulates meat

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Based on the need for a scientific basis for existing requirements in EU legislation on freezing of meat or for its possible amendment, the opinion compares microbial growth of relevant pathogenic, spoilage and indicator microorganisms within five scenarios of chilling, storage and defrosting of bovine, ovine and porcine meat, using predictive microbiology models that considered various conditions of temperature and, where possible, pH and a_w. Results obtained were compared to a reference scenario: storing meat at 7°C, aerobically, until 15 days post-slaughter. Storage of meat for 6 weeks, vacuum-packed immediately after stabilisation or 15 days post-slaughter, resulted in more growth of at least some of the bacteria assessed compared to the reference scenario, both at 3°C (certainty level 66%–90%) and at 7°C (certainty level 95%–99%). Predictions allowed estimating time at which equivalent microbial growth (i.e. ≤ 0.5 $\log_{10}$ difference) to the reference scenario is reached ('equivalence time'), assuming different initial contamination levels of relevant spoilage bacteria. When storing meat at 7°C, vacuum-packed immediately after stabilisation, equivalence time was determined by *Salmonella* and reached in 5–6 days of post-slaughter storage (certainty level 66%–90%). When storing meat at 3°C, equivalence time was determined by spoilage lactic acid bacteria and reached in 29–30 days post-slaughter (certainty level 66%–90%). However, when initial contamination with spoilage bacteria was high (e.g. 5 $\log_{10}$ CFU/cm^2), predicted spoilage levels of 7 $\log_{10}$ CFU/cm^2 were reached after 15–16 days. When considering also expected growth during post-defrosting storage at 4°C for 7 days, equivalence times were of 5–6 days (unchanged) and 13–16 days, respectively, though meat would have to be frozen immediately after stabilisation when initial contamination with spoilage bacteria is high. Predicted levels of indicator microorganisms for verification are provided for different assumed initial contamination levels, representing examples to be further adjusted based on actual measurements in practical settings.

KEYWORDS

frozen meat, meat chilling, microbial growth, predictive modelling, spoilage, thawing

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SUMMARY

Based on the need for a scientific basis for existing requirements in EU legislation related to the freezing of meat or for its possible amendment, the European Commission requested EFSA to provide a scientific opinion on the microbiological safety of ungulates meat intended to be frozen and on the defrosting of frozen ungulates meat.

The opinion compares microbial growth of relevant pathogenic, spoilage and indicator microorganisms during the following scenarios of chilling, storage, defrosting and subsequent post-defrosting storage of ungulates meat:

- Scenario 1 (7°/noVP/15d), which represents the reference scenario: aerobic storage at 7°C until 15 days after slaughter;
- Scenario 2 (7°/VPst/42d): storage at 7°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation;
- Scenario 3 (3°/VPst/42d): storage at 3°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation;
- Scenario 4 (7°/VP15/42d): storage at 7°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter;
- Scenario 5 (3°/VP15/42d): storage at 3°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter.

Considering the data available in the scientific literature on the input variables needed to estimate bacterial growth in meat, only meat from bovine, ovine and porcine animals could be included in the assessment. Considering similarities of meat characteristics among species and of relevant bacteria to be considered, the assessment performed for ovine meat can be extrapolated to caprine meat, and the assessment performed for bovine meat can be extrapolated to equine meat. The assessment cannot be extrapolated to other ungulate species.

The following microorganisms were considered relevant for this assessment, with respect to the five animal species covered. With regard to pathogenic bacteria: *Salmonella* spp. (all animal species), Shiga toxin-producing *E. coli* (bovine, ovine and caprine species), *L. monocytogenes* (all animal species), *Y. enterocolitica* (porcine species) and non-proteolytic *C. botulinum* (all animal species). With regard to spoilage bacteria: pseudomonads, lactic acid bacteria (LAB) and psychrotolerant *Clostridia* (all animal species). With regard to indicator microorganisms: aerobic colony count, *Enterobacteriaceae* and *E. coli* (all animal species).

Predictive microbiology models were used to estimate microbial growth. They were selected and evaluated based on their validated performance in meat matrices and ability to account for temperature, pH, water activity effects and for packaging under vacuum conditions. Models took into account mean and conservative conditions of temperature and, where possible, pH and water activity during chilling, storage and defrosting, defined as Baseline I conditions (mean) and Baseline II conditions (conservative).

Within Term of Reference (ToR) 1.1, the BIOHAZ Panel assessed microbial growth in Scenarios 2, 3, 4 and 5 compared to Scenario 1. Considering the results obtained and the uncertainties identified, it is judged to be 95%–99% certain (extremely likely) that Scenario 2 (7°/VPst/42d) and Scenario 4 (7°/VP15/42d) result in more growth of at least some of the bacteria assessed compared to Scenario 1 (7°/noVP/15d), and to be 66%–90% certain (likely) that Scenario 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d) result in more growth of at least some of the bacteria assessed compared to Scenario 1 (7°/noVP/15d). Therefore, Scenarios 2, 3, 4 and 5 are not microbiologically equivalent to Scenario 1.

Scenario 4 (7°/VP15/42d) inevitably leads to higher contamination levels than Scenario 1 (7°/noVP/15d); therefore, it was not considered further in the assessment. Also, given that Baseline II conditions (conservative) predicted growth substantially higher for all microorganisms considered, making comparisons not informative, they were not considered further in the assessment.

Within ToR 1.2, the BIOHAZ Panel assessed both the storage times leading to microbiological equivalence between Scenarios 2, 3 and 5 and Scenario 1 and the times at which the spoilage threshold of $7\log_{10}$ CFU/cm^2 would be reached for LAB and *Pseudomonas* (time to spoilage).

Considering the results obtained and the uncertainties identified, it is judged to be 66%–90% certain (likely) that Scenario 2 (7°/VPst/42d) allows 5–6 days of storage before microbiological equivalence with Scenario 1 (reference) (7°/noVP/15d) is reached; and it is judged to be 66%–90% certain (likely) that Scenarios 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d) allow 29–30 days of storage before microbiological equivalence with Scenario 1 (7°/noVP/15d) is reached. Overall, LAB were often the limiting bacteria defining practical shelf-lives (spoilage times). It is also important to note that the scenarios for LAB contamination are strongly dependent on the hygiene level. Fresh meat with higher initial contamination was predicted to reach the spoilage threshold of 7 $\log_{10}$ CFU/cm^2 much earlier, which in practice shortens the usable storage time. Based on the initial levels of spoilage bacteria considered in this assessment, it is concluded that, under better hygiene conditions (lower initial load of spoilage bacteria), equivalence time is primarily determined by pathogens, whereas under worse hygiene conditions (higher initial load of spoilage bacteria), reaching the spoilage threshold rather than equivalence time (pathogenic and spoilage bacteria) with Scenario 1 (7°/noVP/15d, reference scenario) defines the practical limit.

Within ToR 2.1, the BIOHAZ Panel assessed the effect of defrosting scenarios on microbial growth, considering several scenarios where freezing occurs at −12°C or −18°C, defrosting at 4°C or 7°C, for short (4–8 h) or long (24–72 h) defrosting duration, and with dynamic or static defrosting applied, in thin (5 cm) or thick (15 cm) meat pieces. Considering the results obtained and the uncertainties identified, it is judged to be 90%–95% certain (very likely) that the defrosting phase under the conditions assessed does not lead to relevant growth of pathogenic and spoilage bacteria.

The impact of post-defrosting storage for 7 days at 4°C or 7°C was also assessed. Storage at 7°C after defrosting led to substantial growth of both pathogenic and spoilage bacteria, with $\log_{10}$ increases nearly as high as those observed under Scenario 1 (7°/noVP/15d). Therefore, storage conditions at this temperature were not further considered in the assessment.

Post-defrosting storage at 4°C was predicted to result in increases of *Y. enterocolitica* (2.8 $\log_{10}$), *L. monocytogenes* (1.5 $\log_{10}$), LAB (2.0 $\log_{10}$) and *Pseudomonas* (3.8 $\log_{10}$).

Within ToR 2.2.1, the BIOHAZ Panel adjusted equivalence times defined under ToR 1.2.1 taking into account the above-described impact of post-defrosting storage and identified the times at pre-freezing stage that would ensure microbiological equivalence among scenarios at the end of post-defrosting storage for 7 days at 4°C. For Scenario 2 (7°/VPst/42d), no adjustment was required since *Salmonella* remained the limiting bacteria, with no significant additional growth during defrosting and post-defrosting storage. For Scenarios 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d), the adjusted equivalence times were shorter for both pathogenic and spoilage bacteria: *L. monocytogenes* (for bovine and ovine meat) and *Y. enterocolitica* (for porcine meat) reached equivalence at approximately 18–23 days, while LAB reached equivalence at approximately 13–16 days.

Finally, within ToRs 1.2.2 and 2.2.2, levels of indicator microorganisms selected as most relevant for verification purposes (aerobic colony counts (ACC), *Enterobacteriaceae*, *E. coli*) were predicted, considering three initial contamination levels, which reflect different possible meat hygiene conditions at post-slaughter chilling. Predicted levels represent examples under the assumption of initial contamination levels and model parameters, which can take a wide range of values and should be further adjusted based on actual measurements in practical settings.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor – Part I: Microbiological safety of ungulates meat intended to be frozen

Regulation (EC) No 853/2004[1] lays down a limited number of specific requirements for the hygiene of frozen meat of ungulates:

- Some information requirements (date of production and date of freezing) are laid down in Section IV of Annex II;
- Fresh meat of ungulates intended to be frozen must be frozen after slaughter without undue delay taking into account, where necessary, a stabilisation period before freezing in accordance with point 4 of Chapter VII in Section I (domestic ungulates) and point 1 of Section III (even-toed farmed game) to Annex III.

Additional European Union (EU) harmonised requirements on the storage and transport temperatures of certain frozen food exist:

- Council Directive 89/108/EEC[2] includes, among others, a maximum temperature of −18°C for storage and transport of quick-frozen food. The percentage of quick-frozen meat of ungulates placed on the market is low compared to frozen meat of such animals;
- For minced meat, meat preparations, mechanically separated meat and fishery products, a maximum temperature for storage and transport of frozen products is laid down in Regulation (EC) No 853/2004 (−18°C, respectively, in Annex III, Section V, Point 2(c)(ii) and 4(f), and Section VIII, Chapter I(C)(1) and Chapter III(B) with the exception of whole fish frozen in brine intended for canning: not more than −9°C, Chapter II(7)).

No maximum temperature limits for the storage and transport are laid down and wordings such as 'undue delay' and 'stabilisation' or 'stabilisation period' are not defined. There is no scientific opinion developed by EFSA or the former Scientific Committees of the Commission providing recommendations to lay down possible additional EU harmonised requirements on frozen meat.

In points 2.1.1 to 2.1.4 of Chapter 2 of Annex I to Commission Regulation (EC) No 2073/2005,[3] some process hygiene criteria (aerobic colony count, Enterobacteriaceae, *Salmonella*) have been laid down for carcasses of ungulates after dressing but before chilling in slaughterhouses. After that stage, some additional process hygiene criteria (aerobic colony count) are only laid down for (fresh) carcasses in specific transport situations in accordance with point (3)(b)(viii) in Chapter VII of Section I of Annex III to Regulation (EC) No 853/2004.

Recently, the Commission updated its staff working document: '*Guidance on the implementation of certain provisions of Regulation (EC) No 853/2004 on the hygiene of food of animal origin*',[4] among others by adding a Section 5.8. This section provides the Commission's interpretation on freezing of fresh meat without undue delay, mainly from a legal perspective by lack of scientific data on the risk. The Commission also recently amended the requirements of Regulation (EC) No 853/2004, providing a derogation on the obligation to freeze meat without undue delay, in case of dry-ageing of beef,[5] allowing freezing of dry-aged bovine meat until 35 days from the end of the stabilisation period upon slaughter.

From a risk point of view, some inconsistency exists within Regulation (EC) No 853/2004. The Regulation allows for the preparation of minced meat, followed by freezing, from chilled meat within no more than 15 days from the slaughter of the animals in case of boned vacuum-packed beef and veal (Point 2(b) and (c) in Chapter III of Section V of Annex III to Regulation 853/2004). Freezing of the same vacuum-packed meat without mincing is not allowed, although this seems to be a safer practice since mincing may represent an increased risk of microbiological growth of the product.

The above illustrates the need for a scientific opinion to provide a risk-based justification of existing or a scientific basis for possible amendments to these EU requirements for the freezing of meat to guarantee the microbiological safety of frozen meat of ungulates.

Terms of Reference (ToR)

In accordance with Art. 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to deliver a scientific opinion on the microbiological safety for the consumer (presence and growth of pathogens and spoilage bacteria after defrosting) by the

[1]Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin (OJ L 139, 30.4.2004, p. 55), as last amended by Commission Delegated Regulation (EU) 2025/1449 of 18 July 2025.

[2]Council Directive 89/108/EEC of 21 December 1988 on the approximation of the laws of the Member States relating to quick-frozen foodstuffs for human consumption (OJ L 40, 11.2.1989, p. 34), as last amended by Council Directive 2013/20/EU of 13 May 2013.

[3]Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (OJ L 338 22.12.2005, p. 1), as last amended by Commission Regulation (EU) 2020/205.

[4]https://food.ec.europa.eu/document/download/ce8efc9d-8750-4b75-9563-7427da889666_en.

[5]Point 3(a)(iv)(1) of the Annex to Commission Delegated Regulation (EU) 2024/1141 of 14 December 2023 amending Annexes II and III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards specific hygiene requirements for certain meat, fishery products, dairy products and eggs (OJ L, 2024/1141, 19.4.2024, ELI: http://data.europa.eu/eli/reg_del/2024/1141/oj).

freezing of fresh meat of ungulates (at least of domestic bovine, ovine, caprine, porcine and equine animals and of wild boars and deer, if sufficient information is available) but excluding offals as defined in Annex I of Regulation (EC) No 853/2004.

For the purpose of the following assessment:

- Steps considered in the assessment: (i) Meat stabilisation of carcass/meat cuts to reach 7°C, (ii) refrigerated storage/transport of fresh carcass/meat cuts at 7°C before freezing (unless alternative temperature requested in the ToR);
- Stabilisation period is defined as the period needed for the carcass/meat cuts to reach a core temperature of 7°C and to stabilise the pH of meat by chilling applied immediately after slaughter (a matter of a few days, so shorter than a possible 'maturation' period);
- The end point of this assessment would be the meat at the end of refrigeration and prior to freezing;
- The safety of the meat product at consumption will also be influenced by the storage conditions of the frozen meat, the thawing conditions, the subsequent storage conditions and the possible further processing. Good hygiene practices during thawing should be assumed. Further steps after thawing at food business operator level are not under the scope of this mandate;
- Pathogenic as well as spoilage bacteria should be considered since spoilage bacteria also render the meat unfit for human consumption in accordance with Article 14(5) of Regulation (EC) No 178/2002.

More specifically, EFSA is asked:

TOR 1.1. To compare the effect on survival and growth of relevant food-borne pathogenic bacteria, indicator organisms and spoilage bacteria in fresh meat of ungulates that has been stored/transported at the following conditions applied between slaughter and freezing:

- core temperature of maximum 7°C **until** 15 days after slaughter;
- core temperature of maximum 7°C **until** 6 weeks after slaughter, vacuum-packed immediately after stabilisation;
- core temperature of maximum 3°C **until** 6 weeks after slaughter, vacuum-packed immediately after stabilisation.
- core temperature of maximum 7°C **until** 6 weeks after slaughter, vacuum-packed 15 days after slaughter;
- core temperature of maximum 3°C **until** 6 weeks after slaughter, vacuum-packed 15 days after slaughter.

TOR 1.2. If differences are identified in the survival and growth of relevant food-borne pathogenic or spoilage bacteria (outcome of ToR 1), then to

- identify refrigeration times/temperatures/use of vacuum packaging scenarios for meat intended to be frozen that would result in a similar load of the relevant bacterial hazards as compared to standard fresh (never frozen) meat (**ToR 1.2.1**); and
- indicate which bacteria would be most relevant to monitor in these scenarios and what bacterial load might be expected just before freezing (**ToR 1.2.2**).

1.2 | Background and Terms of Reference as provided by the requestor – Part II: Microbiological safety of frozen meat of ungulates – Defrosting

On 18 October 2024, the Commission submitted a mandate to EFSA requesting a scientific opinion on the microbiological safety of meat of ungulates intended to be frozen. The mandate more specifically asked to deliver a scientific opinion on the microbiological safety for the consumer related to storage conditions before the freezing of fresh meat of ungulates.

The microbiological safety for the consumer will however also be influenced by the microbiological growth at and after defrosting of the meat. To allow risk managers to consider the need for additional control measures on frozen meat, an additional risk assessment should supplement the initial mandate by evaluating microbiological growth during defrosting and subsequent storage.

Current legal requirements related to frozen meat have been provided as background information in the initial mandate. Information provided by the industry on common practices of defrosting of meat of ungulates has been added to this mandate as an Appendix.[6]

Terms of Reference (ToR)

In accordance with Art. 29 of Regulation (EC) No 178/2002, the Commission asks EFSA to deliver a scientific opinion on the microbiological safety for the consumer (presence and growth of pathogens and spoilage bacteria) during defrosting and subsequent storage of meat of ungulates (at least of domestic bovine, ovine, caprine, porcine and equine animals, and of wild boars and deer, if sufficient information is available) but excluding offals as defined in Annex I of Regulation (EC) No 853/2004.

[6]Appendix to the mandate, available at: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00711.

The baseline scenario for this assessment would be meat of ungulates that has been stored/transported at core temperature of maximum 7°C and for a maximum of 15 days after slaughter and prior to freezing.

The end point of this assessment would be the meat at the end of storage after defrosting. Further industrial processing and/or retail and consumer stages are excluded from this assessment for which good hygienic practices are assumed.

It can be assumed that no bacterial growth will occur during freezing. Different defrosting practices by consumer and at retail are outside the remit of this mandate.

More specifically, EFSA is asked:

TOR 2.1. To compare the effect on survival and growth of relevant food-borne pathogenic bacteria, indicator organisms and spoilage bacteria in defrosting scenarios where freezing occurs at –12 or –18°C, defrosting at 4 or 7°C, for short (4–8 h) or long (24–72 h) duration, dynamic or static defrosting applied, meat is vacuum-packed or not, and subsequent storage for 7 days at 4 or 7°C temperature.

TOR 2.2. Based on the outcome of the mandate on the microbiological safety of ungulates meat intended to be frozen and ToR 1 of this mandate, provide scenarios that consider the pre-freezing, defrosting and storage conditions that would result in a similar load of the relevant bacterial hazards as compared to standard fresh (never frozen) meat (**ToR 2.2.1**).

Indicate which bacteria would be most relevant to monitor in these scenarios and what bacterial load might be expected at the end of storage post defrosting (**ToR 2.2.2**).

1.3 | Interpretation of the Terms of Reference

The following has been clarified with the requestor:

- The first scenario described in ToR 1.1, i.e. 'core temperature of maximum 7°C until 15 days after slaughter' is considered as the 'reference scenario' equivalent to 'standard fresh (never frozen) meat' for comparison purposes in ToR 1.2 and 2.2. It is referred to as 'Scenario 1' or 'reference scenario' throughout the opinion. The relevant duration of this reference scenario can be tailored to the specific ungulate species as considered best until a maximum period of 15 days. For meat from certain species (e.g. porcine or ovine meat), a shorter duration than 15 days may be appropriate. The other scenarios listed in ToR 1.1. are referred to as Scenarios 2–5.
- In order to take into account variability in the possible conditions during carcass chilling and storage, two different baseline conditions are considered for the different scenarios and compared among them, reflecting mean/standard conditions vs. more growth promoting conditions of temperature, pH and a_w. These are referred to in the document as 'Baseline I conditions (mean)' and 'Baseline II conditions (conservative)' and are explained more in detail when defining the parameters used in modelling.
- The stabilisation period is defined as the period needed for the carcass/meat cuts to reach a core temperature of 7°C and to stabilise the pH of meat by chilling applied immediately after slaughter. The surface temperature after the stabilisation period is considered to be the same as the core temperature, i.e. 7°C.
- For ToR 2, the WG defines defrosting time as the period required for the complete disappearance of ice crystals at the core of the meat. This marks the end of the defrosting phase. Bacterial growth on the surface is only considered up to this point.
- The assessment made in this opinion needs to include the stabilisation period, as the aim of the European Commission is to understand how long meat can be stored after slaughter and before freezing to decide how much flexibility can be allowed in the regulations to allow possibly longer storage periods before freezing. At the end of this stabilisation period, depending on the scenario, the meat is either stored at 3°C or at 7°C. At this point, the surface temperature is assumed to instantaneously adjust to the respective storage temperature.
- The assessment focuses on the potential growth and survival (no change) of bacteria only, which are the group of microorganisms being relevant under the different scenario conditions to be evaluated. For scenario comparison with the reference, the growth of either relevant food-borne pathogenic or spoilage bacteria is considered. To establish a microbiological parameter for verification purposes (referred to as 'to monitor' in the formulation of the ToRs),[7] the growth of either hygiene indicator microorganisms or spoilage bacteria is assessed.
- For pathogenic bacteria, the predicted $\log_{10}$ increase is used as a measure of the bacterial growth, whereas for spoilage bacteria and indicator microorganisms, loads together with $\log_{10}$ increase are assessed.
- In the assessment, 'ungulates meat' is used to refer both to ungulates' carcasses and meat cuts.
- Considering practical conditions during defrosting to avoid cross-contamination and the possible presence of foreign bodies originating from packaging material, it is assumed that vacuum-packed meat is always thawed inside their vacuum package and unpacked only at the end of defrosting.

During the assessment, in consultation with the mandate requestor, and considering the data available, it was decided to focus the assessment on meat from bovine, ovine and porcine animals, while excluding caprine, equine and farmed/

[7]The term 'verification' instead of 'monitoring' is used in this opinion in accordance with the general principles of food hygiene (FAO and WHO, 2023) and Commission Notice 2022/C 355/01 (OJ C 355 16.9.2022, p. 1). Within the context of food safety management system, microbiological criteria and the microbiological analysis of food are used to verify the effectiveness of the preventive and control measures. Microbiological determinations do not provide results in a real-time basis to detect deviations/failures enabling the timely application of adjustments to maintain the system within the critical limits.

slaughtered wild boar and deer, for which possible extrapolation from the above species will be considered if possible, based on available data, as explained in detail within Section 3.1.

The ToRs of the two mandates were translated into assessment questions (AQs), as reported below:

ToR 1.1:

- **AQ.1.1.1**: Which are the relevant bacteria and their levels to be considered in ungulates meat?
- **AQ.1.1.2**: Which are the representative surface and core temperature profiles for each ungulate species during the initial carcass cooling phase/stabilisation period?
- **AQ.1.1.3**: Which are the relevant intrinsic and extrinsic factors (excluding temperature) and their values for assessing bacterial growth during the storage/transport conditions?
- **AQ.1.1.4**: Which are the existing predictive microbiology models appropriate for assessing the growth of relevant bacteria (AQ.1.1.1) under the conditions defined in AQ.1.1.2 and AQ.1.1.3?
- **AQ.1.1.5**: Are the tested scenarios different in terms of potential growth and survival of relevant bacteria based on defined equivalence criteria (outcome of ToR 1)?

ToR 1.2:

- **AQ.1.2.1**: Which are the storage/transport times for scenarios (temperatures/use of vacuum packaging) of ungulates meat intended to be frozen that would result in a similar load of pathogenic and spoilage bacteria as compared to standard fresh (never frozen) meat?
- **AQ.1.2.2**: Which are the criteria to identify relevant bacteria to detect and/or quantify in these scenarios?
- **AQ.1.2.3**: Which are the relevant bacteria to detect and/or quantify and the expected loads just before freezing?

ToR 2.1:

- **AQ.2.1.1**: What are the representative surface temperature and core temperature profiles on meat of each ungulate species under the conditions to be evaluated during defrosting?
- **AQ.2.1.2**: What is the potential growth of relevant (AQ.1.1.1) bacteria at the ungulates meat surface temperature during defrosting and subsequent storage according to AQ.2.1.1?

ToR 2.2:

- **AQ2.2.1**: Which are the equivalence thresholds obtained in AQ.1.2.1 that ensure similarity to the reference scenario taking also in consideration the defrosting of ungulates' meat?

2 | DATA AND METHODOLOGIES

The approach to answer the ToRs was defined in advance and is described in the protocol (Annex A). It covers both the problem formulation (i.e. what the assessment aims to address) and which methods are used for addressing the problem. The problem formulation ('what') includes the clarification of the mandate and consists of the steps (1) translation of the mandate into scientifically answerable assessment questions (AQs), and their relationship (conceptual model) and (2) the selection of the approach for the assessment. The planning of the methods for conducting the assessment ('how') consists of (1) specifying the evidence needs and the methods for answering each AQ, including uncertainty analysis, and (2) the methods for integrating evidence across AQs and addressing the remaining and overall uncertainty. Protocol development followed the draft framework for protocol development for EFSA's scientific assessments (EFSA Scientific Committee, 2023).

In the following sections, additional details in relation to data and methodologies used in the assessment are reported, compared to the protocol, where relevant.

2.1 | Scenarios assessed

In accordance with ToR 1, the following scenarios of pre-freezing chilling and storage of meat were assessed and are further referred to in the document as Scenarios 1–5 (Figure 1):

- Scenario 1 (7°/noVP/15d), which represents the reference scenario: aerobic storage at 7°C until 15 days after slaughter;
- Scenario 2 (7°/VPst/42d): storage at 7°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation;
- Scenario 3 (3°/VPst/42d): storage at 3°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation;
- Scenario 4 (7°/VP15/42d): storage at 7°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter;
- Scenario 5 (3°/VP15/42d): storage at 3°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter.

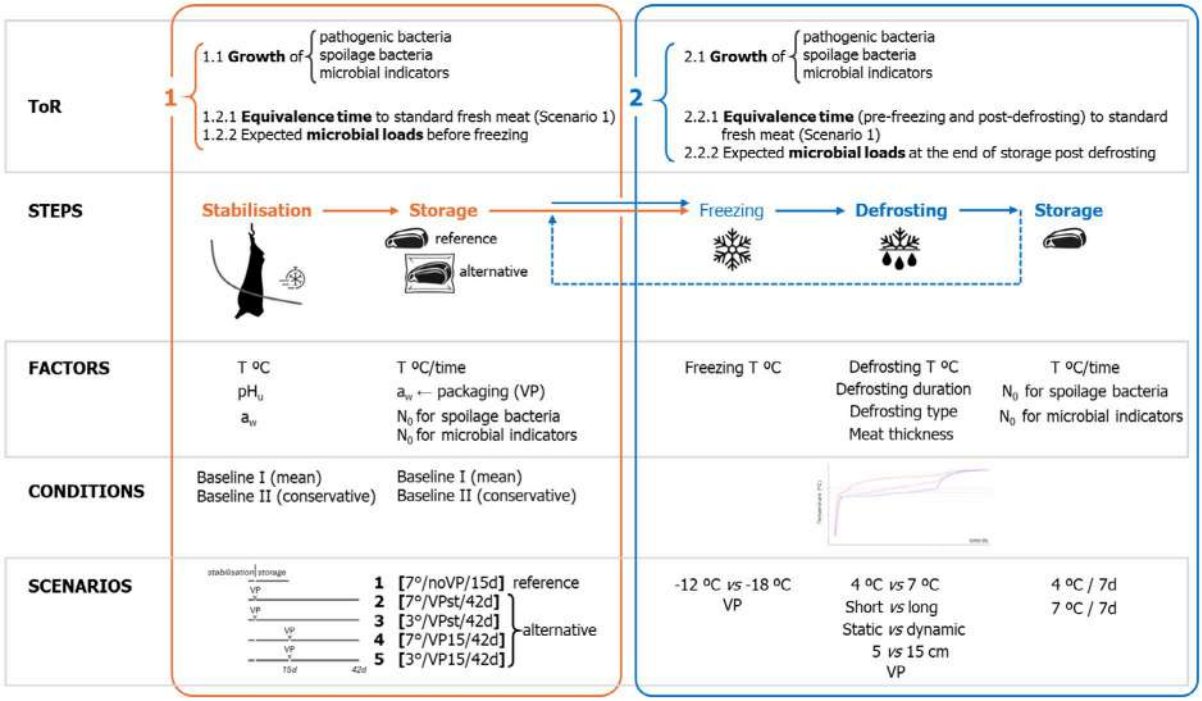

FIGURE 1 Schematic representation of the stages of the assessment and of the specific factors, conditions and scenarios considered in each ToR. a_w = water activity; N_0 = initial contamination level; pH_u = ultimate pH; T = temperature; VP = vacuum-packaged.

Given the lack of exhaustive empirical microbiological data for the above scenarios, the behaviour of pathogenic and spoilage bacteria was generated using time–temperature profiles, pH, a_w data (see Section 3.2) and predictive microbiology models calibrated/validated for fresh meat (see Section 3.3.3).

To assess the amount of growth of the pathogenic, spoilage and indicator microorganisms on ungulates meat carcasses in the different scenarios, baseline conditions for the carcass chilling and subsequent storage had to be developed describing surface temperature, pH and a_w for meat of each animal species. Growth during reference Scenario 1 was the basis for comparison of growth assessed in Scenarios 2–5. A multitude of different carcass chilling curves is compliant with the current legislation, and carcass pH and a_w may vary greatly, making the selection of baseline conditions to some extent arbitrary. To address this, two baseline conditions were defined. 'Baseline I conditions (mean)' are representative of mean values of growth controlling parameters; 'Baseline II conditions (conservative)' represent more extreme conditions that would be more favourable to bacterial growth, i.e. a more conservative scenario. Detailed explanations on the parameters used in Baseline I and II conditions are included in Section 3.2.

2.2 | Time–temperature profiles during chilling and subsequent storage

The surface time–temperature profiles during carcass chilling were developed using the same data and approach as in the EFSA opinion on transport of meat part 1 (EFSA BIOHAZ Panel, 2014a). For comparative purposes, a search for additional data was carried out (Annex B). For ovine meat, the additional data were used to develop Baseline I conditions for temperature, which were missing in the previous opinion (de Oliveira et al., 2004; Dos Santos Costa et al., 2010; Redmond et al., 2001; Sheridan, 1990). In short, exponential decay functions were fitted to the data on the surface temperatures over chilling times needed to reach a core temperature of 7°C.

For bovine and ovine meat, the following exponential decay function (Equation (1)) was used to describe Baseline I and II conditions of current chilling in terms of surface temperature:

$$T = T_0 \times e^{-kt} \quad (1)$$

where T = surface temperature (°C), T_0 = surface temperature at time 0 (°C), i.e. when chilling starts, k = chilling rate (h-1) and t = time (h).

For porcine meat, data from 42 French slaughterhouses were used to fit a modified exponential decay function (Equation 2) (ANSES, 2014):

$$T = T_a + \left(T_0 - T_a\right) \times e^{-kt} \quad (2)$$

where T_a is the asymptotic final temperature (°C), and the other parameters are as described above for Equation (1).

The models were fitted to the data using the nonlinear least squares (*nls*) method for non-linear curve fitting included in the R statistical and modelling software.[8] The resulting functions were used to simulate the time–temperature profiles for each animal species to obtain surface temperature data for the respective baseline conditions during the stabilisation/carcass chilling period.

As indicated above, Scenario 1 was taken to represent a situation where carcasses remain in the chilling room until the core temperature reaches 7°C and are then stored/transported at a constant temperature of 7°C, up to 15 days. Baseline I conditions represent the calculated surface temperature profiles, using the mean of the fitted parameters, during chilling in the slaughterhouse to a core temperature of 7°C and then, assuming that the surface temperature is the same as the surrounding temperature, a constant surface temperature of 7°C. Baseline II conditions are the calculated surface temperature profile, using the 95-percentile of fitted parameters defined in appendix A to the EFSA opinion on transport of meat part 1 (EFSA BIOHAZ Panel, 2014a), during chilling in the slaughterhouse to a core temperature of 7°C and storage/transportation at a surface temperature of 7°C up to 15 days. Baseline II conditions represent a subset of temperature profiles that would support more bacterial growth than Baseline I conditions. In Scenarios 2–5, the same surface temperatures defined in Baseline I and II conditions are used and surface temperatures during subsequent storage are as defined in the ToRs.

2.3 | pH

Data from various published studies were compiled to characterise post-mortem pH dynamics during the stabilisation period in bovine, ovine and porcine carcasses (Annex C). The selection process of relevant studies excluded those in which animals experienced pre-slaughter stress or underwent electrical stimulation, as these factors alter natural pH decline patterns.

To describe the pH dynamics during the stabilisation period, an exponential decay model (Equation 3) was used:

$$pH(t) = \left(pH_0 - pH_u\right) \times e^{(-k \times t)} + pH_u \tag{3}$$

where pH_0 is the initial pH of the carcass, t is the time (h) *post-mortem*, pH_u the ultimate pH and k the rate constant of pH decline ($h-1$).

For establishing k value, an additional filtering process of the studies was implemented: only studies with a minimum of four measurement points were retained, and analysis was limited to measurements taken within the first 48 h post-mortem. An exponential decay model with pH_0 fixed at 7.0 was fitted to each qualified data set. The models were fitted to the data using the nonlinear least squares (*nls*) method for nonlinear curve fitting included in the R statistical and modelling software. A global average of the estimated k values was established for each targeted ungulate species.

In parallel, a separate empirical approach was used to characterise pH_u. For each study with available data at 24 h post-mortem, the mean pH within that interval was first calculated. These study-level mean values were then used to derive two representative pH_u estimates across studies: the mean pH_u, calculated as the average of these study-level means, and a conservative pH_u, defined as the mean plus two standard deviations of the study-level means.

Similar to what was done for temperature, two baseline pH decline conditions were developed to represent typical and conservative patterns of post-mortem acidification during the stabilisation period. This approach was applied to bovine, ovine and porcine carcasses. For each species, a fixed initial pH (pH_0) of 7 was assumed, and the average k value (decay rate constant) was calculated from study-level exponential model fits, where pH_u was fixed. Baseline I conditions were based on the mean pH_u, while Baseline II conditions used the same average k combined with a conservative pH_u, as defined above. The latter, more conservative, approach ensures adequate safety margins when simulating pH decline in diverse production conditions.

2.4 | Determination of initial contamination levels of microbial indicator groups in ungulates meat

To address ToRs 1.2 and 2.2, which require the estimation of microbial loads of spoilage organisms and indicator microorganisms under the proposed scenarios, it was necessary to establish appropriate initial microbial counts. These values were used as starting levels in the modelling approach to assess the impact of the temperature and packaging scenarios on microbial growth. The selection of the food production stage at which to initiate the predictions is crucial to define both suitable initial levels and modelling conditions.

Microbiological indicator data were extracted from peer-reviewed articles and technical reports, focusing exclusively on ungulate carcasses. To ensure consistency and relevance, only data collected directly from carcasses in the European region were included. Studies sampling environmental surfaces or using non-carcass materials were excluded. Furthermore, carcasses submitted to interventions such as chemical sprays or novel packaging techniques were not considered unless a non-treated control group was available.

[8] www.r-project.org.

Each eligible study was reviewed to extract metadata including animal species, anatomical region (e.g. brisket, shank, midline), sampling method, cooling condition, country of origin and any reported seasonality. Sampling directly from the carcass surface, either through swabbing, sponging or excision, was a required inclusion criterion. Entries without this specification were omitted.

The compiled data were structured within a spreadsheet for harmonisation. Only quantitative microbial data were retained, specifically aerobic colony counts (ACC), *Enterobacteriaceae*, *E. coli*, lactic acid bacteria (LAB) and *Pseudomonas* spp.

Several studies reported results using detection limits, especially for *E. coli* and *Enterobacteriaceae*, which were frequently below the quantification threshold. In these cases, values reported as '<' or 'not detected' were treated as left-censored.

Where necessary, microbial counts were converted to a common unit ($\log_{10}$ CFU/cm^2). Only values representing baseline contamination under standard commercial processing conditions were retained. Data from laboratory studies or pilot-scale experiments were excluded.

2.5 | Tools for assessing microbial behaviour

The growth of pathogenic, spoilage and indicator microorganisms during carcass chilling, meat storage and defrosting of frozen meat was predicted through the application of selected predictive models of the maximum specific growth rate (μ_{max}) as a function of temperature and, when possible, pH and a_w. Predictive microbiology models available from the literature applied in the EFSA opinion on the microbiological safety of aged meat (EFSA BIOHAZ Panel, 2023) were also used here. Furthermore, models for other relevant bacteria not covered in such opinion were selected and/or obtained by fitting data extracted from the literature. The predictive performance of mathematical models was evaluated specifically for raw meat stored under aerobic conditions and vacuum-packaged as previously described in the opinion on aged meat. Briefly, growth behaviour and environmental parameters regarding temperature, pH and a_w were collected from experimental trials available in the ComBase Browser of the ComBase portal[9] and/or complemented with additional data from scientific literature. When the pH and a_w values were not reported, they were assumed to be 5.8 and 0.997, respectively, as inputs to get the predicted growth rate from the models. When directly reported the $\log_{10}$ counts were retrieved or digitalised from figures using the WebPlotDigitizer (v4.5) tool.[10] The growth rate was estimated by fitting the Baranyi and Roberts (1994) growth model using the DMFit tool available at the ComBase portal or by using the *nls* Microbio R package (Baty et al., 2024), which provides a suite of non-linear regression functions tailored to predictive microbiology. In some cases, only the growth rate was provided, without the possibility to check the fit made. The observed growth rates were compared with the predictions obtained by the models, enabling the calculation of the Bias (B_f) and Accuracy (A_f) factors according to Baranyi et al. (1999) as Equation (4) and (5), respectively:

$$B_f = \exp\left(\frac{\sum_{i=1}^{n} Ln\ PRED\mu_{max} - Ln\ OBS\mu_{max}}{n}\right) \tag{4}$$

$$A_f = exp\sqrt{\frac{\sum_{i=1}^{n} (Ln\ PRED\mu_{max} - Ln\ OBS\mu_{max})^2}{n}} \tag{5}$$

where $OBS\mu_{max}$ is the observed growth rate, $PRED\mu_{max}$ is the growth rate predicted by the model and n the number of data used to assess the predictive performance. The predictive model performance is considered good or acceptable when growth rates are not over- or underpredicted by more than 43% and 13%, respectively, corresponding to a B_f between 1.43 and 0.87 (Mejlholm et al., 2010; Ross, 1996). When B_f was higher than 1.10 or lower than 0.90, it was used as a calibration factor, allowing for a correction of the predictions provided by the mathematical models used in the simulations of microbial growth. An A_f higher than 1.5 indicates poor model precision (Mejlholm & Dalgaard, 2013).

To estimate microbial growth, the selected and fitted predictive models were simulated using deSolve (Soetaert et al., 2010) and biogrowth (Garre et al., 2023) packages in R statistical and modelling software.

2.6 | Criteria for definition of equivalence among scenarios

Within the assessment, several comparisons are made between microbial growth under different scenarios of meat storage and defrosting conditions.

An increase of 0.5 or 1 $\log_{10}$ unit is generally considered sufficient to define microbiologically relevant growth of bacteria and/or differences between the bacterial levels estimated for two scenarios. The rationale is to distinguish the actual increase of microbial populations from the experimental variability or methodological counting errors. Based on this, the

[9] www.combase.cc.

[10] https://automeris.io/.

level of 0.5 $\log_{10}$ was defined as the threshold value to identify a difference in bacterial growth when comparing different scenarios within ToRs 1.1 and 2.1 and therefore assess equivalence among scenarios, defined as 'microbiological equivalence' for the purpose of this opinion.

ToRs 1.2 and 2.2 require the identification of storage times/conditions resulting in a similar bacterial load. To answer this, the concept of 'equivalence time' was defined, which refers to the storage duration under alternative conditions that results in the same predicted microbial load as that reached in Scenario 1 (7°/noVP/15d, reference scenario).

For pathogenic microorganisms, except *C. botulinum*, the equivalence time corresponds to the storage period required to reach the same predicted $\log_{10}$ increase as in Scenario 1.

For *C. botulinum*, no growth is expected under the reference conditions (7°C, non-vacuum-packed). Therefore, equivalence cannot be established. Possible risks are represented by production of botulinum neurotoxins (BoNT), which have been linked to a 2.2 $\log_{10}$ increase according to the predictive model of Koukou et al. (2021). However, compliance with this threshold value is uncertain to assess, as the predictive model used for estimating growth of non-proteolytic *C. botulinum* showed low accuracy. Considering this uncertainty, the equivalence time was defined pragmatically as the storage duration required to reach a 0.5 $\log_{10}$ increase, corresponding to the predefined threshold distinguishing 'no growth' from 'growth'.

For spoilage and indicator microorganisms, equivalence is defined as the time needed to reach the same final concentration as in the reference, assuming identical initial contamination levels.

Finally, in accordance with the past EFSA opinion on spoilage of meat (EFSA BIOHAZ Panel, 2016) the level of 7 $\log_{10}$ CFU/cm^2 for LAB and Pseudomonas was used as level to identify spoilage, and the predicted time for these bacteria to grow and reach this level was defined as 'time-to-spoilage'.

When concluding on equivalence times between alternative and reference scenarios, consideration was given to the shortest time among (i) equivalence time for pathogenic bacteria, (ii) equivalence time for spoilage bacteria and (iii) time to spoilage.

2.7 | Uncertainty analysis

As recommended by the EFSA guidance and related principles and methods on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018a, 2018b), an uncertainty analysis was implemented.

To assess the level of uncertainty associated with predictive microbiology models used in this opinion, a structured evaluation framework was developed. The uncertainty classification is based on four key criteria: (i) the number of environmental factors considered in the model (e.g. temperature, pH, a_w), (ii) the accuracy factor (A_f), which reflects the average deviation between model predictions and observed data, (iii) the quantity and quality of data used to fit the model and estimate its parameters and (iv) the quantity of independent data used for model validation and/or calibration.

Each criterion was scored qualitatively as good, medium or poor based on predefined thresholds.

Considering the first criterion, models accounting for three environmental factors were rated as 'good', while those including only one were considered 'poor'. Similarly, for the second criterion, an A_f value ≤ 1.5 was rated 'good', values between 1.5 and 2.0 as 'medium' and values ≥ 2.0 as 'poor'.

For the last two criteria – the quality and quantity of data used to fit the model and the quantity of independent data used for validation and/or calibration – the scoring was established by expert judgement within the working group drafting this opinion. The assessment was based on the number and robustness of available data sets, which was deemed higher when data came from multiple publications, involved diverse strains or encompassed a wide range of experimental conditions representative of the target meat products. Models supported by extensive and diverse data sets were rated as 'good', those relying on a moderate amount of data or restricted to a limited number of strains or conditions as 'medium' and models derived from very scarce data or theoretical extrapolations as 'poor'.

To convert these individual assessments into a single overall measure of model reliability, each rating is assigned a numerical score (good = 2, medium = 1, poor = 0). A total score was then calculated for each model. This integrated approach allowed comparing the uncertainty levels across different predictive models. Results of the evaluation are reported in Table 5.

In the case of indicators comprising different microbial species (*Enterobacteriaceae* and ACC), specific predictive models are typically lacking, and their growth is therefore inferred from the behaviour of representative surrogate species. As these proxies do not capture the full diversity or physiological behaviour of the indicator groups, a default confidence level of 'low' was assigned to the models used to represent their growth.

In addition to the specific factors and uncertainties affecting the confidence in the predictive models used within the assessment, a number of additional uncertainties affecting the assessment were identified. Table F.1 (Annex F) provides an overview of the uncertainty sources affecting the different ToRs/AQs and their impact on the conclusions.

The appraisal of the impact of the overall uncertainty on the conclusions for the different ToRs was established through consensus judgement among the experts of the working group drafting this opinion. For the different conclusions, experts first quantified independently the degree of overall uncertainty associated with selected conclusions of the assessment according to the approximate probability scale recommended within the EFSA Guidance on Uncertainty Analysis (EFSA Scientific Committee, 2018a) and then discussed together the individual judgements, agreeing on a consensus probability range. This evaluation integrated the reliability of the predictive models as described above and the effect of the

uncertainties identified in Table F.1. In addition, in the case of ToRs 1.1 and 2.1, the magnitude of the difference of the predicted microbial growth between each assessed scenario and the reference scenario was also taken into account.

3 | ASSESSMENT

3.1 | Scenarios and animal species assessed

Meat from bovine, ovine and porcine animals (i.e. the three main ungulate species) is considered in the assessment. Figure 6 provides an overview of the meat a_w, pH and temperature conditions over time that were assessed for the different animal species.

Caprine, equine as well as farmed and slaughtered wild boar and deer are excluded from the assessment due to the lack of data and the reasons presented below. However, bovine and ovine meat can be considered as proxy for equine and caprine meat, respectively.

Caprine meat is not widely consumed in Europe, and in many countries, it is restricted to consumption of younger animals. Meat from older goats is often considered as a 'by-product' of dairy goats; it is less acceptable in comparison to younger animals (Kerth, 2024) as it tends to be very tough while meat from older male goats can have an unpleasant odour. According to 2025 Eurostat data[11] (2025), there were around 10.5 million goats in the EU in 2024, which is much lower than the number of animals belonging to each of the three main ungulate species (i.e. roughly 5, 7 and 13 times less than sheep, cattle and pigs, respectively). The assessment for ovine meat may be extrapolated to caprine meat as the input factors for predicting microbial growth are very similar for these two species. The main difference compared to ovine meat is the greater leanness of caprine meat (Devine & Gilbert, 2024) and usually a somewhat higher ultimate pH (pH_u) in caprine meat (see Section 3.2.1). Relevant microorganisms including meat-borne microbiological hazards are the same for the two species (EFSA BIOHAZ Panel, 2013c).

Equine meat is also less consumed in comparison to the three main species while deliberate breeding of horses for meat production is generally concentrated in a limited number of countries in Europe or their regions (EFSA BIOHAZ Panel, 2013d). Input factors for predicting microbial growth in bovine and equine meat are similar; therefore, the assessment for bovine meat can be assumed for relevant microorganisms in equine meat.

Consumption of farmed game meat is also less frequent compared to the three main meat species (EFSA BIOHAZ Panel, 2013b; Needham et al., 2023) and has often regional importance (e.g. farmed reindeer in Nordic countries). Wild boar belongs to the same taxonomic species as domestic pig; however, there are differences between them in pH decline dynamics that seem to be more gradual in wild boar (Marchiori & Felício, 2003). Also, wild boar carcasses are more often skinned at the slaughter-line than scalded (EFSA BIOHAZ Panel, 2013b), in contrast to domestic pigs' carcasses that usually remain with skin-on. Skinned wild boar carcasses might exhibit different cooling dynamics as compared to domestic pigs. Therefore, the input factors for predicting microbial growth on meat from farmed wild boar would be more similar to ovine meat than to porcine meat. Also, due to the size of carcasses (usually between the size of bovine and ovine), temperature decline kinetics and pH decline kinetics appear to correspond to ovine meat; therefore, the input factors in farmed deer would probably be similar to ovines. Given the lack of data to justify these assumptions and the consequential large uncertainties, the assessment for relevant microorganisms in the three main species cannot be extrapolated to wild boar and deer.

3.2 | Matrix-related parameters for the different animal species

Rationale

Meat provides a nutrient-rich environment that supports the growth of various bacteria. The ability of bacteria to proliferate is influenced by intrinsic meat characteristics, such as pH and water activity (a_w). Therefore, understanding these parameters is crucial for assessing microbial growth on meat.

3.2.1 | pH

At the time of slaughter, the pH of muscle tissue is usually 7.0–7.2. Following death, glycolysis continues in the absence of oxygen, leading to the accumulation of lactic acid and a subsequent decline in pH. At the end of the stabilisation period, pH of meat of ungulates drops to a range of 5.4–5.9 that is considered as ultimate pH (pH_u) in normal conditions. The speed of acidification differs between species: It is most rapid in porcine meat (i.e. pH_u is reached in 4–12 h), followed by ovine meat (12–24 h), then bovine and equine meat (24–48 h) (Sen et al., 2004; Teixeira et al., 2017). The rate of pH decline in caprine meat is similar to ovine, although their pH_u is usually somewhat higher – elevated pH levels are attributed to the excitable nature of goats, resulting in low muscle glycogen reserves and insufficient lactic acid production (Webb et al., 2005). Based

[11] https://ec.europa.eu/eurostat/databrowser/view/apro_mt_lsgoat/default/table?lang=en.

on a limited number of studies investigating farmed and slaughtered wild boar and deer, the pH decline rate seems also to be similar to ovine meat (i.e. pH_u usually reached in 12–24 h). During storage, the pH of meat can continue to change. It often slightly increases due to the production of nitrogenous compounds by proteolysis (Zhang et al., 2020). The pH values of meat species considered in this Opinion at different points of stabilisation and further cold storage of meat are shown in Annex C.

The rate of pH decline is crucial for meat quality. If the pH drops too quickly while the temperature remains high (i.e. to ≤ 5.8 within an hour), and/or pH decline is extensive ($pH_u \approx 5.3$), protein denaturation can occur, leading to pale, soft and exudative (PSE) meat. This defect is most common in porcine meat and is associated with genetics, nutrition, environmental conditions and ante-mortem handling (Moreno et al., 2020). Contrarily, if the pH decline is insufficient (i.e. remains ≥ 6, with some as high as 6.8), the meat may become dark, firm and dry (DFD), a defect more frequent in bovine meat but also prevalent in certain breeds such as Iberian pigs (Moreno et al., 2020). The condition is linked to low muscle glycogen reserves at slaughter, often resulting from long-term pre-slaughter stress (Bowker et al., 2000). The higher pH and higher water-holding capacity of DFD meat provide more favourable conditions for bacterial growth, thus affecting both meat quality and safety.

In vacuum-packed meat (wet aging), the surface pH typically ranges from 5.1 to 5.9 for bovine meat, 5.4 to 6.3 for porcine meat, 5.5 to 5.9 for ovine meat (EFSA BIOHAZ Panel, 2023) and 5.9–6.1 for equine meat (Bonilauri et al., 2004).

Regarding microbial growth, not all bacteria are equally sensitive to pH. Figure 2 depicts the growth rate at 7°C for *Salmonella, L. monocytogenes* and LAB as a function of pH, according to the predictions provided by the models described in Section 3.3.3. While *L. monocytogenes* is relatively sensitive to pH and the growth rate at pH 5.6 is 23% slower than at pH 7, LAB are much less affected (i.e. only a 4% reduction of the growth rate when the pH decreases from 7.0 to 5.6). Similarly, *Salmonella* growth rate is only reduced by 5% when the pH drops from 7.0 to 5.0. Despite the growth of pathogens being little affected by the variation of the meat pH within the foreseeable range (ca. 5.8–7) of fresh meat of different ungulate species, this factor will be considered for assessing the microbial behaviour when the predictive model has pH as an input parameter.

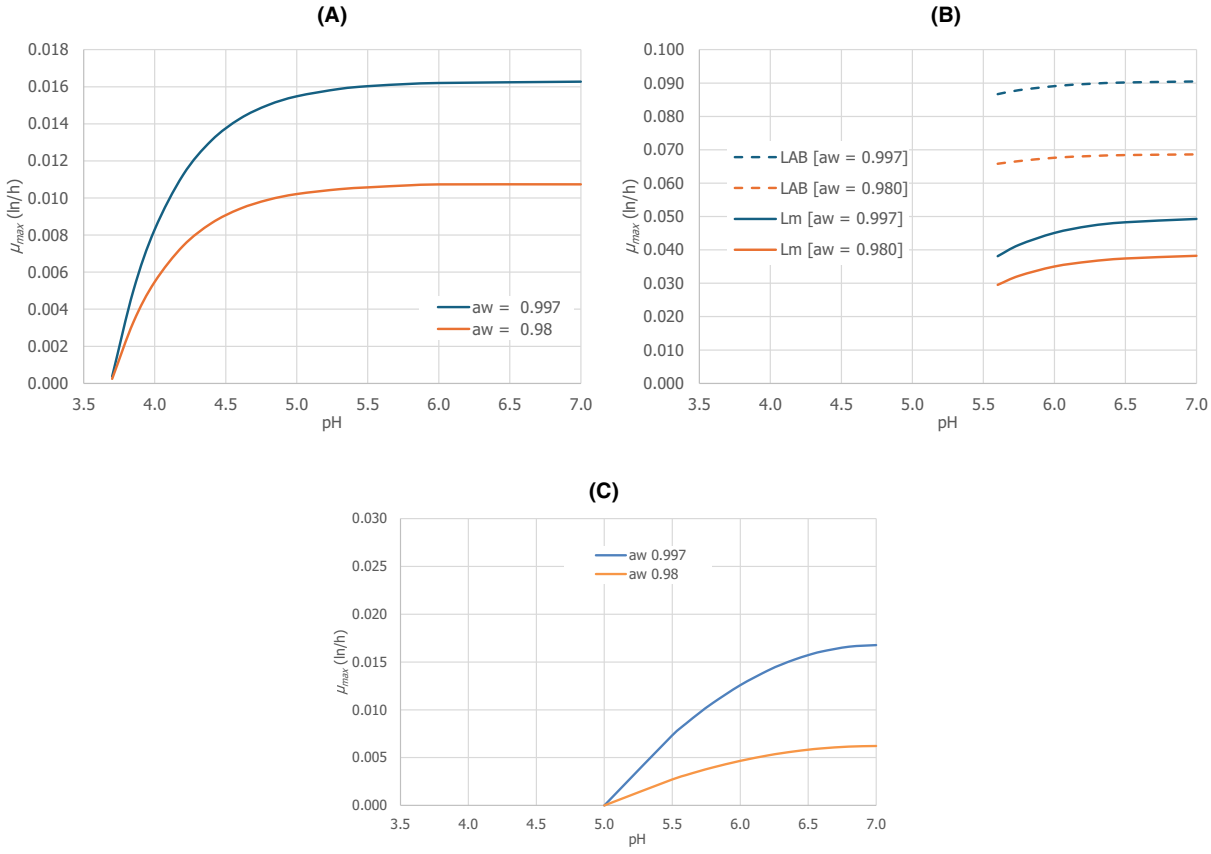

FIGURE 2 Effect of pH and aw on the growth rate (μmax) of Salmonella [A], *L. monocytogenes* (Lm) and lactic acid bacteria (LAB) [B] and non-proteolytic *C. botulinum* [C] at 7°C according to the predictive models used in this opinion (Table 5).

For bovine, ovine and porcine meat, the exponential decay equation indicated in Section 2.3 (Equation 3) was used to describe Baseline I (mean) and II (conservative) conditions for post-mortem pH decline during the stabilisation period, where the initial pH (pH_0) was fixed at 7.0 (see Table 1 with parameter values). Table 1 summarises the parameters of the equations describing the pH decline during the stabilisation period of bovine, ovine and porcine meat.

TABLE 1 Summary of the pH baseline conditions for bovine, porcine and ovine meat in terms of the equations describing pH decline with time during the stabilisation period. Baseline I conditions use the mean values of pH_u and k, while Baseline II conditions use the mean value of k and an elevated pH_u (mean + 2 standard deviations) to account for biological variability.

Meat species	Baseline I conditions	Baseline II conditions
Bovine	$pH = (7 - 5.72) \times e^{-0.35t} + 5.72$	$pH = (7 - 6.20) \times e^{-0.35t} + 6.20$
Ovine	$pH = (7 - 5.76) \times e^{-0.27t} + 5.76$	$pH = (7 - 6.14) \times e^{-0.27t} + 6.14$
Porcine	$pH = (7 - 5.70) \times e^{-0.44t} + 5.70$	$pH = (7 - 5.99) \times e^{-0.44t} + 5.99$

Figure 3 presents the post-mortem pH decline curves for the three studied species (bovine, ovine, and porcine) according to the two baseline conditions developed (see Section 2.3). The models are represented over a 30-h post-mortem period, corresponding to the pH stabilisation phase.

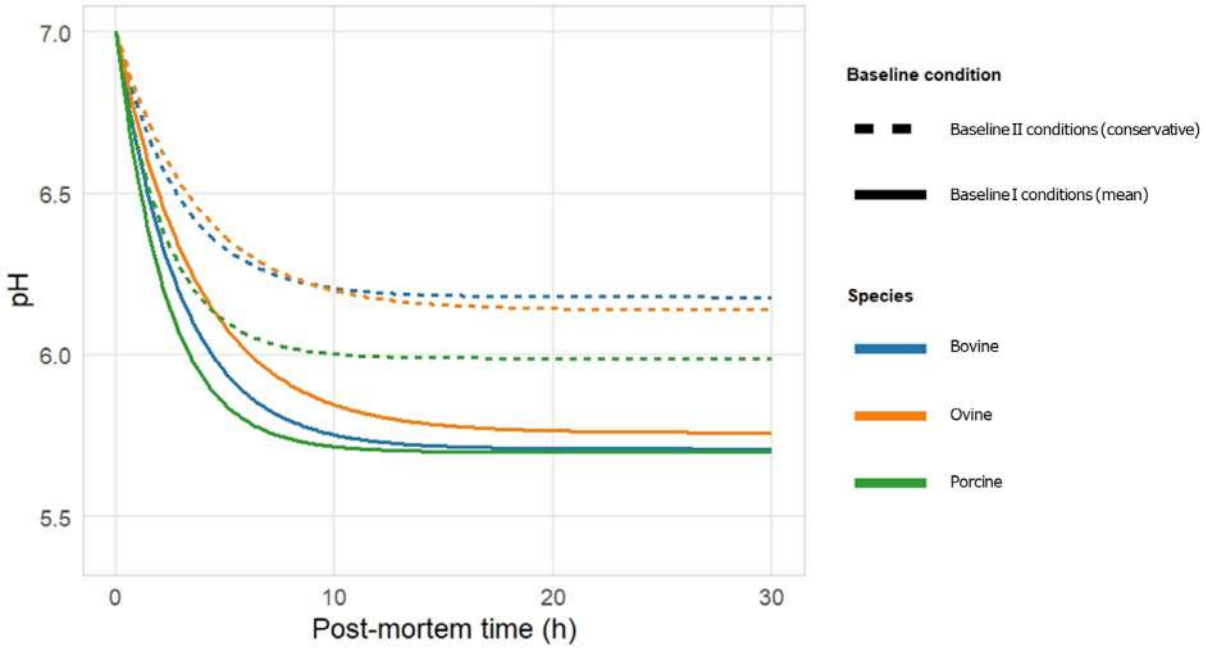

FIGURE 3 Post-mortem pH decline models during the stabilisation period for bovine (blue), ovine (orange) and porcine (green) carcasses. Solid lines represent Baseline I conditions using mean pH_u and k parameter values. Dashed lines represent Baseline II conditions using mean k values with elevated pH_u (mean + 2 standard deviations). Models are based on Equation (3), with parameters indicated in Table 1.

3.2.2 | Water activity (a_w)

The a_w of fresh meat (muscle) is generally high, around 0.99 (ICMSF, 2005). The surface a_w of carcasses and meat cuts is heterogeneous as they may have fat, connective tissue and muscle with different water binding capacity. During carcass chilling, the surface is exposed to chilled air flow and the a_w is expected to decrease due to moisture loss, typically reaching values of 0.96–0.97 (Reid et al., 2017). This fact is rarely reflected in the scientific literature, possibly related to the technical limitations for obtaining accurate surface-specific a_w values on meat carcasses or cuts. It is difficult to sample and measure only the most superficial layer, without including more internal parts with a higher a_w that may bias the analytical results. Due to the lack of accurate a_w values for meat of the different ungulate species, for the purpose of assessing microbial growth during ungulates meat stabilisation and storage through predictive microbiology models, the a_w scenarios used were based on those established for standard fresh meat in the EFSA opinion on aged meat (EFSA BIOHAZ Panel, 2023), with additional supporting bibliographical references as indicated in Annex D (Figure 4). Irrespectively of the animal species, meat just after slaughter was considered to have an a_w value of 0.990. After stabilisation, the a_w at the surface of the meat carcass decreases to 0.970 and continues to decrease to 0.950 at the end of 15 days of storage. All changes were assumed to be linear along storage time. Except for Scenario 1 (reference scenario, 7°/noVP/15d), the other scenarios include vacuum-packaging, either immediately after stabilisation (Scenarios 2 and 3) or after 15 days (Scenarios 4 and 5). For vacuum-packed meat, the a_w was assumed to be 0.980 immediately after packaging. The above-mentioned a_w values were considered for Baseline I conditions, while a conservative value of a_w of 0.990 (e.g. muscle surface of a just cut meat piece) till the end of the 6 weeks of storage was considered for Baseline II conditions.

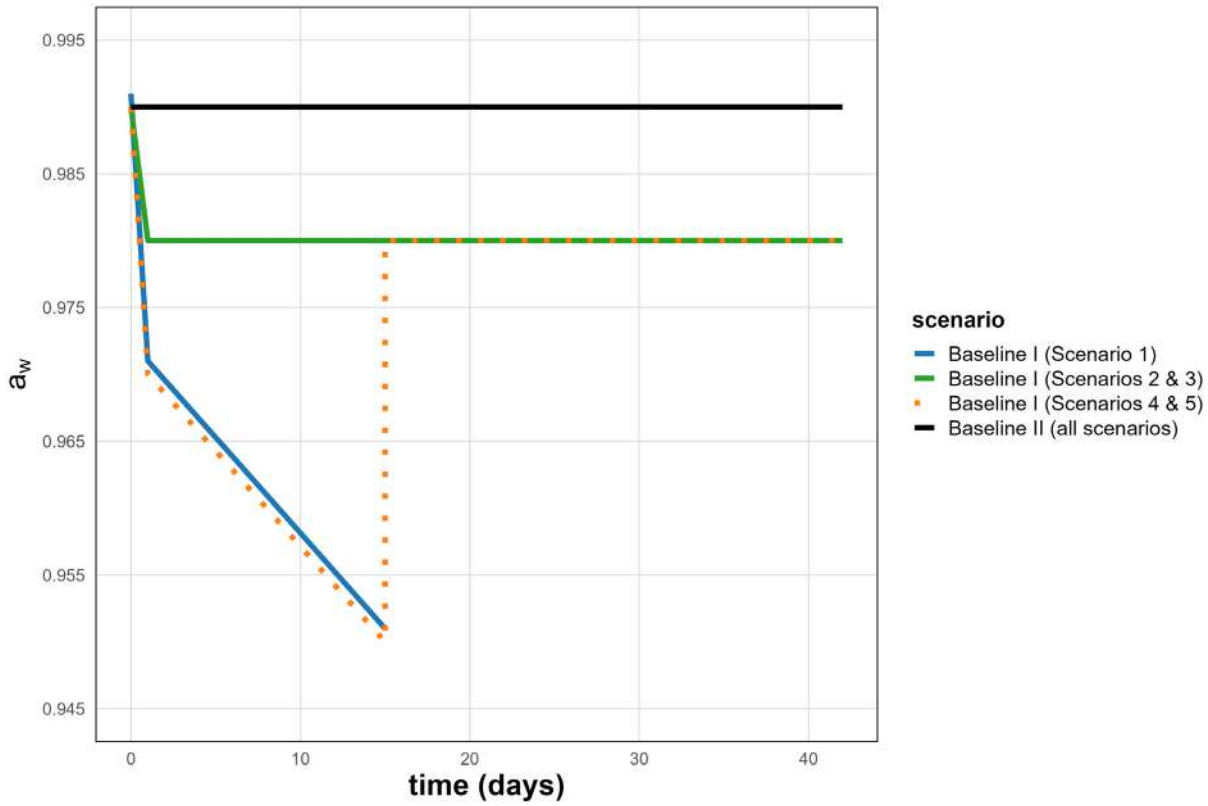

FIGURE 4 Meat a_w values along storage time considered for the different storage scenarios: Baseline I conditions for Scenario 1 (7°/noVP/15d), for Scenarios 2 (7°/VPst/42d) and 3 (3°/VPst/42d), and for Scenarios 4 (7°/VP15/42d) and 5 (3°/VP15/42d). An a_w of 0.990 along the storage was used as Baseline II conditions for all scenarios.

The a_w represents the availability of free water for microbial growth, and most bacteria require high a_w values (> 0.91) for proliferation. The slight reduction in a_w during chilling can decrease microbial growth but has only a limited effect on inactivation of microorganisms considered in this opinion (see also EFSA BIOHAZ Panel, 2023). However, not all bacteria are equally sensitive to a_w decreases. As shown in Figure 2, for a slight reduction of a_w, from 0.997 to 0.980, a reduction of growth rate by 34% is predicted for *Salmonella*, by 23% for *L. monocytogenes*, and by 22% for LAB (at 7°C within the pH range ca. 5.6–7). Given 10% of change of the growth rate is generally considered to be a microbiology relevant difference (Métris et al., 2006); therefore, these results indicate that, within the range of physicochemical characteristics of the ungulates meat, the impact of a_w is higher than the impact of pH.

3.2.3 | Representative surface and core temperature profiles for each ungulate species during the initial carcass chilling phase/stabilisation period

Microbial growth is mainly related to carcass surface and not core temperatures. Since current legislation is based on core temperatures, few data are available on carcass surface temperature decline during chilling, and even fewer on the core and surface temperatures on the same carcass during chilling. There is no simple relationship between carcass surface and core temperatures. The approach used was to fit exponential decay functions to carcass surface temperature data and determine the time to reach 7°C from the corresponding measurement of carcass core temperature data. The decay functions used to develop the baseline conditions during carcass chilling are slightly different for the different species due to the type and amount of input data that were available.

For bovine and ovine meat, the exponential decay function (Equation (1)) was used to estimate parameters to describe Baseline I and II conditions of carcass chilling in terms of surface temperatures (Figure 5, Table 2). The storage time indicated in Table 2 is the time for Scenario 1 (reference scenario, 7°/noVP/15d). The other scenarios employed the same parameters for the carcass chilling baseline conditions, but with the storage times defined in the ToRs.

Bovine meat cooling curves were compared with newer data showing that the mean time–temperature cooling curve for three weight classes of bovine meat was between Baseline I and II conditions curves based on the opinion on transport of meat (Annex B, Figure B1). For ovine meat, additional data were retrieved which was used to develop the temperature profiles for Baseline I conditions. This baseline was omitted from the opinion on transport of meat since data reporting both core and surface temperature from the same animals were missing. Some such data were found (Redmond et al., 2001; Sheridan, 1990) and complemented with data reporting either core or surface temperature (see Annex B).

For porcine meat, data from 42 French slaughterhouses were used to estimate parameters of Equation (2), a modified exponential decay function (ANSES, 2014) to describe carcass chilling Baseline I and II conditions (Table 2).

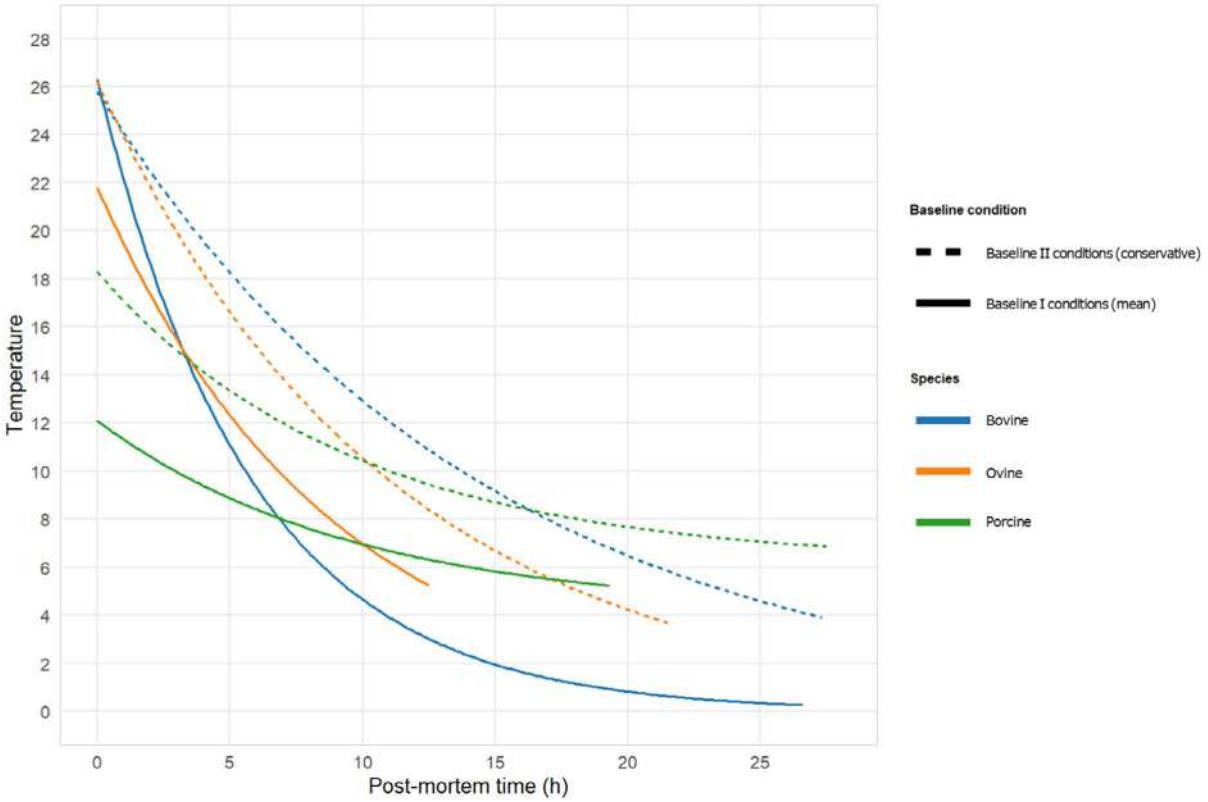

FIGURE 5 Post-mortem temperature decline models during the stabilisation period for bovine (blue), ovine (orange) and porcine (green) carcasses. Solid lines represent Baseline I conditions (mean) and dashed lines represent Baseline II conditions as defined by the parameter values in Table 3 and Equations (1) and (2).

TABLE 2 Summary of the temperature Baseline I and II conditions for bovine, ovine and porcine meat in terms of the equations describing surface temperature decline with time, and the time to reach a core temperature of 7°C (time in slaughterhouse) and the temperature during up to 15 days of storage/transport, i.e. Scenario 1. Baseline II conditions represent a subset of temperature profiles that would support more bacterial growth, i.e. a more conservative scenario than in Baseline I conditions which is considered representative of a chilling process based on estimated mean values.

	Baseline I conditions			Baseline II conditions		
Meat species	Surface temperature during chilling (°C)	Time to core temperature of 7 (°C) during chilling in slaughterhouse (hours)	Surface temperature up to 15 days storage/ transport (i.e. scenario 1) (°C)	Surface temperature during chilling (°C)	Time to core temperature of 7 (°C) during chilling in slaughterhouse (hours)	Surface temperature up to 15 days storage/transport (i.e. scenario 1) (°C)
Bovine	$T = 26.3 \times e^{-0.17t}$	26.6	7	$T = 25.8 \times e^{-0.07t}$	27.3	7
Ovine	$T = 21.8 \times e^{-0.11t}$	12.5	7	$T = 26.2 \times e^{-0.09t}$	21.5	7
Porcine	$T = 4.2 + (12.1 - 4.2) \times e^{-0.11t}$	19.3	7	$T = 6.2 + (18.3 - 6.2) \times e^{-0.11t}$	27.5	7

3.2.4 | Overview of parameters used in modelling

Figure 6 gives an overview of the parameters used for the five different scenarios and both Baseline conditions I and II. It should be noted that not all parameters are included in every model, and a detailed overview of which parameters were used in each model is provided in Table 5.

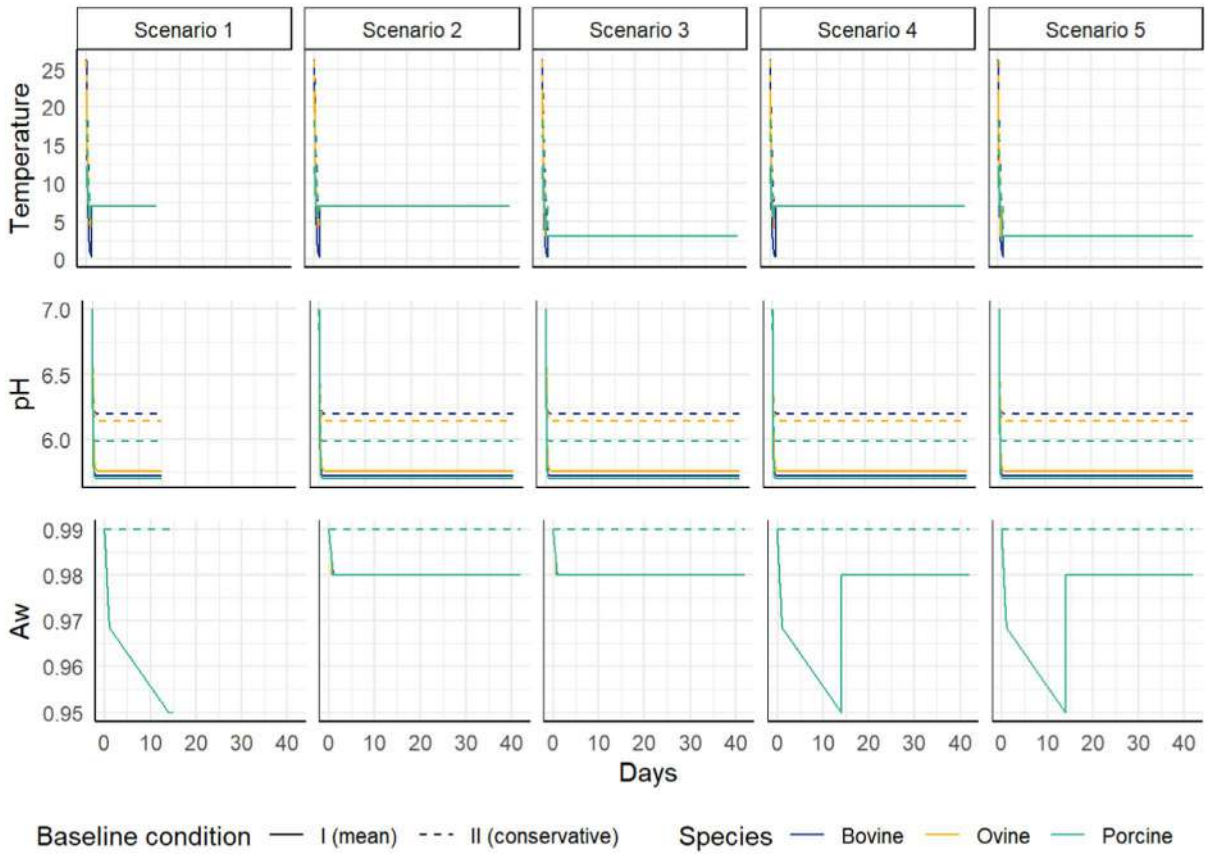

FIGURE 6 Overview of the temperature, pH and a_w values used in the models for the different scenarios for bovine (blue), ovine (orange) and porcine (green) carcasses/meat. Solid lines represent Baseline I conditions (mean) and dashed lines represent Baseline II conditions (conservative). The temperature decline on carcasses during the initial hours differs between animal species and baseline conditions, but this is not visible at the scale of this figure (see Figure 5 in Section 3.2.3 to see the differences). After the stabilisation period, the same temperature is used for all species, so only one line is shown. Similarly, for a_w, only one line is displayed, as the values are identical across animal species.

3.3 | Microorganisms and associated models to assess their growth on meat

3.3.1 | Relevant microorganisms

Only bacteria are considered relevant for the purpose of this opinion. Their relevance for the different animal species is summarised in Table 3 and briefly explained in the following sections.

Parasites and viruses do not grow in/on the carcasses/meat and therefore are excluded from any consideration. Yeasts and moulds may be part of the meat spoilage microbiota when conditions permit during aerobic storage, i.e. only when bacterial growth is prevented, because they grow far more slowly than the spoilage bacteria (Yang & McMullen, 2024). Furthermore, moulds such as *Aspergillus* spp. and *Penicillium* spp. are not capable of producing mycotoxins on meat at temperatures between −0.5 and 3°C, a relative humidity of 75%–85% and an airflow of 0.2–0.5 m/s (EFSA BIOHAZ Panel, 2023), so they are not considered hazards in meat stored at these chilling parameters.

TABLE 3 Relevant pathogenic, spoilage and hygiene indicator microorganisms in ungulates meat intended to be frozen.

Microbial group/species		Animal species[a]						
		Bovine	Ovine	Porcine	Caprine	Equine	Wild boar (farmed)	Deer (farmed)
Pathogens	*Salmonella*	√	√	√	√	√	√	√
	STEC	√	√	–	√	–	–	√
	L. monocytogenes	√	√	√	√	√	√	√
	Y. enterocolitica	–	–	√	–	–	√	√
	Only for vacuum-packaging scenarios: *Cl. botulinum* non proteolytic (psychrotrophic)	√	√	√	√	√	√	√
Spoilage	Pseudomonads (aerobic)	√	√	√	√	√	√	√
	Lactic acid bacteria (aerobic, anaerobic)	√	√	√	√	√	√	√
	Only for vacuum-packaging scenarios: *Clostridium* spp. (anaerobic)	√	√	√	√	√	√	√
Indicator	Aerobic colony count	√	√	√	√	√	√	√
	Enterobacteriaceae	√	√	√	√	√	√	√
	E. coli	√	√	√	√	√	√	√

[a]Bovine, ovine and porcine animals: assessed within the opinion; equine and caprine animals (shaded in light grey): extrapolation assumed from bovine and ovine animals, respectively; farmed wild boar and farmed deer (shaded in dark grey): not assessed within the scientific opinion.

3.3.1.1 | *Pathogens*

Salmonella can be attributed to bovine, ovine and porcine meat and is considered a relevant hazard for all animal species assessed in this opinion. Process hygiene criteria for *Salmonella* are set for dressed carcasses of all domestic species according to Regulation (EC) No 2073/2005 and are considered a priority for meat inspection/safety of bovine (EFSA BIOHAZ Panel, 2013a), porcine (EFSA BIOHAZ Panel, 2011) and farmed wild boar (EFSA BIOHAZ Panel, 2013b). *Salmonella* criteria are also set for chilled carcasses in many overseas countries, such as Australia, including wild boar and deer (DAFD, 2023) and the USA (FSIS, 1996). The limits for *Salmonella* are set in the EU as food safety criteria for meat (minced meat, meat preparations, mechanically separated meat (MSM), meat products) on the market.

Shiga toxin-producing *Escherichia coli* (STEC) is selected to be assessed in bovine and ovine meat. The hazard is considered relevant also for caprine and farmed deer. Meat of ruminants is among the most important sources of STEC human infections (EFSA BIOHAZ Panel, 2013a, 2013c; Sauvala et al., 2023); this hazard is also prioritised for inspection of bovine, ovine and caprine meat (EFSA BIOHAZ Panel, 2013a, 2013c). Although STEC is occasionally found in/on porcine, equine and wild boar and their meat/carcasses (EFSA BIOHAZ Panel, 2011, 2013b, 2013d), it is not considered as particularly important in these species due to low attribution to human disease.

Listeria monocytogenes is selected to be assessed in bovine, ovine meat and porcine meat. This hazard is relevant for all animal species considered in this scientific opinion as it can be attributed to all the respective meats. It is a ubiquitous and psychrotrophic hazard implying it is often present and able to grow on meat during cold storage (Eglezos, 2024).

Yersinia enterocolitica is selected to be assessed in porcine meat. In addition to pigs, it is considered relevant in wild boar and deer because this psychrotrophic microorganism often occurs in these animals and their meat (Altissimi et al., 2023; Aschfalk et al., 2008; Fredriksson-Ahomaa et al., 2009; Martínez et al., 2011; Van Damme et al., 2015). It is also assessed as a priority hazard for meat inspection of porcine (EFSA BIOHAZ Panel, 2011). *Yersinia* occasionally occurs in other ungulate species and their meat/carcasses (Łada et al., 2023; Sierra et al., 1995), but these are not considered as particularly important sources of human yersiniosis, as usually non-pathogenic strains are isolated.

Non-proteolytic *Clostridium botulinum* is selected to be assessed in vacuum-packed bovine, ovine and porcine meat. Given the ubiquitous nature of spores of this strict anaerobic and psychrotrophic microorganism (Austin, 2014), it is a relevant hazard for all species considered in this scientific opinion.

3.3.1.2 | *Spoilage bacteria*

Pseudomonas is selected for assessment in aerobically stored bovine, ovine meat and porcine meat. *Pseudomonas* is relevant for all animal species considered in this scientific opinion because it is generally recognised as the main spoilage bacteria of meat stored under aerobic low temperature conditions (EFSA BIOHAZ Panel, 2016, 2023).

LAB are selected for assessment in vacuum-packed bovine, ovine and porcine meat. LAB are recognised as major meat spoilage bacteria under anaerobic and chilled storage conditions and are thus considered relevant in all animal species being assessed (EFSA BIOHAZ Panel, 2016, 2023).

Blown-pack spoilage of vacuum-packed meat is sometimes caused by strict anaerobic *Clostridium* spp. strains that can grow even below 3°C (Bolton et al., 2015; Húngaro et al., 2016). Therefore, this microorganism is also considered relevant in vacuum-packed meat of all animal species in this scientific opinion and assessed in bovine, ovine and porcine meat.

3.3.1.3 | *Indicator microorganisms*

Aerobic colony count (ACC), sometimes referred to as aerobic plate count (APC) or total viable count (TVC), is an indicator of the general microbiological condition of meat (Schaffner & Smith-Simpson, 2014) and relevant for all animal species considered in this Opinion. Process hygiene criteria for ACC are set for dressed carcasses of all domestic (i.e. not for wild boar and deer) ungulate species as well as for minced meat and MSM by the EU legislation (Regulation EC No 2073/2005),[12] and in Australia for chilled carcasses exported to the EU (DAFD, 2023). Many different microbial species grow on meat and contribute to ACC (Schaffner & Smith-Simpson, 2014). However, *Pseudomonas* spp. is found to dominate in growth (and spoilage) of chilled meat stored aerobically (Dorn-In et al., 2024; Harrison et al., 1981; Koutsoumanis et al., 2006), while LAB dominate in chilled meat stored anaerobically (Chen et al., 2020; Jääskeläinen et al., 2016; Rodriguez-Caturla et al., 2025). Therefore, *Pseudomonas* spp. and LAB are used in subsequent sections to model ACC levels in aerobically and anaerobically (vacuum-packaged) chilled meat intended for freezing, respectively.

The family Enterobacteriaceae is relevant for all species because it contains genera that are important meat-borne pathogens (such as *Salmonella*, STEC), while some psychrotrophic members are causing meat spoilage during cold storage, particularly if there is temperature abuse (Nychas et al., 2008). Process hygiene criteria for Enterobacteriaceae are set for dressed carcasses of all domestic ungulate species by the EU legislation.

Escherichia coli is selected to be assessed in bovine, ovine meat and porcine meat. *E. coli* is relevant for all species as this microorganism is considered to be a specific indicator of faecal contamination of meat (Schaffner & Smith-Simpson, 2014). Process hygiene criteria for *E. coli* are set in the EU legislation for minced meat, MSM and meat preparations, although not for carcasses. However, *E. coli* microbiological criteria exist for ungulates' carcasses in other countries, such as Australia (including also wild boar and deer; DAFD, 2023) and the USA (FSIS, 1996).

3.3.2 | Initial contamination levels of microbial indicator groups in ungulates meat

Bacterial loads observed on carcasses show considerable variability (Alvseike et al., 2019). For instance, Alonso-Calleja et al. (2017) reported levels ranging from 0.7 to 6.19 $\log_{10}$ CFU/cm^2 for ACC and 0.7 to 5.54 $\log_{10}$ CFU/cm^2 for *Enterobacteriaceae* on ovine meat carcasses within a single slaughterhouse. This heterogeneity is influenced by several factors, including differences between slaughterhouses and their hygienic practices (Alonso-Calleja et al., 2017; Alvseike et al., 2019; Antic et al., 2021; Blagojevic et al., 2011; Lenahan et al., 2009; Salmela et al., 2013; Tsitsos et al., 2022), animal characteristics such as weight/age (Tsitsos et al., 2022) and cleanliness (Serraino et al., 2012), sampling location on the carcass (Biasino, De Zutter, Mattheus, et al., 2018; Biasino, De Zutter, Woollard, et al., 2018) and sampling method (swabbing/sponge vs. excision/destructive method) (Salmela et al., 2013). Specific practices, such as the use of lactic acid on bovine carcasses, result in a reduction of 0.9–3.8 $\log_{10}$ aerobic bacteria and 0.4–1.0 $\log_{10}$ *Enterobacteriaceae*, and 0.1–1.8 $\log_{10}$ *E. coli* counts (Antic et al., 2021). Hot water surface pasteurisation and steam vacuum have also been shown to reduce ACC, *Enterobacteriaceae* and *E. coli* levels by 0.5–0.95 $\log_{10}$ (Omer et al., 2015).

Conditions applied during carcass chilling affect bacterial levels to a variable extent. Rapid chilling of bovine carcasses can reduce bacterial counts by more than 1 $\log_{10}$ (Antic et al., 2021; McSharry et al., 2021). However, effects are not consistent as Lenahan et al. (2009) reported a reduction in ACC on 40% of ovine meat carcasses post-chilling, but an increase on 58% of the carcasses. Similarly, on porcine carcasses, ACC and *Enterobacteriaceae* counts can be both higher and lower after chilling (Lenahan et al., 2009; Tomovic et al., 2011). Reported reductions may depend not only on actual microbial inactivation but also on other factors such as differences in attachment of bacteria before vs. after cooling, or on the difficulty of recovering stressed cells due to chilling, resulting in an overestimation of the inactivation effects. Given this variability and the fact that only microbial growth is considered, it was decided to start the assessment for indicator microorganisms from the post-chilling stage. This approach avoids introducing uncertain assumptions about microbial behaviour during chilling and ensures a more robust and transparent simulation framework.

The values and study methodology reported in different studies reporting counts of indicator microorganisms on carcasses were very diverse (Annex E). Studies originated from a limited number of countries and often had a primary objective other than estimating the distribution of microbial loads. Some are based on relatively small sample sizes, a restricted number of slaughterhouses or specific animal groups. Furthermore, study protocols differ with respect to sampling site of the carcass, timing during/after chilling, sampling technique and microbiological methods. Also, results are reported in different ways, e.g. as arithmetic or geometric mean, median, range, percentiles or confidence intervals. Because of this heterogeneity, it is not possible to define a representative mean microbial count for carcasses.

Due to the scarcity of information across the different animal species and the high variability observed among studies, it was decided to adopt a scenario-based approach. Scenarios were designed to capture a plausible range of initial counts

[12]Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria on foodstuffs. OJ L 338, 22.12.2005, pp. 1–26, as amended by Commission Regulation (EU) 2020/205 of 14 February 2020.

for each indicator group, considering the diversity in hygienic practices and process parameters. These ranges were in accordance with the reported values on chilled carcasses in Annex E. The minimum and maximum of the reported ranges on chilled carcasses are summarised in Table 4 for ACC, *Enterobacteriaceae* and *E. coli*.

The reported ranges across studies varied substantially, often ranging from below the detection limit to over 6 $\log_{10}$ CFU/cm^2, confirming that microbial contamination on carcasses spans several $\log_{10}$ units. For those indicators covered by the EU process hygiene criteria, the upper bound of the range for bovine, ovine and porcine carcasses corresponded to the value M established in the microbiological criteria for carcasses after dressing. Based on these criteria and the reported ranges, three levels (low, medium, high) per indicator and animal species were selected to represent the possible range of initial contamination and used as input values in the modelling.

Low values were set close to the lowest detectable concentrations, while high values were aligned with the upper value of the reported ranges and the EU process hygiene criteria (5 $\log_{10}$ CFU/cm^2 for ACC and 3 $\log_{10}$ CFU/cm^2 for *Enterobacteriaceae*). For *Enterobacteriaceae* and *E. coli*, the lower bound was often reported as below the detection limit, so the minimum values were set to 0.0 $\log_{10}$ CFU/cm^2.

For *E. coli*, data are scarce, with most publications reporting the proportion of positive samples rather than enumeration results. Since there are no microbiological criteria for *E. coli* on carcasses and the limited number of studies reporting *E. coli* counts, arbitrary values of 0, 1 and 2 $\log_{10}$ CFU/cm^2 were chosen for all three animal species.

Enumeration data are particularly scarce for spoilage bacteria on carcasses. Two Irish studies reported *Pseudomonas* and LAB levels on chilled bovine carcasses. McSharry et al. (2021) found *Pseudomonas* and LAB mean counts of 0.9–3.8 $\log_{10}$ CFU/cm^2 and 0.7–1.8 $\log_{10}$ CFU/cm^2, respectively, depending on the chilling treatment and slaughterhouse after 24–48 h of chilling. Reid et al. (2017) reported *Pseudomonas* and LAB numbers varying from 0.3 to 1.8 $\log_{10}$ CFU/cm^2 and from 0.99 to 1.03 $\log_{10}$ CFU/cm^2 on bovine carcasses, respectively, after 24 h of chilling. As relatively few data are available, wider ranges were defined for modelling purposes to account for potential variability, aligned with the levels used for ACC and the levels used in the EFSA opinion on growth of spoilage bacteria during storage and transport of meat (EFSA BIOHAZ Panel, 2016).

TABLE 4 Summary of reported ranges of ACC, *Enterobacteriaceae* and *E. coli* on chilled carcasses in scientific literature (in $\log_{10}$ CFU/cm^2), EU process hygiene criteria for dressed carcasses (Regulation (EC) No 2073/2005) and selected values of initial counts for modelling of ACC, *Enterobacteriaceae* and *E. coli*.

Indicator	Species	Reported range in literature	Process hygiene criteria for dressed carcasses	Selected values for modelling
ACC*	Bovine	−1.2; 5.5	3.5 (m); 5.0 (M)	1.0; 3.0; 5.0
	Ovine	1.3; 6.7	3.5 (m); 5.0 (M)	
	Porcine	2.0; 5.3	4.0 (m); 5.0 (M)	
Enterobacteriaceae	Bovine	−3.5; 3.0	1.5 (m); 2.5 (M)	0.0; 1.5; 3.0
	Ovine	−1.3; 5.6	1.5 (m); 2.5 (M)	
	Porcine	< −0.4; 3.1	2.0 (m); 3.0 (M)	
E. coli	Bovine	−3.6; 2.0	Not applicable	0.0; 1.0; 2.0
	Ovine	−0.5; 2.0	Not applicable	
	Porcine	< −0.4; 2.4	Not applicable	

**Pseudomonas*/Lactic acid bacteria are used as proxies for ACC in the modelling.

No count data for psychrotolerant *Clostridium* spp. were found in scientific literature. This paucity of data is mainly a consequence of the limitations of the available analytical methods, including the difficulty of distinguishing psychrotolerant Clostridia populations from other Clostridia groups, the need for strict anaerobic conditions and their typically low population levels (Húngaro et al., 2016; Mang et al., 2021).

Despite the scarcity of quantitative data, the literature generally indicates that psychrotolerant Clostridia are present at low levels in meat, either as spores or vegetative cells. The presence of its spores on carcasses has been reported, likely due to their ability to withstand stringent conditions, such as exposure to oxygen, chemicals and high temperatures (Moschonas et al., 2011; Moschonas & Bolton, 2013). In addition, there is scientific evidence demonstrating that vegetative forms can survive up to 7 days in refrigerated environments (Adam et al., 2013).

Current knowledge suggests that carcasses contaminated with psychrotolerant Clostridia are likely to show low contamination levels, especially for vegetative cells. Although contamination by vegetative cells rather than spores represents a worst-case scenario, it remains plausible under certain conditions, such as persistent contamination sources from the environment, ventilation systems or inadequate cleaning. Consequently, in such scenarios, concentrations may be as low as or close to the typical limit of quantification of culture-dependent methods, generally ranging between 1 and −1 $\log_{10}$ CFU/cm^2.

3.3.3 | List of relevant predictive microbiology models

Previous EFSA scientific opinions addressing the behaviour of relevant pathogenic and spoilage bacteria associated with meat storage and transport (EFSA BIOHAZ Panel, 2014a, 2014b, 2016, 2023) consistently applied predictive microbiology models to estimate bacterial growth under various time–temperature scenarios. These models were either developed from laboratory experiments using culture media and/or derived or calibrated from data on meat matrices. Assumptions regarding environmental factors used as input of the predictive models (e.g. pH and a_w) often reflected worst-case scenarios – highly permissive to bacterial growth – thus leading to conservative outputs. Importantly, to support conclusions of the opinions, the primary aim of these models varied: some served to compare the log increase of pathogens across scenarios and assess the equivalence of conditions, while others aimed to predict absolute concentration levels of specific pathogenic or spoilage bacteria and/or estimate time to reach a target level of acceptability.

The primary objective of the current assessment is to estimate and compare the levels of microorganisms potentially present at the time of freezing, during defrosting and after post-defrosting storage, in different scenarios. To support this aim, predictive microbiology models should be selected based on their demonstrated predictive performance in meat matrices and their ability to reflect the influence of realistic ranges of extrinsic factors (i.e. temperature) and physico-chemical characteristics of the meat surface (such as pH and a_w). Preference should be given to models calibrated or validated using independent empirical growth data on raw fresh meat since these models better reflect the ecological environment in the scenarios relevant to chilled fresh meat prior to freezing.

Following the approach taken in the EFSA opinion on aged meat (EFSA BIOHAZ Panel, 2023), the selection of growth models prioritised cardinal parameters type models (CPM) that include parameters for key environmental factors (e.g. pH, a_w and/or temperature). Despite some of the available models being validated for a variety of food products, these products do not include fresh meat of different ungulate species. For this purpose, the predictive performance of the models for each microorganism was assessed for fresh meat by calculating the bias factor (B_f) and accuracy factor (A_f), i.e. comparing the model predictions with observed growth rates from independent experiments in fresh meat (making sure that they were not used to generate the model parameters). When relevant, the model was calibrated applying the specific correction factor (i.e. the B_f). Where available, models implemented in the abovementioned EFSA opinion on aged meat were also used in the present opinion (i.e. for *L. monocytogenes*, *Y. enterocolitica*, psychrotolerant LAB and pseudomonads), as the underlying conditions (matrix, temperature, pH, a_w) match those in the current assessment. For further information, see Annex C of EFSA opinion on aged meat. The models for *Y. enterocolitica* and pseudomonas were reassessed with some further data at higher temperatures (i.e. up to 30°C for *Y. enterocolitica* and 20°C for pseudomonas) compared to those used in the EFSA opinion on aged meat. The model of psychrotolerant LAB were also reassessed considering aerobic storage of meat.

For non-proteolytic *C. botulinum*, a model was generated based on the gamma concept (Zwietering et al., 1996) accounting for pH, a_w and temperature and using the growth cardinal parameters described in ICMSF (1996). The calibration factor was calculated from the few available growth rates on fresh meat-based matrixes.

For pathogens (STEC and *Salmonella*) not covered in the EFSA opinion on aged meat, additional models were identified from scientific literature, with preference for those with published validation data or performance metrics on fresh meat matrices. For spoilage psychrotrophic clostridia, a model was generated from growth rate data found in literature.

For STEC/*E. coli*, the mathematical model developed by Tamplin et al. (2005) was used. The model takes into consideration the effect of storage temperature (from 5 to 42°C) and was obtained by fitting the extended Ratkowsky square root model to growth data of 10 strains of *E. coli* (including STEC) on ground bovine meat stored at 5–42°C.

For *Salmonella*, the model developed by Pin et al. (2011) was used, which is based on the gamma concept of temperature, pH and a_w; the parameters were obtained from *Salmonella* growth data available in the ComBase portal and validated in ground porcine meat and fermented sausages.

The predicted growth rates by the models were graphically compared with growth rates values in fresh meat for *E. coli* (e.g. bovine meat and mutton) and/or *Salmonella* (porcine and bovine meat) at different storage temperature, which were extracted from the ComBase portal and scientific articles, to calculate the predictive performance indexes.

For psychrotrophic clostridia associated with blown-pack spoilage of vacuum-packaged meat, growth data of two strains of *Clostridium estertheticum* in broth from Yang et al. (2009) were used to derive a gamma concept model considering temperature (from –2 to 17°C) and pH (5.5–7.5). Subsequently, the predictive performance was evaluated by comparing the predictions with the growth observed in anaerobically stored juice from bovine meat (from –2 to 17°C) and ovine meat (–2 and 8°C).

To estimate the expected log-increase and/or final concentration, under the conditions (static and dynamic) associated with each time–temperature scenario, the growth rate derived from the selected CPM was coupled with the Baranyi and Roberts primary model (Baranyi & Roberts, 1994). In this approach, no lag time was assumed, and the maximum population density (MPD) was fixed at 9 $\log_{10}$ CFU/cm^2.

TABLE 5 Overview of microorganisms and predictive models used to evaluate the growth behaviour of pathogens and spoilage bacteria in fresh meat during carcass stabilisation and meat storage prior to freezing and during post-defrosting storage, and overview of the assessment of the reliability of the models (see also Section 2.7).

				Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
Microorganism	**Predictive model type [input factors considered]**	**Original source (model and/or data to generate it)**	**Model parameters**	**Fresh meat matrix[b]**	**Calibration factor[c]**	**Bias and accuracy factors[d]**	**(I) Factors**	**(II) A_f**	**(III) data to fit and generate the model**	**(IV) data to validate/ calibrate**	**Score: Overall [each criterion]**
Pathogenic bacteria											
Listeria monocytogenes	CPM/Gamma [T, pH, a_w]	Equation provided in Mejlholm and Dalgaard (2009) and Mejholm et al. (2010), simplified for the input factors considered	T_{min}= –2.83°C T_{ref}=25°C pH_{min}=4.97 $a_{w\,min}$=0.923 $\mu_{max_{ref}}$ =0.419	Aerobically stored bovine and porcine meat	0.74	B_f=1.00 A_f=1.43	3 (12 factors plus interaction term in the original model)	1.43	60 growth rate values in seafood for the original model, plus large number of experiments in broth to derive cardinal parameters for extending the model to new factors and assessing the $\mu_{max_{ref}}$with ready-to-eat products	66 growth rates on: • fresh bovine and porcine meat (grown, steak, loin) • stored aerobically • at temperatures ranging from 0 to 35°C • estimated from challenge tests with different strains *Notes*: For 63 values, input for a_w had to be assumed (0.997).	8 [2 + 2 + 2 + 2]

(Continues)

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
				Anaerobically (vacuum-packaged) stored bovine meat	1	$B_f = 0.96$ $A_f = 1.53$	3 (12 factors plus interaction term in the original model)	1.53	As above	12 growth rates on • fresh bovine and porcine meat (grown, steak) under vacuum-packaging • stored at temperatures ranging from 0 to 10°C, • estimated from challenge tests with different strains *Notes*: Input for a_w had to be assumed (0.997). For pH, four values had to be assumed (5.8). Several other references showing no growth at all (not being used for assessing the predictive performance)	6 [2 + 1 + 2 + 1]

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
Non-proteolytic *Clostridium botulinum*	CPM/Gamma [T, pH, a_w]	Equation based on Gamma concept (Zwietering et al., 1996)	T_{min} = 3°C T_{opt} = 30°C pH_{min} = 5.0 pH_{max} = 9.0 $a_{w\,min}$ = 0.970 $a_{w\,opt}$ = 0.997 μ_{opt} = 1.3	Unknown type of meat and chicken skin	1.20	B_f = 1.00 A_f = 3.05	3	3.05	None (growth domain limits as cardinal parameters)	12 growth rates on: • on poultry skin and meat (unknown type or other than bovine, porcine or poultry meat) under vacuum-packaging • stored at temperatures ranging from 3 to 30°C • estimated from challenge test though 10 values corresponding to the same strain cocktail *Notes:* Growth rates estimates could not be verified (no raw data available)	3 [2 + 0 + 0 + 1]

(Continues)

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
Yersinia enterocolitica	Square root model [T]	Model fitted to growth rates of Y. enterocolitica on bovine meat stored aerobically from −2°C to 10°C, extracted from Gill and Reichel (1989)	T_{min}= −5.58°C b=0.00041	Aerobically and anaerobically stored bovine, porcine and poultry meat[e]	1	B_f= 1.02 A_f= 1.68	1	1.68	5 growth rate values in bovine meat cubes (striploins with pH>6)	28 growth rates on: • bovine, porcine and poultry meat (covering wide range of pH) • stored aerobically and vacuum-packaging, • at temperatures ranging from 0 to 25°C • data included different strains	2 [0 + 0 + 1 + 1]
Shiga toxin producing *E. coli* (O157)	Extended square root Ratkowsky model [T]	Equation provided in Tamplin et al. (2005)	T_{min}= 3.79°C T_{max}=47.16°C b=0.028 c=0.7524	Aerobically and anaerobically stored bovine and ovine (mutton[e]) meat	1	B_f= 1.02 A_f= 1.45	1	1.45	20 growth rates in sterile ground bovine meat at 5–46°C	45 growth rates on: • fresh bovine and ovine (mutton) meat • stored aerobically and vacuum-packaging at temperature ranging from 8 to 35°C • data included different strains	6 [0 + 2 + 2 + 2]

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
Salmonella	Square root model [T, pH, a_w]	Equation provided in Pin et al. (2011)	T_{min} = 4.27°C pH_{min} = 3.69 $a_{w\,min}$ = 0.947 b = 0.209	Aerobically and anaerobically stored (5 to 30°C) fresh bovine, porcine and ovine (mutton[e]) meat	1	B_f = 0.95 A_f = 1.49	3	1.49	276 growth rates in broth for different serovars	78 growth rates on • fresh bovine, porcine and ovine (mutton) meat • stored aerobically (66) and vacuum-packaging (12) • at temperatures from 5 to 20°C • data includes different strains *Notes*: Input for a_w had to be assumed (0.997) for 70 growth rates. For pH, 22 values had to be assumed (5.8)	8 [2 + 2 + 2 + 2]
Spoilage bacteria											
Psychrotolerant LAB	CPM/Gamma [T, pH, a_w]	Equation provided in Mejlholm and Dalgaard (2007, 2013), simplified for the input factors considered	T_{min} = −5.25°C T_{ref} = 25°C pH_{min} = 4.24 $a_{w\,min}$ = 0.928 $\mu_{max_{ref}}$ = 0.583	Anaerobically (vacuum packaged) stored bovine, ovine and porcine meat	2.21	B_f = 1.00 A_f = 1.85	3 (12 factors plus interaction term in the original model)	1.85	96 growth rates in seafood (30) and meat products (66) with different strains	56 growth rates on • fresh bovine, ovine and porcine meat stored under vacuum-packaging • at temperatures ranging from −1.5 to 20°C • data include different strains *Notes*: Input for a_w had to be assumed (0.997)	7 [2 + 1 + 2 + 2]

(Continues)

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
				Aerobically stored bovine and porcine meat	1.40	B_f = 1.00 A_f = 1.68	3 (12 factors plus interaction term in the original model)	1.68	Original model plus experiments in broth to derive cardinal parameters for extending the model to new factors	16 growth rates on • bovine, ovine meat and porcine meat • aerobically stored • at temperature ranging from −0.5 to 15°C • data include different strains *Notes*: Input for a_w had to be assumed (0.997) for 12 values, while pH was assumed (5.8) for two	6 [2 + 1 + 2 + 1]
Pseudomonads	Square root model [T, a_w]	Equation provided in Neumeyer et al. (1997)	T_{min} = −7.6°C $a_{w\,min}$ = 0.947 b = 0.154	Aerobically stored bovine, porcine and ovine meat	0.73	B_f = 1.00 A_f = 1.55	2	1.65	Large data set (376) growth rates in lab media 0–30°C and a_w > 0.945 (using different strains)	81 growth rates on • bovine, porcine and ovine meat • aerobically stored • at temperatures ranging from 0 to 20°C *Notes*: Input for a_w had to be assumed (0.997) for 33 values	6 [1 + 1 + 2 + 2]

TABLE 5 (Continued)

Microorganism	Predictive model type [input factors considered]	Original source (model and/or data to generate it)	Model parameters	Assessment of predictive performance for fresh meat[a]			Appraisal assessment				Model reliability
				Fresh meat matrix[b]	Calibration factor[c]	Bias and accuracy factors[d]	(I) Factors	(II) A_f	(III) data to fit and generate the model	(IV) data to validate/ calibrate	Score: Overall [each criterion]
Gas producing psychrotrophic clostridia (blown-pack spoilage)	CPM/Gamma [T, pH]	Model fitted to growth rates on broth media under anaerobic conditions from Yang et al. (2009)	T_{min}= −9.17°C T_{max}= 19.68°C T_{opt}= 13.62°C pH_{min}= 4.79 pH_{max}= 8.94 pH_{opt}= 6.37 μ_{opt} = 0.09	Anaerobically stored (vacuum) bovine meat juice, ovine and bovine meat	1	B_f= 1.06 $A_{f=}$1.27	2	1.27	A limited data set for growth rate in lab media from −2 to 30°C and pH =5.5–7.5 using 2 different strains of *Clostridium estertheticum*	20 growth rates on • bovine meat juice, bovine and ovine meat • anaerobically stored (vacuum packaged) • at temperatures ranging from −2 to 20°C • data include 2 different strains	5 [1 + 2 + 1 + 1]

[a]Comparison of predicted vs. observed growth rates from independent studies.

[b]Fresh meat in the format of cuts, pieces, steak, ground, etc.; sterilised or not.

[c]Calibration factor equal to 1 means that the original model without correction was implemented.

[d]Predictive performance indexes after the application of the calibration factor. To be considered good or acceptable, growth rates should not be over- or underpredicted by more than 43% and 13%, respectively, corresponding to a bias factor of between 1.43 and 0.87 (Mejlholm et al., 2010; Ross, 1999). Accuracy factors higher than 1.5 indicate poor model precision (Mejlholm & Dalgaard, 2013).

[e]No clear difference was observed between aerobically stored meat and vacuum-packaged, in agreement with similar growth reported by Shenoy and Murano (1996) and Özbaş et al. (1996). See uncertainty table for further discussion.

For indicator organisms, such as ACC and Enterobacteriaceae, growth behaviour is more challenging to predict as they are heterogeneous microbial groups and dependent on the storage conditions. As in the EFSA opinion on spoilage bacteria (EFSA BIOHAZ Panel, 2016), growth of total microbiota under aerobic conditions is generally approximated by that of *Pseudomonas* spp., whereas under vacuum packaging or modified atmospheres, LAB are more appropriate as a proxy. For mesophilic and psychrotrophic Enterobacteriaceae, growth can be described by prediction from species such as *E. coli* or *Y. enterocolitica*, respectively, for mesophilic and psychrotrophic Enterobacteriaceae. Although *Yersinia* has been reclassified from the family Enterobacteriaceae to Yersiniaceae, it was retained as a representative psychrotrophic member of the group. This is justified because current legislation (EC No. 2073/2005) still refers to ISO 21528-2 for the enumeration of Enterobacteriaceae on carcasses, which also covers *Y. enterocolitica*. The choice of indicators and associated growth models aligns with the storage context (aerobic vs. anaerobic) and will follow the assumptions established in previous EFSA modelling works (EFSA BIOHAZ Panel, 2016, 2023).

Figure 7 below summarises the different approaches for assessing microbial dynamics. For pathogens, the focus is placed on their growth potential. In contrast, spoilage bacteria are evaluated in relation to the concentration they can reach, with the critical point being the time required to attain a predefined spoilage threshold that marks the onset of sensory deterioration. For indicator microorganisms, the assessment concentrates on the final concentration achieved at the end of the storage period. For both spoilage and indicator microorganisms, the impact of the initial levels was evaluated by assuming different initial levels in the scenarios.

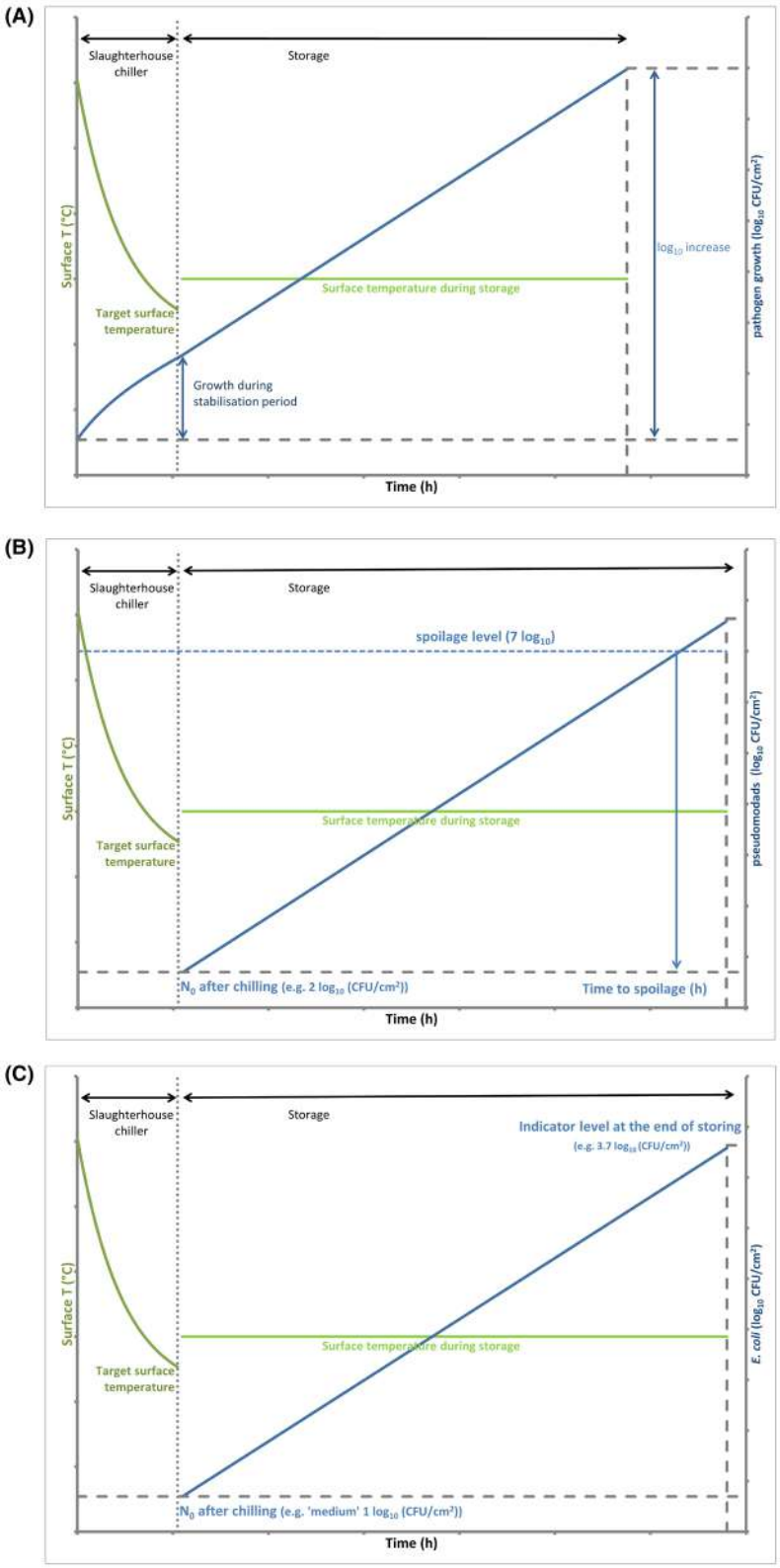

FIGURE 7 Approaches for assessing microbial dynamics in pathogenic bacteria (A), spoilage bacteria (B) and indicator microorganisms (C).

3.4 | Development of scenarios to compare the effect on survival and growth of relevant microorganisms in frozen meat of ungulates during defrosting

Frozen foods have been implicated in food-borne illness outbreaks (Kase et al., 2017; Lund, 2000; Sarno et al., 2021), which illustrates that human food-borne pathogens are not totally inactivated by freezing. Bacterial spores are very resistant to freezing, even repeated freezing and defrosting cycles (Lund, 2000). The effects of freezing on vegetative bacterial cells, yeasts and moulds are mixed, with survival affected by the presence of chemicals in the food matrix that may serve as protectants or can induce stress and potentially cross protection (Archer, 2004). Gram-positive bacteria are more freeze resistant than Gram-negative, but Gram-negative bacteria may survive well in frozen foods, and differences may exist both

between and within species. For protozoan parasites such as *Cryptosporidium parvum*, *Giardia* spp., *Toxoplasma* spp. and nematode parasites such as *Trichinella spiralis* or cestode parasites like *Taenia* spp. infectivity appears to be lost if freezing is carried out at sufficiently low temperatures and long times (EFSA BIOHAZ Panel, 2004; Erickson & Ortega, 2006; McEvoy et al., 2004; Mirza Alizadeh et al., 2018; Noeckler et al., 2019).

The rate of freezing has a large impact on the quality of frozen products as well as on the reduction of bacteria. The damaging effect of freezing and defrosting on bacterial cells is related to extracellular and intracellular ice formation, the concentration of extracellular and intracellular solutes and the low temperature (Archer, 2004). Even though extracellular ice is formed in the food during freezing, this ice cannot enter through the bacterial plasma membrane. The bacterial cell contents remain unfrozen at temperatures above −5°C and often above −10°C, a state that is termed supercooled. In the supercooled state, the vapour pressure is higher inside than outside the bacterial cell. This, together with the extracellular ice formation making the fluid in the food hypertonic, leads to an outward flow of water, i.e. to dehydration and an increased concentration of intracellular solutes in the bacterial cell. At lower freezing rates, for *E. coli* lower than around 8°C per minute (in water or saline), dehydration and high solute concentrations are the main inactivation mechanisms (Calcott & MacLeod, 1975). At higher freezing rates, internal ice formation causing mechanical cell damage and death is the important mechanism (Calcott & MacLeod, 1975). Defrosting may inflict additional damage as ice crystals can grow during this process since it generally takes longer time than freezing, and the food remains longer in a temperature zone which may favour recrystallisation during melting of small ice crystals that melt and refreeze on larger ice crystals (El-Kest & Marth, 1992).

The longer the cells remain in the dehydrated state, the more microbial damage will occur. This will have an impact on $\log_{10}$ reduction and/or subsequent lag times and growth rates. It is difficult to make general predictions on the extent of bacterial $\log_{10}$ reduction or the prolongation of lag times since the extent of inactivation is affected by several factors, not least the presence of components in the food that may act as bacterial cryoprotectants (El-Kest & Marth, 1992). The freeze/thaw process can result in sublethal damage that may render the bacteria unable to multiply and thus difficult to quantify on cultural media. This makes estimations of the $\log_{10}$ reduction during freezing uncertain. The bacterial cells may be able to repair the damage and subsequently become viable and infectious (Archer, 2004). Thus, despite freezing being able to reduce the number of bacteria in food, inactivation during freezing and defrosting was not considered in the assessment.

3.4.1 | Meat defrosting modelling approach

3.4.1.1 | *General element related to meat defrosting*

The mathematical modelling of meat defrosting requires consideration of heat and mass transfer mechanisms, which is challenging due to the significant changes in thermophysical properties that occur as the product transitions from frozen to thawed state. Various modelling approaches exist, ranging from analytical solutions of the heat conduction equations which provide closed-form expressions under simplifying assumptions to more empirical methods derived from experimental observations. In contrast, numerical methods solve the governing differential equations by discretising space and time.

Heat transfer at the surface is particularly complicated as it involves convection, radiation and mass transfer phenomena, including frost formation on the product surface at temperatures below the dew point. Several key factors influence defrosting time predictions, including the product's shape, size, composition, initial temperature, defrosting medium temperature and surface heat transfer coefficient. Both numerical methods and simplified empirical equations have been developed (Cleland et al., 1986; Pham, 1984).

For predicting defrosting times accurately, it is possible to determine when the centre of a meat block has reached the end of the latent heat plateau by measuring surface temperature changes.

3.4.1.2 | *Modelling approach used to assess surface temperature during defrosting*

A model was developed based on the Lind model (Lind, 1991) and included the development of an R code that implements a one-dimensional, explicit finite difference model for simulating heat transfer with phase change during the defrosting of frozen foods. The model numerically solves the general heat conduction equation (Fourier equation):

$$\rho(t)C_{p,app}(t)\frac{\partial_t}{\partial_\theta} = \frac{\partial_t}{\partial_x}\left(k(t)\frac{\partial_t}{\partial_x}\right) \tag{6}$$

where

- The thermal conductivity (k), density (ρ) and specific heat capacity (Cp) are temperature-dependent.
- Phase change is accounted for through an effective specific heat approach.
- In the numerical implementation, convective boundary conditions are applied at the product surface (Ts), where the convective heat transfer coefficient (h) links the conductive heat flux inside the meat to the external air temperature (Tair) according to: $-k\partial x\partial T = h(Ts - Tair)$

The model handles the challenge of the phase transition by using an apparent specific heat term ($C_{p,app}$) that incorporates the latent heat of fusion within a specified temperature range (between Tf_{min} and Tf_{max}).

The model makes several key assumptions:

- One-dimensional heat transfer: Heat flow is assumed to occur only in the thickness direction, which is reasonable for slab-shaped meat products where thickness is much smaller than length and width.
- Homogeneous material properties: The meat is assumed to have uniform composition throughout.
- No mass transfer effects: Unlike Lind's more comprehensive model, this implementation does not include mass transfer effects (condensation, frost formation and evaporation) at the food surface.
- Constant ambient conditions: Air temperature and heat transfer coefficient remain constant throughout the defrosting process.

3.4.1.3 | *Numerical simulation*

The modelling code uses an explicit finite difference scheme where:

- Spatial discretisation: The spatial domain is discretised into N nodes (typically 20 nodes for adequate resolution). Heat conduction is represented through a tridiagonal coefficient matrix, corresponding to a central difference scheme for internal nodes and first-order finite differences for the boundary nodes.
- Time discretisation: A forward difference approximation is used for the time derivative with small time steps (1 s) to ensure numerical stability.
- Spatial derivatives: A central difference approximation is used for the spatial derivatives.
- Boundary conditions: Convective boundary conditions are simulated using Newton's law of cooling at both food surfaces. These are introduced as additional source terms via a boundary vector.
- Property updates: Temperature-dependent properties are updated at each time step. In practice, thermal properties were implemented as piecewise constant values for solid and liquid states, with linear interpolation in the freezing range (between Tf_{min} and Tf_{max}) to incorporate the latent heat of fusion through an apparent heat capacity formulation.
- Output tracking: model outputs were extracted at different depths (surface, quarter-thickness and core) to represent defrosting kinetics. Reference lines at −3°C, −1.5°C and 0°C were added to the plots in order to identify the freezing range and practical defrosting completion thresholds.

The explicit finite difference scheme requires attention to numerical stability. The Courant–Friedrichs–Lewy (CFL) condition must be satisfied to prevent numerical instability:

$$F_0 = \alpha \times \frac{\Delta t}{\Delta x^2} \pi r^2 \leq 0.5 \quad (7)$$

where *Fo* is the Fourier number, α is thermal diffusivity, Δ*t* is the time step and Δ*x* is the spatial step. In the present implementation, the spatial resolution (20 nodes across the different thicknesses) and time step (1 s) lead to Fourier numbers well below the critical threshold (*Fo* ≤0.5), ensuring numerical stability of the explicit finite difference scheme across the whole simulation domain.

The model parameters used for the simulations, including thermophysical properties and numerical settings, are summarised in Table 6.

TABLE 6 Parameters of the model used for predicting surface temperature during defrosting according to air conditions and meat characteristics.

Symbol in code	Meaning	Value	Comment and references
N	Number of nodes	20	Set for this work
ρ_s	Density of the solid phase (kg/m^3)	917	Density of ice
ρ_l	Density of the liquid phase (kg/m^3)	1000	Density of water
Cp_s	Specific heat capacity in solid phase (J/kg/K)	2347	Based on Lind (1991), Dromenko et al. (2021) and Tavman et al. (2007) (mean values measured for porcine and bovine meat at value close to −18°C)
Cp_l	Specific heat capacity in liquid phase (J/kg/K)	3347	Based on Lind (1991), Dromenko et al. (2021) and Tavman et al. (2007) (mean values measured for porcine and bovine meat at values > 4°C)
la_s	Thermal conductivity in solid phase (W/m/K)	1.6	Thermal conductivity at −18°C in meat (Lind, 1991)
la_l	Thermal conductivity in liquid phase (W/m/K)	0.71	Thermal conductivity at 4–7°C in meat (Lind, 1991)

(Continues)

TABLE 6 (Continued)

Symbol in code	Meaning	Value	Comment and references
Tf_{min}	Minimum freezing temperature (°C)	−3	Defrosting region (defrosting time is defined for T reached −1.5°C)
Tf_{max}	Maximum freezing temperature (°C)	0	
l_f	Latent heat of fusion (J/kg)	334,000	Heat fusion of water (Van der Sman, 2008).
dt	Time step (s)	1	Set for this work

3.4.1.4 | *Model validation*

To illustrate the behaviour of the developed model and demonstrate its ability to predict surface temperature evolution during defrosting, we tested a representative condition of a controlled defrosting process in a refrigerated environment (Figure 8). This case study aims to highlight the three characteristic phases of defrosting (EFSA BIOHAZ Panel, 2021) and validate the physical consistency of the model predictions.

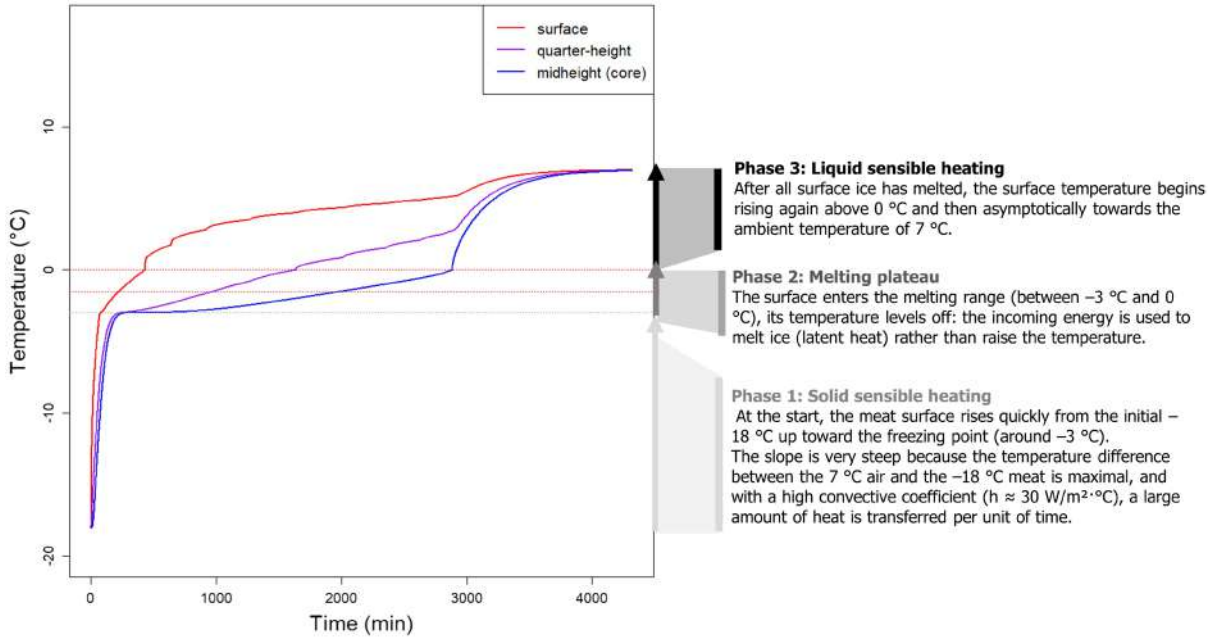

FIGURE 8 Illustration of predicted kinetics of temperature of the developed defrosting model, with parameters $T_0 = -18°C$ (initial temperature representative of freezer storage); $T_{air} = 7°C$ (refrigerated air ambient temperature); $h = 30\ W/m^2 \cdot °C$ (convective heat transfer coefficient corresponding to moderate forced air circulation; $ep = 15$ cm (selected thick cut); $td = 72$ h (extended simulation duration to ensure complete defrosting – defrosting is considered ended after 33.2 h when the core reached −1.5°C).

The surface-temperature curve shows the three typical distinct phases (Figure 8):

The model validation was then performed using two experimental data sets to ensure accuracy and reliability. To evaluate the predictive capability of the defrosting model, two independent experimental data sets were used. The first data set, from Lind (1991), provides defrosting times for minced bovine meat under controlled conditions, while the second data set, from Flores et al. (1993), reports defrosting kinetics for mutton. For each condition, the model was run with the corresponding air temperature, initial product temperature, convective heat transfer coefficient and thickness. The predicted time to reach −1.5°C at the product centre was then compared with the observed defrosting time. Results are presented in Figure 9 as a scatterplot of predicted versus observed values, with a ± 15% acceptance band around the line of equality. Overall, the model showed good agreement with experimental data, with the majority of predictions falling within the ±15% interval.

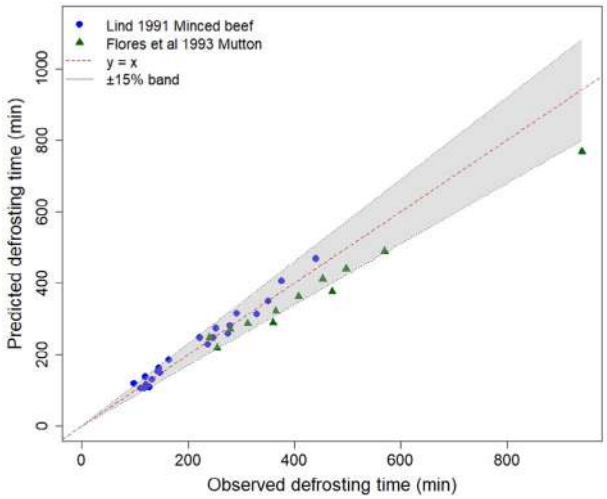

FIGURE 9 Observed versus predicted defrosting times for two validation data sets. Blue circles correspond to data from Lind (1991, minced bovine meat) and green triangles to data from Flores et al. (1993, mutton). The dashed red line indicates the line of equality ($y = x$). The shaded grey area represents the ±15% acceptance interval.

3.4.2 | Scenarios of defrosting

In line with ToR 2.1, the defrosting scenarios were defined as a full factorial design combining two freezing temperatures (–12°C and – 18°C), two defrosting temperatures (4°C and 7°C), two defrosting methods (static and dynamic) and two values of meat thickness (5 cm and 15 cm), selected based on expert knowledge as representative of two typical thicknesses of meat pieces. Dynamic defrosting involves forced air circulation and operates with an air-blowing system in several phases, first with tempered air, until the surface temperature of the meat reaches zero degrees or below and then with cold air. Static defrosting involves no forced-air circulation. Air circulation in dynamic defrosting reduces the time required to reach a uniform core temperature in the product compared to static defrosting.[13] The defrosting methods were associated with two different h values 10 (static) and 30 (dynamic) that represent typical heat transfer coefficients for natural and forced convection (Erickson & Hung, 2012). The endpoint of the defrosting process was defined as the time at which the core temperature reached –1.5°C. Thermal simulations were used to estimate, for each condition, the defrosting time required to reach –1.5°C at the core. Following defrosting, a 7-day storage phase was simulated at the same temperature as used during defrosting, in line with the cold chain continuity principle (4°C or 7°C, respectively), under conditions of high a_w (0.99) associated with exudate formation.

This design resulted in 16 defrosting scenarios that cover a broad range of practical conditions (see Table 7).

TABLE 7 Defrosting scenarios and predicted defrosting times under different conditions.

Defrosting scenario	Freezing temperature (°C)	Defrosting temperature (°C)	Defrosting method	Thickness (cm)	Defrosting time (h)	Example of practical defrosting conditions
1	–12	4	Static	5	22.81	Overnight defrosting in refrigerator of small pieces
2	–18	4	Static	5	23.34	
3	–12	7	Static	5	16.04	
4	–18	7	Static	5	16.50	
5	–12	4	Dynamic	5	9.46	Rapid defrosting of small pieces
6	–18	4	Dynamic	5	9.65	
7	–12	7	Dynamic	5	6.82	
8	–18	7	Dynamic	5	6.99	
9	–12	4	Static	15	85.12	Long defrosting of large pieces
10	–18	4	Static	15	86.83	
11	–12	7	Static	15	61.43	
12	–18	7	Static	15	62.91	
13	–12	4	Dynamic	15	43.98	Forced-air defrosting of large pieces
14	–18	4	Dynamic	15	44.70	
15	–12	7	Dynamic	15	32.58	
16	–18	7	Dynamic	15	33.20	

[13]See also information provided with the mandate for this scientific opinion (Appendix to the mandate, available at: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00711).

3.5 | Bacterial growth before freezing: Comparing growth at the end of scenarios 1–5 (ToR 1.1)

3.5.1 | Pathogenic microorganisms

The predicted growth of different pathogens in meat from different animal species across the alternative storage scenarios is presented in Table 8 (filterable data table also available in Annex G), and summarised below. Predicted growth (log_{10} increase) was compared at the defined time points, i.e. 15 days after slaughter for Scenario 1 (reference scenario, 7°/noVP/15d) and 6 weeks after slaughter for Scenarios 2–5, under the two baseline conditions described in Section 2.1: Baseline I conditions with representative mean temperature, pH and a_w, and Baseline II conditions with conservative values favouring growth (as long as the predictive model for the assessed microorganisms includes this among the input factors, see Table 5). Colours indicate the magnitude of deviation from the reference Scenario 1 (7°/noVP/15d, with dynamically changing a_w). Red denotes cases where the log_{10} increase was ≥ 0.5 log_{10} higher than that observed in the reference scenario, whereas green indicates ≥ 0.5 log_{10} lower log_{10} increase compared to the reference scenario.

Under Baseline I conditions, Scenarios 3 and 5, applying low temperature (3°C) and combining air and vacuum conditions, resulted in 0.5 log_{10} lower predicted increases for *Salmonella* as compared to the reference Scenario 1. In comparison, the same temperatures (7°C) for longer storage times, represented by Scenarios 2 and 4, led to higher predicted increases than in the reference scenario, i.e. 3–4 log_{10} higher in all cases. These differences were even more pronounced when Baseline II conditions were considered. Low-temperature scenarios resulted in up to 2 log_{10} lower predicted levels compared to reference Scenario 1, whereas the predicted same-temperature scenarios resulted in approximately 4 log_{10} higher increase.

A similar pattern was observed for STEC. Under Baseline I conditions, predicted increases for Scenarios 3 and 5 of 0.8–0.9 $log_{10,}$ were lower than for Scenario 1 (3.6 log_{10}), while Scenarios 2 and 4 reached 8.6 log_{10} increase. Also, under Baseline II conditions (for STEC considering conservative conditions only for temperature), the predicted log_{10} increase for Scenarios 3 and 5 (1.6–2.1 log_{10}) was lower compared to Scenarios 1, 2 and 4 within each animal species.

For *L. monocytogenes*, the predicted log_{10} increase under Scenarios 2 and 4 reached the maximum population levels (9.0 log_{10} increase) under Baseline I conditions, compared with 4.3–4.8 log_{10} increase in the reference scenario. Predicted increases for Scenarios 3 and 5 (5.0–5.6 log_{10}) also exceeded those of the reference scenario, albeit to a lesser extent. Under Baseline II conditions, all scenarios approached or reached the maximum population levels (predicted increases of 7.4–9.0 log_{10}).

For *Y. enterocolitica*, predicted increases were consistently close to or at the maximum of 9 log_{10} across all scenarios and both baseline conditions. Note that for *Y. enterocolitica*, pH and a_w were not considered in the model; thus, the potential impact of the reduction of the a_w at the meat/carcass surface (Baseline I conditions) was underestimated.

TABLE 8 Predicted growth (log_{10} increase) of pathogenic bacteria in bovine, ovine and porcine meat under five storage scenarios, considering two baseline conditions, i.e. Baseline I conditions (mean), Baseline II conditions (conservative). Values represent the predicted log_{10} increase at the final time point of each scenario. Text colour reflects the relative difference between each scenario and Scenario 1 (reference scenario) as follows: Green indicates ≥ 0.5 log_{10} lower than the reference; black indicates 0–0.5 log_{10} lower/higher than the reference; red indicates ≥ 0.5 log_{10} higher than the reference. A filterable data table with results is also available in Annex G.

		Baseline I conditions (mean)					Baseline II conditions (conservative)				
Microorganism (model confidence score*)	Animal species	Scenario 1 (Reference) 7°C noVP 15d	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 4 7°C VP15 42d	Scenario 5 3°C VP15 42d	Scenario 1 (Reference) 7°C noVP 15d	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 4 7°C VP15 42d	Scenario 5 3°C VP15 42d
Salmonella (8)	Bovine	1.4	5.4	0.8	4.5	0.8	4.1	7.9	2.0	7.9	2.0
	Ovine	1.3	5.4	0.8	4.4	0.7	3.6	7.6	1.6	7.6	1.6
	Porcine	0.8	4.8	0.2	3.9	0.2	3.0	6.9	1.0	6.9	1.0
STEC (6)	Bovine	3.6	8.6	0.9	8.6	0.9	4.8	9.0	2.1	9.0	2.1
	Ovine	3.6	8.6	0.8	8.6	0.8	4.4	8.9	1.7	8.9	1.7
L. monocytogenes (8, 6)	Bovine	4.5	9.0	5.5	9.0	5.3	9.0	9.0	8.1	9.0	8.6
	Ovine	4.8	9.0	5.6	9.0	5.4	8.9	9.0	7.7	9.0	8.3
	Porcine	4.3	9.0	5.2	9.0	5.0	8.7	9.0	7.4	9.0	8.0
Y. enterocolitica (2)	Porcine	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
C. botulinum non proteolytic (3)	Bovine	NG	2.0	NG	1.4	NG	NG	5.7	NG	3.9	NG
	Ovine	NG	2.1	NG	1.4	NG	NG	5.5	NG	3.8	NG
	Porcine	NG	2.0	NG	1.3	NG	NG	5.0	NG	3.4	NG

Abbreviations: NG, no growth of non-proteolytic *C. botulinum* was assumed due to air conditions (reference Scenario 1) or storage temperature below the minimum growth temperature (Scenarios 3 and 5); noVP, non-vacuum-packed; VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

*Confidence score of each predictive model (see Table 5). For *L. monocytogenes*, the first and second values refer to the score of the model under aerobic and anaerobic conditions, respectively.

Growth of non-proteolytic *C. botulinum* was predicted only at 7°C in vacuum-packaged meat (Scenarios 2 and 4). Storage temperature of 3°C (Scenarios 3 and 5, under either baseline condition) was below the minimum growth temperature for this pathogen.

The predicted $\log_{10}$ increase under Baseline II conditions may lead to concentrations potentially associated with the production of BoNTs. The predicted growth is more limited under Baseline I conditions, but still close to the 2.2 $\log_{10}$ increase proposed by Koukou et al. (2021) as a threshold for toxin formation. In accordance with the criteria for equivalence defined in Section 2.6, any predicted growth of *C. botulinum* (i.e. > 0.5 $\log_{10}$ increase) was considered a potential risk of toxin formation.

Temperature was the primary driver of predicted $\log_{10}$ increase of the assessed pathogens: storage at 3°C (Scenarios 3 and 5) consistently predicted lower $\log_{10}$ increases than storage at 7°C (Scenarios 2 and 4). The timing of vacuum packaging (immediately after stabilisation vs. after 15 days) had limited influence, which was mainly associated with the higher a_w assumed for vacuum packaged meat compared with aerobically stored meat, with only minor differences between Scenarios 2 versus 4 and Scenarios 3 versus 5 under both baseline conditions. After 6 weeks of storage, the 7°C Scenarios 2 and 4 consistently resulted in predicted bacterial $\log_{10}$ increases of more than 2 $\log_{10}$ above the day−15 reference, whereas the 3°C Scenarios 3 and 5 were generally close to or below the reference.

Under Baseline II conditions, while overall trends in relation to reference Scenario 1 remained unchanged, the predicted growth ($\log_{10}$ increase) was substantially higher across microorganisms and the differences between storage at 7°C versus 3°C were magnified (Table 8). This highlights that cooling conditions (temperature) and animal-related stress factors (pH decline until the ultimate pH of meat) and the meat surface a_w during storage are critical determinants of microbial growth and should be carefully taken into account. Under these more favourable conditions, both the reference and alternative scenarios, the predicted growth of the pathogens often reached the stationary phase (MPD) or close to that, which makes direct comparisons between scenarios of the predicted $\log_{10}$ increase more difficult. Thus, the conservative baseline conditions II will not be retained for ToR 1.2. Since Scenario 4 (7°/VP15/42d) extends reference Scenario 1 (7°/noVP/15d) with an additional vacuum-packaged storage phase, and thus will always result in more predicted growth, it will also be excluded from the equivalence assessments performed to address ToRs 1.2 and 2.2.

3.5.2 | Indicator and spoilage microorganisms

The impact of the different storage scenarios on the predicted growth of indicator and spoilage bacteria in bovine meat is shown in Table 9 (filterable data table, also including results for ovine and porcine meat, is available in Annex H). The predicted final concentrations ($\log_{10}$ CFU/cm^2) at the defined time points – day 15 for Scenario 1 and 6 weeks for Scenarios 2–5 – were compared under two baseline conditions (Baseline I and Baseline II), but in addition, across different initial contamination levels (N_0 from −1 to 5 $\log_{10}$ CFU/cm^2, depending on the microorganism). For illustrative reasons, first the growth of LAB is described in more detail before other spoilers and indicators are presented. When the Baseline I (mean) was applied, results indicated larger increases for LAB in the alternative storage scenarios with respect to the reference scenario, with levels reaching the maximum population in storage Scenarios 2 and 4, which represent storage at 7°C. Maximum population levels (9 $\log_{10}$ CFU/cm^2) were reached in all cases for Scenarios 2 and 4. Only for Scenarios 3 and 5, predicted levels remained just below the cut-off associated with spoilage of 7 $\log_{10}$ CFU/cm^2 when the lowest initial levels (1 $\log_{10}$ CFU/cm^2) were used. Under Baseline II conditions, levels close or at the maximum population level were reached in most of the scenarios, except in Scenario 3 using an initial inoculum of 1 $\log_{10}$ CFU/cm^2. In all cases, no marked differences in final concentrations were observed between animal species (see complete results in Annex H). The impact of vacuum packaging could be assessed for LAB because anaerobic vs. aerobic growth conditions could be simulated with different models (as listed in Table 5), both including a_w as an input factor, which was assumed to be higher in vacuum packaged meat compared with aerobically stored meat. However, as the LAB reached or approached the maximum population levels before the end of the storage, no differences (in most of the cases) or minimal differences (only for initial levels of 1 $\log_{10}$ CFU/cm^2 and 3°C storage temperature) were found between scenarios representing vacuum package just after stabilisation (Scenario 3) and combining air and vacuum packaging after 15 days (Scenario 5). Overall, temperature was the dominant driver for predicted levels: storage at 7°C (Scenarios 2 and 4) consistently yielded higher final levels than storage at 3°C (Scenarios 3 and 5).

TABLE 9 Predicted final concentrations (log_{10} CFU/cm^2) of indicator and spoilage bacteria on bovine meat under five storage scenarios, and different initial levels (N_0, expressed in log_{10} CFU/cm^2) considering two baseline conditions (Baseline I conditions (mean); Baseline II conditions (conservative)). Text colour reflects the relative difference between each scenario and Scenario 1 (reference scenario) as follows: Green: ≥ 0.5 log_{10} lower than the reference; black: 0–0.5 log_{10} lower/higher than the reference; red: ≥ 0.5 log_{10} higher than the reference. A filterable data table, also including results for ovine and porcine meat, is available in Annex H.

		Baseline I conditions (mean)					Baseline II conditions (conservative)				
Microorganism (model confidence score*)	N_0	Scenario 1 (reference) 7°C noVP 15d	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 4 7°C VP15 42d	Scenario 5 3°C VP15 42d	Scenario 1 (reference) 7°C noVP 15d	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 4 7°C VP15 42d	Scenario 5 3°C VP15 42d
Lactic acid bacteria (6, 7)	1	5.0	9.0	6.8	9.0	6.7	8.9	9.0	8.1	9.0	8.9
	3	7.0	9.0	8.6	9.0	8.5	9.0	9.0	9.0	9.0	9.0
	5	8.7	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Pseudomonas (6)	1	5.3	NG	NG	5.3	3.1	9.0	NG	NG	9.0	8.2
	3	7.3	NG	NG	7.3	5.1	9.0	NG	NG	9.0	9.0
	5	8.8	NG	NG	8.8	7.1	9.0	NG	NG	9.0	9.0
E. coli (6)	0	2.7	7.9	NG	7.9	NG	2.7	7.9	NG	7.9	NG
	1	3.7	8.6	NG	8.6	NG	3.7	8.6	NG	8.6	NG
	2	4.7	9.0	NG	9.0	NG	4.7	9.0	NG	9.0	NG
Enterobacteriaceae (2)	0	8.9	9.0	9.0	9.0	9.0	8.9	9.0	9.0	9.0	9.0
	1.5	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
	3	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Psychrotolerant Clostridia (5)	−1	NG	0.1	−0.4	−0.3	−0.6	NG	0.2	−0.3	−0.2	−0.5
	0	NG	1.1	0.7	0.7	0.4	NG	1.2	0.7	0.8	0.5
	1	NG	2.1	1.7	1.7	1.4	NG	2.2	1.7	1.8	1.5

Abbreviations: NG: no growth was assumed for *E. coli* (due to storage temperature below the minimum growth temperature in Scenarios 3 and 5), psychrotolerant Clostridia (due to air conditions in Scenario 1) and *Pseudomonas* (due to anaerobic conditions in Scenarios 2 and 3); noVP, non-vacuum-packed; VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

Growth of *E. coli* was predicted in Scenarios 2 and 4, which were above the levels predicted for the reference scenario. For Scenarios 3 and 4, no growth was registered as it was below the minimum temperature for growth.

Enterobacteriaceae numbers were predicted to reach the maximum population density set in all scenarios. Note that *Enterobacteriaceae* growth was proxied by the *Y. enterocolitica* model, which only incorporates temperature as input in addition to showing psychrophilic characteristics.

Pseudomonas spp. typically attained high counts during the aerobic storage period at 7°C of the reference Scenario 1 and Scenario 4. As no growth was assumed once meat was vacuum-packaged, the predicted *Pseudomonas* growth was lower than the reference scenario when vacuum-packaged immediately after stabilisation (Scenarios 2 and 3) and/or under 3°C (Scenario 5). Psychrotolerant *Clostridia* displayed limited growth overall, assumed to occur only during vacuum-packaging storage conditions, i.e. no growth under reference Scenario 1 (7°/noVP/15d) and generally reduced under 3°C storage in the scenarios applying vacuum-packaging.

Under Baseline II conditions, differences in predicted levels of spoilage and indicator microorganisms between 7°C and 3°C were greater, but the qualitative trends compared with the reference Scenario 1 remained unchanged. Yet, predicted growth curves in both the reference and alternative scenarios often reached the stationary phase (MPD), which makes direct comparisons between scenarios more difficult. Thus, as for pathogens, Baseline II conditions will not be retained for ToR 1.2 and the determination of equivalence times.

3.5.3 | Considerations with respect to identified uncertainties

In order to assess the impact of the uncertainties identified on the outcome of the assessment and express the overall uncertainty in the answer to the terms of reference, the following considerations were taken into account:

- Baseline II conditions (conservative) combine the highest chilling temperature profile with high pH and a_w values for meat that are unlikely to occur simultaneously in all cases. Such highly favourable conditions for bacterial growth are expected to considerably overestimate bacterial growth, and therefore, the magnitude of the predicted increase in bacterial loads. Similarly, not explicitly considering microbial competition, other than using data from naturally contaminated meat in the assessment, also contributes to an overestimation of the growth of pathogens, although the impact on the comparative magnitude of growth across the scenarios remains uncertain.

- In contrast, Baseline I conditions (mean) represent average carcass chilling (stabilisation in temperature and pH) and typical meat characteristics (pH and, particularly, surface a_w) during storage. Two assumptions may lead to a potential underestimation of bacterial growth compared with other reasonably foreseeable conditions (e.g. cuts of meat or lean muscles, which show higher a_w and are less prone to surface drying):
  - the assumption of isothermal storage temperature (7°C or 3°C, depending on the scenario) instantaneously reached on the surface after stabilisation, which is not realistic under commercial conditions;
  - the neglect of heterogeneity in surface temperature and humidity (and thus the a_w of meat surface) across carcasses or cuts.

  The underestimation of the growth will generally cause an underestimation of the differences between reference and alternative scenarios, making the 'no equivalence' output more certain than when the predictions indicate 'equivalent' growth.
  However, other model assumptions could conversely lead to an overestimation of growth also under Baseline I conditions, particularly because no lag phase was included, while a growth delay can occur after chilling.
  Taken together, these opposite factors may partly counterbalance each other. However, experts considered that, overall, the model assumptions are more likely to lead to an overestimation of bacterial growth under Baseline I conditions.
- Magnitude or growth difference to judge the equivalence between scenarios:
  Less than 0.5 $\log_{10}$ difference in the magnitude of the predicted bacterial growth or bacterial levels was considered not relevant making the compared scenarios 'equivalent'. The certainty of the 'non-equivalence' between the reference scenario and the alternative scenarios increases with the increase of the magnitude of the predicted $\log_{10}$ difference.
- The reliability of the predictive models used to estimate bacterial growth:
  The confidence level associated with the predictive microbiology models varied substantially among microorganisms. The models for *Salmonella* and *L. monocytogenes* take the three main factors (temperature, pH and a_w) into consideration as inputs to predict the growth rate. Besides being based on a well-established, validated model across a broad range of meat products, in this opinion they were further calibrated for fresh meat stored under a wide range of storage temperatures, both aerobically and/or anaerobically (vacuum-packaged); thus, they are considered to provide reliable estimates under chilled conditions. In contrast, predictions for *Y. enterocolitica* and non-proteolytic *C. botulinum* rely on models including temperature only or temperature and pH, respectively, obtained and validated with fewer data sets and this introduces greater uncertainty. Any predicted growth > 0.5 $\log_{10}$ was conservatively considered as growth able to allow toxin production.
  For spoilage bacteria, the model for LAB included three factors (temperature, pH, a_w) and was calibrated for aerobic and anaerobic conditions, and the one for *Pseudomonas* included two factors (temperature and a_w) calibrated for aerobic conditions with a considerable number of independent fresh meat data, providing reliability to the models to predict the growth of specific spoilage organisms of vacuum-packaged and aerobically stored meat.
  Psychrotrophic *Clostridia* models, based on sparse literature data, provide only indicative trends rather than reliable quantitative predictions.

3.6 | Bacterial growth before freezing: Equivalence times for growth compared to 'standard fresh meat' (ToR 1.2.1)

As explained in more detail in Section 2.6, the concept of *equivalence time* refers to the storage duration under alternative conditions that results in the same predicted microbial load as that reached in the reference scenario. For pathogenic microorganisms except for non-proteolytic *C. botulinum*, the equivalence time corresponds to the storage period under a given alternative scenario required to reach the same predicted $\log_{10}$ increase as in the reference scenario. For *C. botulinum*, no growth is expected under the reference conditions, and therefore, a direct equivalence cannot be established. In this specific case, the equivalence time was defined pragmatically as the storage duration required to reach a 0.5 $\log_{10}$ increase, corresponding to the predefined threshold distinguishing 'no growth' from 'growth'. For spoilage and indicator microorganisms, it is defined as the time needed to reach the same *final concentration* as in the reference scenario, assuming the same initial contamination levels.

3.6.1 | Pathogenic microorganisms

Equivalent storage times for pathogens are summarised in Table 10 (filterable data table also available in Annex I). Results are shown for the different animal species across the three remaining alternative storage scenarios, considering Baseline I conditions.

The results for *Salmonella* spp. are presented in more detail in Figure 10 to illustrate the approach used for determining equivalence times.

In Scenario 2, with storage at 7°C, the equivalence time for *Salmonella* is around 5 days, shorter than the 15 days of the reference Scenario 1. Because the temperature is identical to the reference scenario, the shorter time is explained by higher

a_w assumed for the storage of meat under vacuum packaging conditions. This fact favours *Salmonella* growth and enables the final population level to be reached more quickly. Figure 10 illustrates this higher growth rate and the procedure for the determination of this equivalence.

In Scenarios 3 and 5, with storage at 3°C, no growth for *Salmonella* during the storage was assumed, as it is below the *minimum* growth temperature for the pathogen (e.g. Tmin = 4.27°C according to the predictive model used). Figure 10 shows that the curves for these two scenarios remain below the reference line throughout the storage period, illustrating a non-equivalent (NE) situation where no adjustment is required.

Figure 11 shows similar curves for L. monocytogenes, showing that equivalence is reached around 12 days for Scenario 2 and about 32–35 days for Scenarios 3 and 5.

TABLE 10 Equivalence times (days) for the pathogen growth of alternative storage scenarios (Scenarios 2, 3 and 5) as compared to Scenario 1 (reference scenario, 7°/noVP/15d), considering Baseline I conditions. Values indicate the time (days) required to reach the same predicted final increase as in Scenario 1 (see Table 4). Values in bold represent the shortest time for equivalence for each assessed scenario, indicating the limiting microorganisms for that scenario. Equivalence times for Scenario 4 and under Baseline II conditions were not considered further in the assessment (see Section 3.5.1), although they were calculated and available in Annex I. A filterable data table with results is also available in Annex I.

Microorganism (model confidence score*)	Animal species	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 5 3°C VP15 42d
Salmonella (8)	Bovine	**5.4**	> 42	> 42
	Ovine	**5.2**	> 42	> 42
	Porcine	**5.4**	> 42	> 42
STEC (6)	Bovine	14.0	> 42	> 42
	Ovine	14.0	> 42	> 42
L. monocytogenes (8, 6)	Bovine	12.4	**33.2**	**35.5**
	Ovine	12.5	**34.7**	**36.8**
	Porcine	12.5	33.9	36.2
Y. enterocolitica (2)	Porcine	14.0	**29.0**	**29.0**
C. botulinum non proteolytic (3)**	Bovine	11.6	NG	NG
	Ovine	10.6	NG	NG
	Porcine	11.4	NG	NG

Abbreviations: NG, no growth assumed under the evaluated conditions; VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

*Confidence score of each predictive model (see Table 5). For *L. monocytogenes*, the first and second values refer to the score of the model under aerobic and anaerobic conditions, respectively.

**For *C. botulinum*, since no growth is expected under Scenario 1 (reference scenario), equivalence times indicate the time required to reach a 0.5 $\log_{10}$ increase (see Section 2.6).

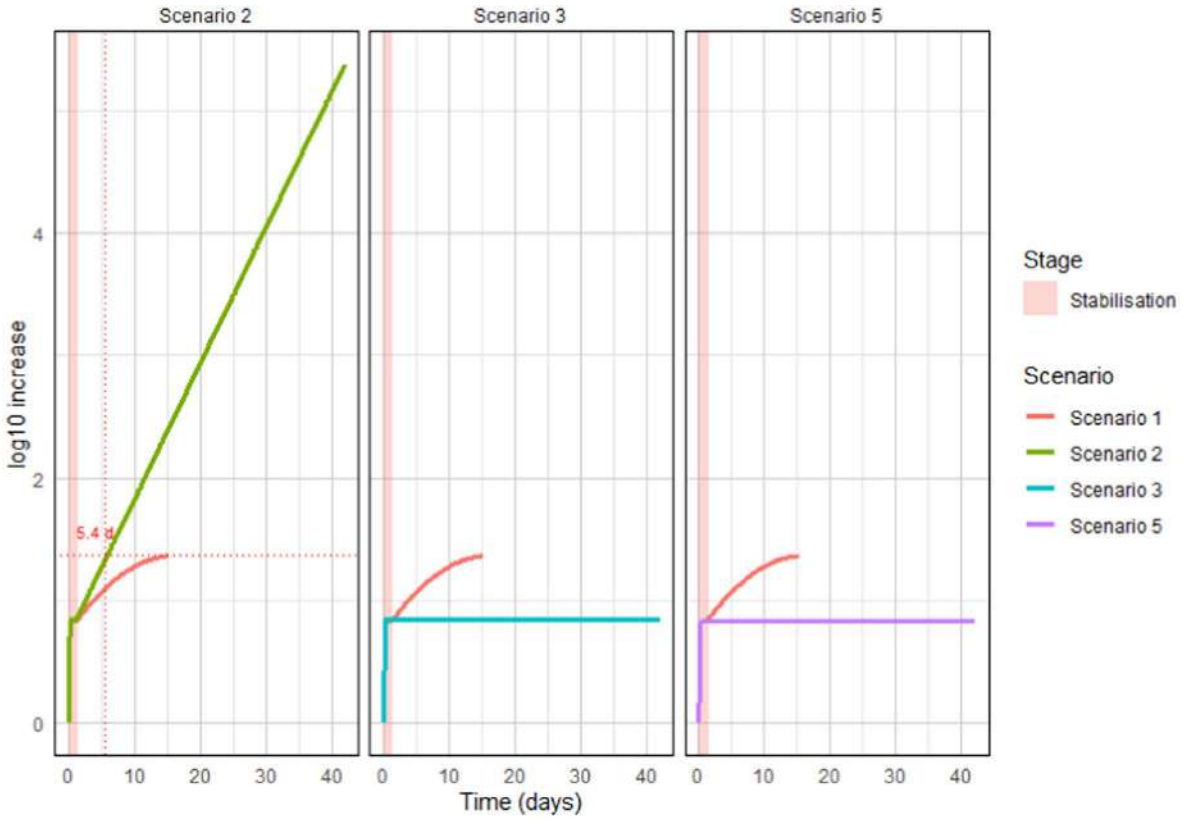

FIGURE 10 Predicted growth of *Salmonella* spp. in bovine meat under Scenario 1 (reference scenario) compared with alternative Scenarios 2, 3 and 5, considering Baseline I conditions.

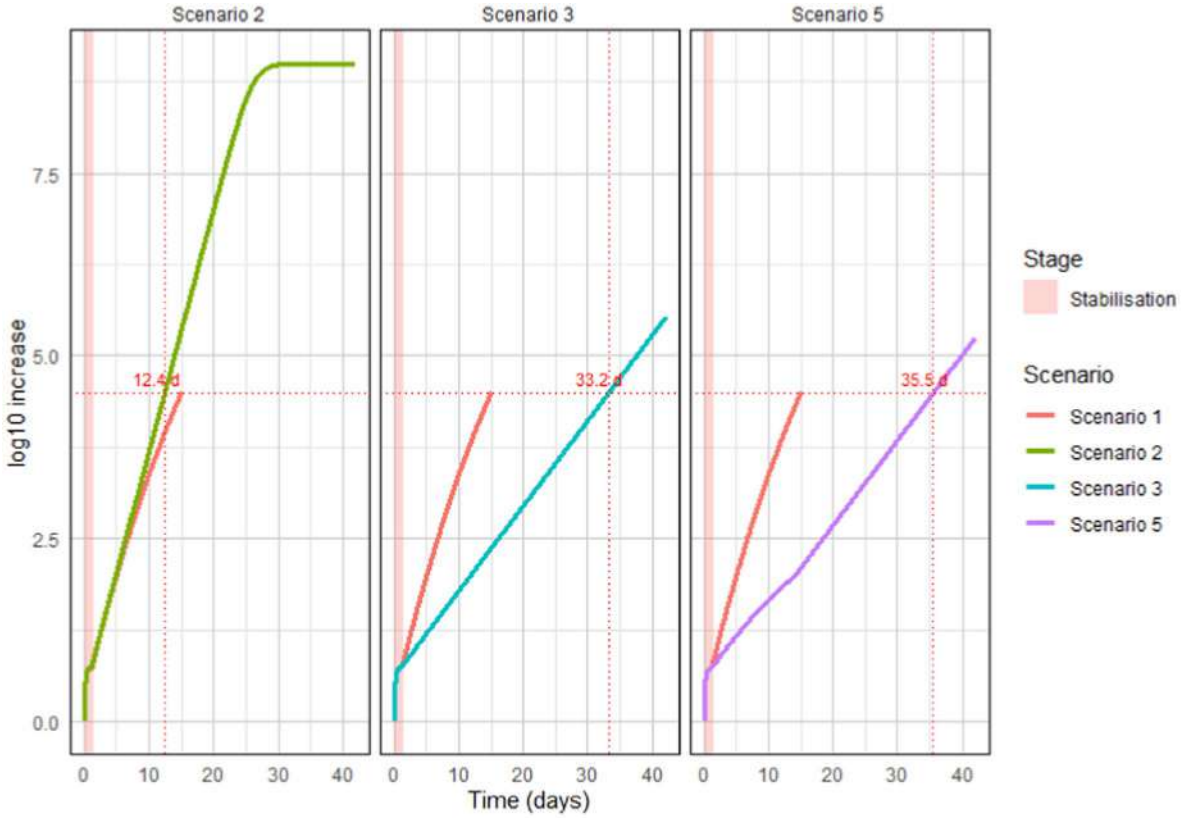

FIGURE 11 Predicted growth of *L. monocytogenes* in bovine meat under Scenario 1 (reference scenario) compared with alternative Scenarios 2, 3 and 5, considering Baseline I conditions.

Across all pathogens and animal species, the shortest equivalence times were consistently observed for Scenario 2 (7°/VPst/42d), confirming that this condition promotes faster bacterial growth compared with the reference. Under this scenario, *Salmonella* spp. showed the shortest time to equivalence (5 days), making it the limiting microorganism.

For Scenario 3 (3°/VPst/42d), equivalence times > 15 days were reached only for *L. monocytogenes* in bovine and ovine meat (33–34 days) and for *Y. enterocolitica* in porcine meat (29 days), indicating that temperature strongly controls bacterial growth under chilled conditions.

Similarly, for Scenario 5 (3°/VP15/42d), *L. monocytogenes* in bovine and ovine meat was again the limiting microorganism for this scenario, with equivalence observed at around 35–36 days.

No growth was assumed for non-proteolytic *C. botulinum* in Scenarios 3 and 5 (as 3°C is below the minimum growth temperature for this pathogen), and only limited growth in Scenario 2 (12 days to increase by 0.5 $\log_{10}$). This organism was therefore not considered limiting for equivalence under any of the tested conditions.

3.6.2 | Indicator and spoilage microorganisms

Equivalent storage times for the different indicator and spoilage microorganisms on bovine meat are summarised in Table 11 (filterable data table, also including results for ovine and porcine meat, is available in Annex J). Results are shown across the three remaining alternative storage scenarios, considering Baseline I conditions. The results for LAB are presented in more detail in Figure 12 to illustrate the approach used for equivalence for these bacteria as well as the notion of spoilage time.

Under Scenario 2, with storage at 7°C, the equivalence time for LAB was slightly shorter than 15 days. These results are consistent with faster LAB growth at higher storage temperatures and favourable a_w associated with the vacuum packaging effect in those scenarios.

Importantly, the relationship between equivalence time and time-to-spoilage depends on the initial levels:

- $N_0 = 1$ $\log_{10}$ CFU/cm^2 or g: spoilage time (20 days) is well after the equivalence time (i.e. equivalence is reached long before sensory rejection).
- $N_0 = 3$ $\log_{10}$ CFU/cm^2 or g: spoilage time (13.9 days) is close to the equivalence time (i.e. equivalence and sensory limit are reached nearly at the same time).
- $N_0 = 5$ $\log_{10}$ CFU/cm^2 or g: spoilage time (7.5 days) is shorter than the equivalence time (i.e. the product spoils before it can reach the same final population as in Scenario 1).

Scenarios 3 and 5, with storage at 3°C displayed longer equivalence times, reflecting slower LAB growth at lower temperatures. In several combinations, the predicted spoilage time is either much later than equivalence or is not reached within the prediction time (i.e. > 42 days), emphasising the strong inhibitory effect of refrigeration on LAB kinetics.

Across all comparisons, no remarkable differences in growth were observed between animal species. Besides that, no practically relevant differences were observed for LAB between scenarios representing vacuum conditions and those combining air and vacuum.

TABLE 11 Equivalence times for *E. coli* and spoilage bacteria (LAB, *Pseudomonas* and psychrotolerant *Clostridia*) in bovine meat under alternative Scenarios 2, 3 and 5 in comparison to Scenario 1 (reference scenario, 7°/noVP/15d), for Baseline I conditions. Values indicate the time (days) required to reach the same final concentration as in Scenario 1. Values in parentheses show the time (days) to reach the spoilage level. If the time to spoilage level is shorter than the equivalence time, the product would spoil before equivalence is achieved. Values in bold represent the shortest time for equivalence for each assessed scenario, indicating the limiting microorganisms for that scenario. Equivalence times for Scenario 4 and under Baseline II conditions were not considered further in the assessment (see Section 3.5.1), although they were calculated and available in Annex J. A filterable data table, also including results for ovine and porcine meat, is available in Annex J.

Microorganism (model confidence score*)	N_0	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 5 3°C VP15 42d
LAB (6,7)	1	**14 (20.2)**	**29.3 (NS)**	**30 (NS)**
	3	**14 (13.9)**	**29.3 (29.2)**	**30 (30.0)**
	5	**13.9 (7.5)**	**29.2 (15.2)**	**30 (16.0)**
Pseudomonas (6)	1	NG (NS)	NG (NS)	> 42 (NS)
	3	NG (NS)	NG (NS)	> 42 (NS)
	5	NG (NS)	NG (NS)	> 42 (13.1)
E. coli (6)	0	**14.8**	NG	NG
	1	**14.8**	NG	NG
	2	**14.8**	NG	NG
Psychrotolerant Clostridia (5)	–1	NA (NS)	NA (NS)	NA (NS)
	0	NA (NS)	NA (NS)	NA (NS)
	1	NA (NS)	NA (NS)	NA (NS)

Abbreviations: NA, not applicable, comparison was not performed since there was no growth in Scenario 1; NG, no growth assumed under the evaluated conditions; NS, no spoilage; VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

*Confidence score of each predictive model (see Table 5). For LAB, the first and second values refer to the score of the model under aerobic and anaerobic conditions, respectively.

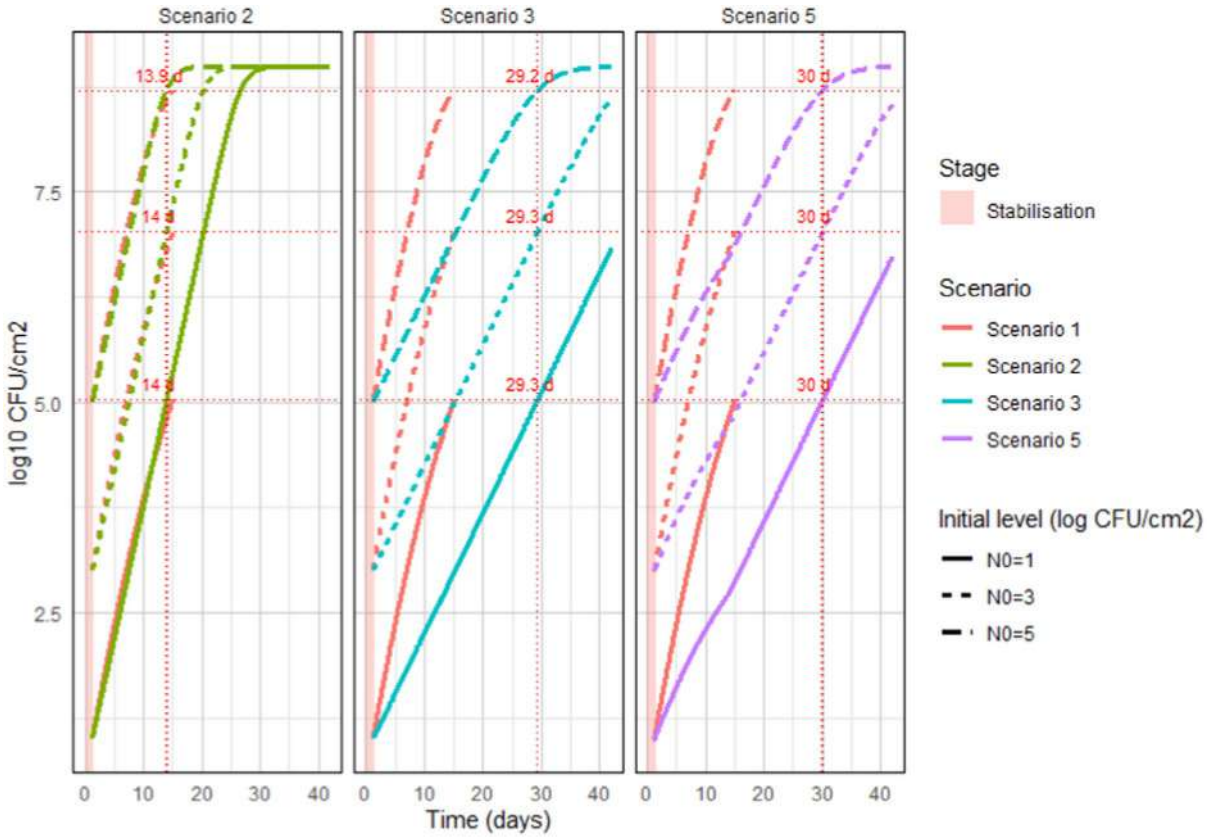

FIGURE 12 Predicted growth of lactic acid bacteria (LAB) in bovine meat under different storage scenarios, considering Baseline I conditions. Each plot represents the predicted bacterial population over time, with Scenario 1 (7°/noVP/15d, with dynamic a_w profile) serving as the reference and Scenarios 2, 3 and 5 as alternative storage scenarios. Curves above the reference indicate faster growth (shorter time to reach the same final concentration), while curves below indicate slower growth. Equivalence time is signalled in the graph by a vertical dotted line, with the corresponding equivalence time value shown.

Under Scenario 2 (7°/VPst/42d), LAB were the limiting organism, with equivalence times around 14 days, slightly shorter than the 15-day reference. *E. coli* equivalence time was slightly longer.

Under Scenario 3 (3°/VPst/42d), LAB again determined the shortest equivalence times (30 days), while *E. coli* did not grow at this temperature. *Pseudomonas* was assumed not to grow under vacuum-packaging conditions.

For Scenario 5 (3°/VP15/42d), LAB were again the limiting microorganism, with equivalence times around 30–31 days and spoilage predicted to occur before or near equivalence depending on the initial contamination level.

Across all comparisons, for psychrotrophic *Clostridia* no spoilage was predicted to happen within the 42 days, and LAB resulted in the dominant spoilage bacterial group defining both the equivalence time and the onset of spoilage. Depending on the initial contamination level (N_0), spoilage sometimes occurred before equivalence was reached: These results indicate that, under high initial contamination, the maximum practical storage duration would be shorter than the equivalence time, due to the actual spoilage of the meat.

3.6.3 | Considerations with respect to identified uncertainties

In order to assess the impact of the uncertainties identified on the outcome of the assessment and express the overall uncertainty in the answer to the terms of reference, besides the considerations described in Section 3.5.3, the following additional considerations were taken into account:

- The limiting microorganism of the equivalence time of an alternative scenario with respect to the reference scenario: Not considering microbial interactions between background microbiota and pathogens, which also depends on the initial contamination ratio, adds uncertainty to the relationship between equivalence time and the onset of spoilage. This is particularly relevant for *L. monocytogenes* and LAB, the limiting factors driving the 'equivalence time' at 3°C (Scenarios 3 and 5) compared to the reference scenario (7°C for 15 days). Actually, the predicted equivalence time for this pathogen and this spoilage bacteria in Scenarios 3 and 5 is very similar when considering the same initial contamination. However, the initial contamination of LAB is expected to be higher than that of *L. monocytogenes*, reinforcing the fact that spoilage by LAB will most probably occur before *L. monocytogenes* reaches levels of concern.

3.7 | Growth during defrosting and subsequent storage (ToR 2.1)

3.7.1 | Growth during defrosting

3.7.1.1 | *Pathogens*

Figure 13 illustrates the predicted pathogen growth during defrosting under the 16 scenarios.

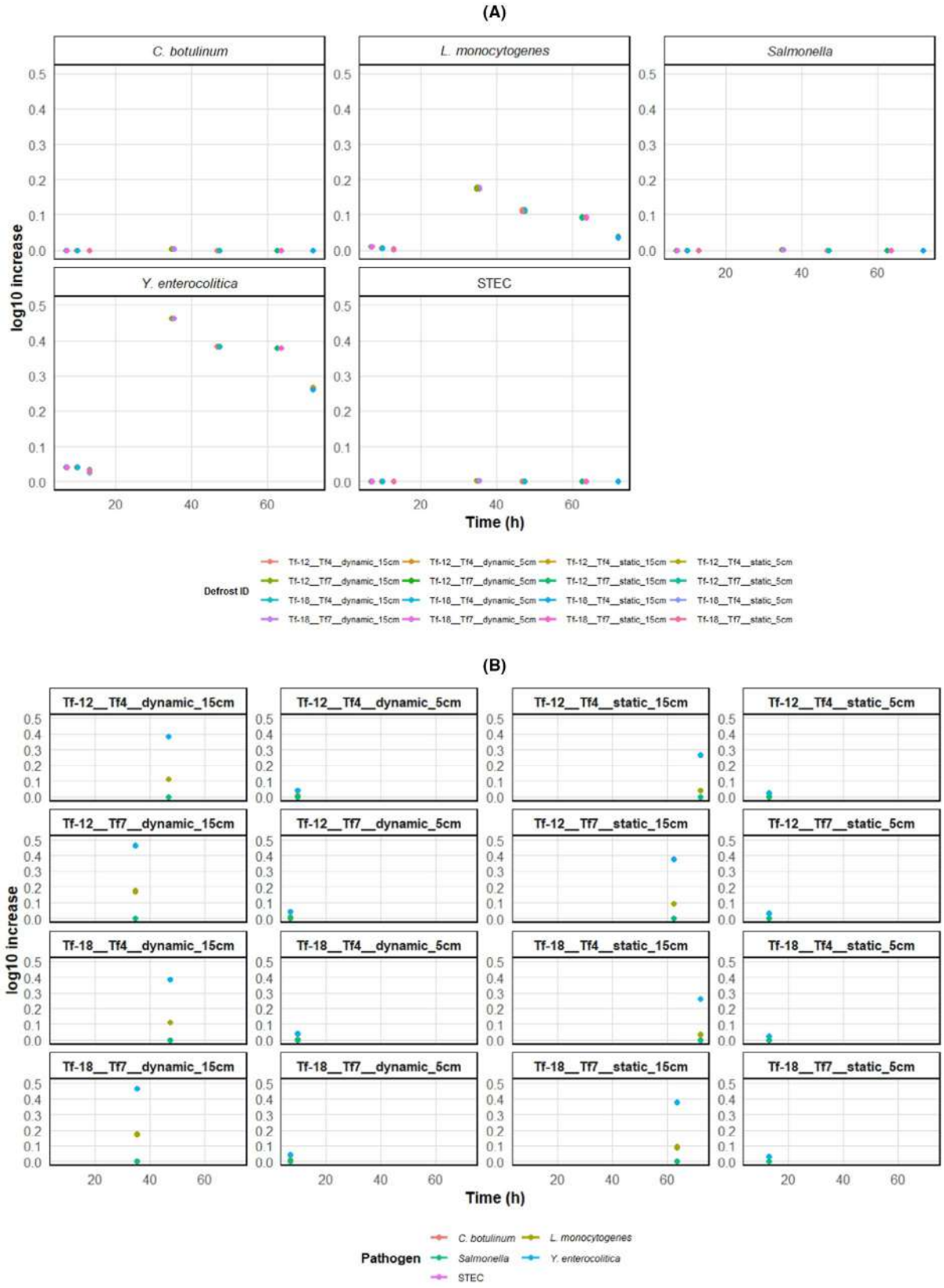

FIGURE 13 Predicted $\log_{10}$ increase of pathogens during defrosting under 16 different scenarios. Each panel in (A) shows growth for a specific pathogen across all defrosting conditions. Each panel in (B) shows growth for all pathogens under a specific defrosting condition. Defrosting conditions are identified in the figure with freezing temperature of –12° or –18°C (Tf-12, Tf-18), defrosting temperature of 4° or 7°C (Tf4, Tf7), defrosting method static or dynamic, thickness of 5 or 15 cm.

No increase was predicted for *Salmonella*, STEC or non-proteolytic *C. botulinum* under any defrosting scenario (Figure 13A). In contrast, limited growth of *Y. enterocolitica* and *L. monocytogenes* (up to 0.18 $\log_{10}$ for *L. monocytogenes* and 0.46 $\log_{10}$ for *Y. enterocolitica*) was predicted only under scenarios corresponding to larger meat pieces, where defrosting durations exceeded approximately 20 h (Figure 13B).

Across all defrosting scenarios, predicted increases remained below 0.5 $\log_{10}$, which corresponds to the predefined threshold used to define 'no growth'. Considering model uncertainty and the assumptions applied, particularly the absence of a lag phase following freezing, it is concluded that the defrosting phase does not lead to any effective pathogen growth and therefore does not contribute to bacterial increase in the overall assessment.

3.7.1.2 | *Spoilage organisms*

Figure 14 presents the predicted growth of spoilage bacteria (LAB and psychrotrophic *Clostridia*) during the defrosting phase across the 16 scenarios. Predicted increases were minimal. Psychrotrophic *Clostridia* showed virtually no growth (< 0.05 $\log_{10}$) under any defrosting scenario. LAB exhibited limited increases, always below 0.2 $\log_{10}$ and mainly under the longest defrosting scenarios corresponding to large meat pieces (defrosting duration exceeding 1 day). No growth of *Pseudomonas* was considered due to the anaerobic environment created by vacuum packaging.

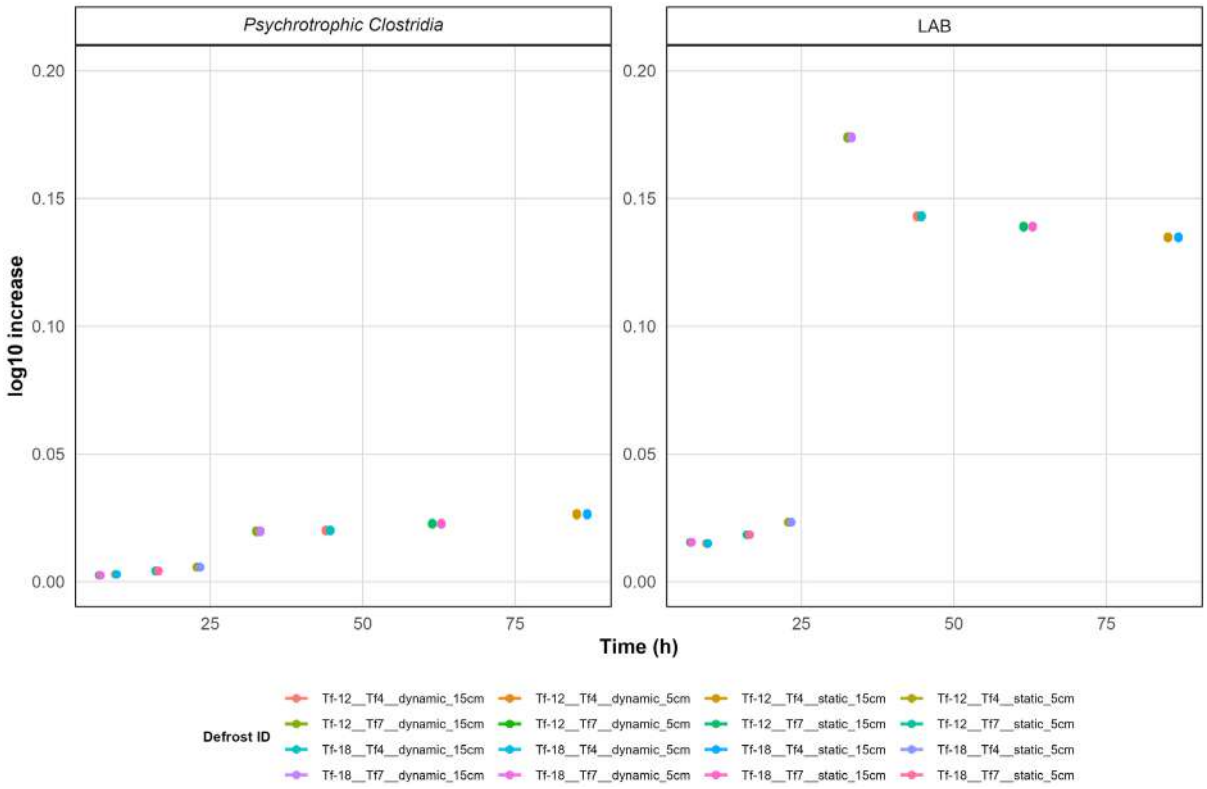

FIGURE 14 Predicted $\log_{10}$ increase of spoilers during defrosting under 16 different scenarios. Each panel shows growth for a specific spoiler across all defrosting conditions. Defrosting conditions are identified in the figure with freezing temperatures of −12°C or −18°C (Tf-12, Tf-18), defrosting temperatures of 4°C or 7°C (Tf4, Tf7), defrosting methods static or dynamic, thicknesses of 5 or 15 cm.

Overall, predicted increases remained below the predefined 0.5 $\log_{10}$ 'no growth' threshold. Given the low magnitude of these changes and considering model uncertainty, defrosting is not considered to contribute to spoilage development in the overall assessment.

3.7.2 | Subsequent storage

3.7.2.1 | *Pathogens*

After defrosting, meats were assumed to be stored for 7 days under two temperature conditions (4°C and 7°C). Figure 15 presents the predicted $\log_{10}$ increase for the different pathogens during this post-defrosting storage period. No growth was assumed for *C. botulinum* as this storage is carried out on unpacked meat where anaerobic conditions are not met.

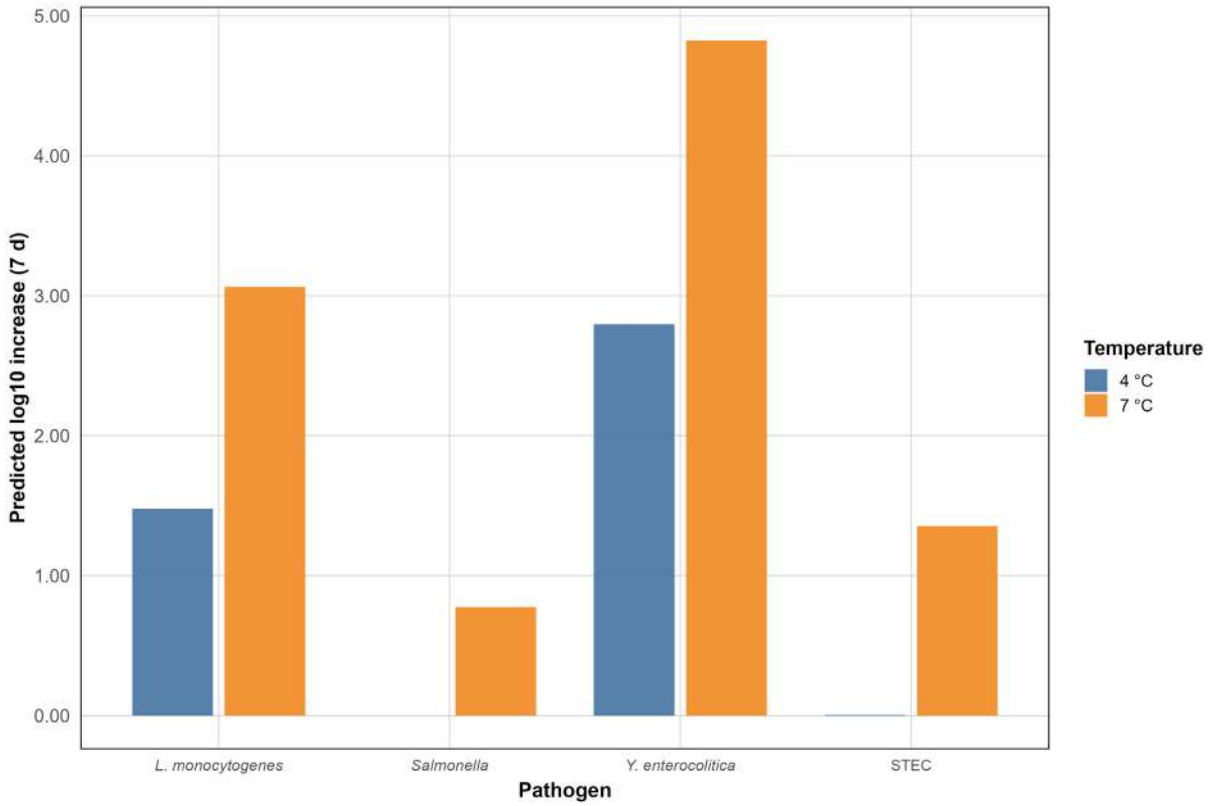

FIGURE 15 Predicted $\log_{10}$ increase of pathogens during post-defrosting storage at 4°C and 7°C after 7 days.

Storage at 7°C after defrosting leads to substantial predicted growth, with $\log_{10}$ increases nearly as high as those observed under the Scenario 1 (7°/noVP/15d, reference scenario). In contrast, post-defrosting storage at 4°C results in markedly smaller bacterial increases. Therefore, storing defrosted meat at 7°C can hardly be combined with pre-freezing storage times identified in any of the scenarios assessed to provide an equivalent storage time to the reference scenario from the safety perspective. In this respect, only storage at 4°C was considered for the estimation of equivalent storage durations relative to the reference condition.

For subsequent adjustment of equivalence times in ToR 2.2 (see Section 3.8), the following increases during post-defrosting storage were retained for the microorganisms identified as limiting pathogenic bacteria for definition of equivalence time for Scenarios 3 and 5: *Y. enterocolitica*: 2.8 $\log_{10}$ and *L. monocytogenes*: 1.5 $\log_{10}$ (see Section 3.6).

3.7.2.2 | *Spoilage organisms*

The growth of spoilage bacteria (LAB and *Pseudomonas*) was assessed under the same post-defrosting storage conditions (4°C and 7°C) after 7 days. No growth was predicted for gas-producing psychrofile clostridia as storage is considered only for unpacked meat where anaerobic conditions are not met.

At 7°C, both *Pseudomonas* and LAB showed substantial growth. Figure 16 presents the predicted $\log_{10}$ increase for these spoilage bacteria during this post-defrosting storage period. For *Pseudomonas*, this $\log_{10}$ increase is particularly high (exceeding 6 $\log_{10}$), meaning that spoilage levels would be reached within the 7-day period even when starting from the lowest initial loads considered (e.g. 1–3 $\log_{10}$ CFU/cm^2). Thus, simply storing defrosted meat at 7°C would lead to spoilage within the evaluated timeframe. In contrast, storage at 4°C markedly limits the growth of both groups. Under this condition, maintaining acceptable microbiological quality during the post-defrosting period remains feasible, provided that pre-freezing levels do not already approach spoilage thresholds (e.g. remain below approximately 5 $\log_{10}$ CFU/cm^2).

For subsequent adaptation of equivalence times in ToR 2.2 (see Section 3.8), a 2.0 $\log_{10}$ increase during post-defrosting storage was retained for LAB, identified as limiting spoilage bacteria for definition of equivalence time for Scenario 2, 3 and 5.

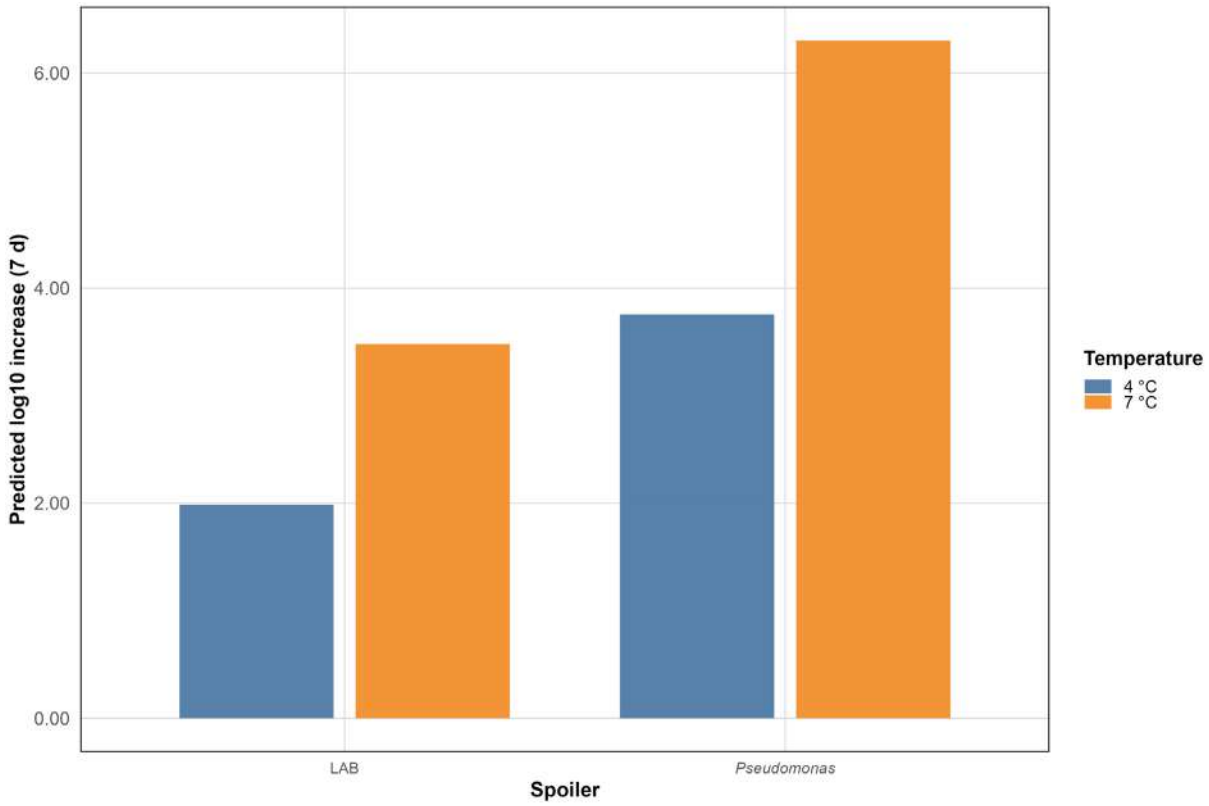

FIGURE 16 Predicted log_{10} increase of spoilage bacteria during post-defrosting storage at 4°C and 7°C after 7 days.

3.7.3 | Considerations with respect to identified uncertainties

In order to assess the impact of the uncertainties identified on the outcome of the assessment and express the overall uncertainty in the answer to the terms of reference, besides the considerations described in Section 3.5.3 and 3.6.3, the following additional considerations were taken into account:

- Lag phase in post-defrosting storage:
  Freezing imposes physiological stress on bacterial cells, which typically induces a lag phase before growth resumes upon defrosting; since this lag phase was not considered in the modelling, the predicted post-thaw growth is therefore likely overestimated.
- Magnitude of growth during defrosting to judge no relevant growth across different defrosting scenarios:
  According to the temperature profiles considered during defrosting, growth was only predicted for the psychrotrophic pathogens L. monocytogenes (< 0.2 log_{10} increase) and Y. enterocolitica (< 0.5 log_{10} increase). The magnitude was considered not to be relevant (considering the usual experimental variability or methodological error of bacterial plate counting). Moreover, the growth prediction was implemented following a conservative approach, i.e. not considering lag phase, probably occurring when bacteria are submitted to the stress of freezing temperatures, which would delay the bacterial growth thus making the log_{10} increase even smaller than the one predicted in the current assessment. Moreover, the a_w (0.99) values used in the prediction cover reasonably foreseeable conservative conditions. These considerations increase the certainty of the assumption that the defrosting scenarios do not contribute to the bacterial increase before the subsequent storage as long as appropriate temperature control practices are implemented during defrosting.

  - Overall, as discussed for bacterial growth before freezing, the magnitude of growth predicted during the storage at 4°C for 7 days after defrosting (used to adjust the storage time before defrosting) was considered more certain for *L. monocytogenes* (1.5 log) and LAB (2 log) due to the robustness of the predictive models used for the estimation than for *Y. enterocolitica*. In all cases, the magnitude of the predicted log_{10} increases is conservative/overestimated since the lag phase was not considered, which would delay the bacterial growth, thus making the log_{10} increase magnitude even smaller than predicted in the current assessment. Moreover, the assumption of no inactivation (no reduction) during freezing may also contribute to an overestimation of the overall change of bacterial loads from before freezing to the end of subsequent storage after defrosting.
  - For *Salmonella*, no growth was assumed because the isothermal storage at 4°C is below the minimum temperature for growth (T_{min}) of this pathogen. However, under real commercial conditions, the temperature of the storage room will most probably oscillate around 4°C (e.g. due to compressor cycles, defrosting cycles, door openings and air circulation patterns) and the *Salmonella* on the meat surface will be exposed to temperatures above the T_{min} allowing

pathogen growth. This may result in an underestimation of the growth potential of *Salmonella* during the subsequent storage after freezing and defrosting and an overestimation of the equivalence time for Scenario 2 (not adjusted).

3.8 | Adjusted storage time before defrosting to account for growth during defrosting and subsequent storage (ToR 2.2.1)

3.8.1 | Calculation of adjusted equivalence times accounting for growth during defrosting and subsequent storage

In Section 3.6, equivalence and spoilage times were estimated based on growth occurring before freezing, assuming that freezing and subsequent defrosting did not modify microbial levels. However, as shown in Section 3.7, non-negligible bacterial growth can occur during the post-defrosting storage phase even at 4°C. Therefore, when these additional growth phases contribute measurably to the overall bacterial increase, the previously estimated equivalence and spoilage times must be adjusted downwards to reflect the actual microbial levels reached in thawed products.

For Scenario 2, with storage at 7°C, *Salmonella* was identified as the limiting microorganism, and its growth is negligible during both defrosting and post-defrosting storage. The pre-freezing equivalence time previously determined for Scenario 2 – a maximum of 5 days at 7°C – already represents a shortened duration compared with the 15-day reference scenario. Since no further growth occurs during defrosting or subsequent storage, this value does not require additional adjustment. Thus, the 5-day pre-freezing period remains sufficient to ensure equivalence with the reference scenario when followed by freezing, defrosting and 7 days of storage at 4°C.

In contrast, for Scenarios 3 and 5, with storage at 3°C, for which LAB, *Y. enterocolitica* and *L. monocytogenes* have been found to be the limiting microorganisms, predicted increases during subsequent storage are not negligible. Consequently, the equivalence times and spoilage limits calculated previously need to be revised to ensure two objectives are met:

1. Equivalence with the reference scenario, that is, the same final microbial levels as in non-frozen meat stored under reference conditions, and
2. Prevention of spoilage at the end of storage for thawed meat.

In practical terms, this means that the total storage time for frozen-defrosted products should remain equivalent to the duration of Scenario 1 (7°/noVP/15d, reference scenario), taking into account the additional growth occurring after freezing. To achieve this, the pre-freezing storage time must be shortened accordingly, so that the cumulative growth (before freezing plus during defrosting and subsequent storage) matches the reference scenario. This adjustment ensures that defrosted meats remain microbiologically comparable to unfrozen reference products at the end of the storage.

3.8.2 | Adjusted equivalence time

The adjusted pre-freezing equivalence and spoilage times, taking into account the predicted growth (log_{10} increase) during post-defrosting storage at 4°C for 7 days, are presented in Tables 12 and 13.

Table 12 presents the adjusted equivalence times for pathogenic bacteria across the alternative storage Scenarios 2, 3 and 5. The adjusted equivalence time for Scenario 2 remains unchanged (5 days), since *Salmonella* does not grow during defrosting or subsequent storage. For *L. monocytogenes* (relevant for bovine and ovine meat) and *Y. enterocolitica* (relevant for porcine meat), the adjusted equivalence times are shorter than the initial estimates for Scenarios 3 and 5. This reduction reflects the additional growth predicted during the post-defrosting storage phase at 4°C.

TABLE 12 Equivalent and adjusted (accounting for growth during post-defrosting storage) equivalence times (days) providing the same growth (log_{10} increase) for pathogens under Scenarios 2, 3 and 5 compared with Scenario 1 (7°/noVP/15d, reference scenario).

Microorganism (model confidence score*)	Non-adjusted equivalence time before freezing			Adjusted equivalence time before freezing[a]		
	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 5 3°C VP15 42d	Scenario 2 7°C VPst 42d	Scenario 3 3°C VPst 42d	Scenario 5 3°C VP15 42d
Salmonella (8)	**5.2**	> 42	> 42	**5.0**	> 42	> 42
L. monocytogenes (8)	12.4	33.2	35.5	7.9	20.3	22.6
Y. enterocolitica (2)	14.0	**29.0**	**29.0**	8.8	**18.0**	**18.0**

Abbreviations: VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

*Confidence score of each predictive model (see Table 5). For *L. monocytogenes*, the value refers to the score of the model under aerobic conditions.

[a]Taking into account the predicted growth (i.e. no growth for *Salmonella*, 1.5 log_{10} increase for *L. monocytogenes*, 2.8 Log_{10} increase for *Y. enterocolitica*) during storage at 4°C for 7 days after freezing and defrosting (see Section 3.7.2.1).

TABLE 13 Non-adjusted and adjusted (accounting for growth during post-defrosting storage) equivalence times (days) for LAB under Scenarios 2, 3 and 5 compared to Scenario 1 (7°/noVP/15d, reference scenario). Values in parentheses indicate the duration (days) of the pre-freezing conditions in order that the levels associated with spoilage (7 $\log_{10}$ CFU/cm^2) are not reached at the end of the subsequent storage after defrosting for different initial levels of contamination (N_0, $\log_{10}$ CFU/cm^2).

Microorganism (model confidence score*)	N_0	Non-adjusted equivalence time before freezing			Adjusted equivalence time before freezing[a]		
		Scenario 2	Scenario 3	Scenario 5	Scenario 2	Scenario 3	Scenario 5
		7°C VPst 42d	3°C VPst 42d	3°C VP15 42d	7°C VPst 42d	3°C VPst 42d	3°C VP15 42d
LAB (6)	1	14 (20.2)	29.3 (NS)	30 (NS)	7.7 (12.5)	15.5 (26.2)	16.2 (26.9)
	3	14 (13.9)	29.3 (29.2)	30 (30.0)	7.6 (7.5)	15.4 (15.2)	16.1 (15.9)
	5	13.9 (7.5)	29.2 (15.2)	30 (16.0)	6.6 (1.2)	13.2 (1.2)	13.8 (1.2)

Abbreviations: NS, no spoilage; VPst, vacuum-packed at stabilisation; VP15, vacuum-packed at 15 days after slaughter.

*Confidence score of each predictive model (see Table 5). The value refers to the score of the model under aerobic conditions.

[a]Taking into account the predicted growth (i.e. 2 $\log_{10}$ increase of LAB) during storage at 4°C for 7 days after freezing and defrosting (see Section 3.7.2.2).

For LAB, the adjusted equivalence times, accounting for growth occurring during defrosting and subsequent storage, are presented in Table 13. For each scenario, the first value indicates the equivalence time before freezing, and the second (in parentheses) corresponds to the duration of pre-freezing conditions to reach the spoilage threshold (7 $\log_{10}$ CFU/cm^2) at the end of the subsequent storage period.

After adjustment, equivalence times are systematically reduced compared with pre-freezing estimates. This reduction reflects the additional LAB growth predicted during the post-defrosting storage phase (2 $\log_{10}$ as explained in Section 3.7.2.2). For Scenario 2, with storage at 7°C, the adjusted equivalence time decreases from about 14 days to 7 days, indicating that the same microbial level as in the reference scenario would be reached approximately 1 week earlier. However, note that, for Scenario 2, *Salmonella* limits the equivalence time to 5 days.

For Scenarios 3 and 5, with storage at 3°C, the adjusted equivalence times are also shorter (around 13–15 days instead of 29–30 days before freezing).

Overall, these results confirm that microbial growth after defrosting cannot be neglected and that pre-freezing storage time must therefore be shortened to maintain microbiological equivalence.

Related to spoilage, higher initial loads result in shorter times before the levels associated with spoilage are reached. For instance, when the initial contamination is low ($N_0 = 1$ $\log_{10}$ CFU/cm^2), spoilage levels are reached after the equivalence time, whereas for higher initial loads ($N_0 = 5$ $\log_{10}$ CFU/cm^2), spoilage levels are reached before and become the limiting factor as the duration is shorter than the equivalence time.

When the pre-freezing storage duration required to be below the spoilage limit becomes extremely short (i.e. limited to the stabilisation period), as observed for the adjusted values in all three scenarios for the highest initial contamination level (N_0), immediate freezing would be required after stabilisation. Maintaining such a short storage time before freezing may be hardly compatible with standard processing and logistics operations.

3.9 | Loads of indicator microorganisms before freezing and at the end of post-defrosting storage

3.9.1 | Loads of indicator microorganisms (ToRs 1.2.2 and 2.2.2)

For Scenario 2 (7°/VPst/42d), a maximum pre-freezing storage duration of 5 days was considered, as limited by the growth of *Salmonella*. Under these conditions:

- The predicted growth of LAB (considered as the main bacterial group responsible for the increase of ACC) was 1.3 $\log_{10}$. Consequently, the ACC levels at the end of this pre-freezing period are estimated to be 2.3, 4.3 and 6.3 $\log_{10}$ CFU/cm^2 for initial contamination levels (N_0) of 1, 3 and 5 $\log_{10}$ CFU/cm^2, respectively.
  When accounting for the additional 2 $\log_{10}$ increase in LAB predicted during the subsequent post-defrosting storage phase, the final levels at the end of subsequent storage would reach approximately 4.3, 6.3 and 8.3 $\log_{10}$ CFU/cm^2, respectively.
- The levels of *E. coli* before freezing were also estimated based on the assumed initial contamination levels ($N_0 = 0$, 1, or 2 $\log_{10}$ CFU/cm^2). Considering growth during 5 days of storage under Scenario 2 conditions, the predicted growth for *E. coli* was 0.8 $\log_{10}$ resulting in levels before freezing of 0.8, 1.8 and 2.8 $\log_{10}$ CFU/cm^2, respectively. The levels would remain without changes at the end of the post-defrosting subsequent storage at 4°C as no growth is expected to occur at storage below the *Tmin* for *E. coli*.
- For *Enterobacteriaceae*, assuming growth behaviour similar to that of *Y. enterocolitica* as a proxy for psychrotrophic enterobacteria, an increase of 3.1 $\log_{10}$ was predicted during the pre-freezing storage phase. Thus, for initial levels of 0, 1.5 and 3 $\log_{10}$ CFU/cm^2, the predicted concentrations before freezing would be 3.1, 4.6 and 6.1 $\log_{10}$ CFU cm^2,

respectively. Considering the additional growth predicted during the post-defrosting subsequent storage phase at 4°C, final *Enterobacteriaceae* levels would reach approximately 5.9, 7.4 and 8.9 $\log_{10}$ CFU/cm^2, depending on the initial contamination level.

For Scenario 3 (3°/VPst/42d), the pre-freezing storage duration is primarily constrained by LAB reaching the spoilage associated levels (7 $\log_{10}$ CFU/cm^2); assuming the intermediate initial level of LAB (3 $\log_{10}$ CFU/cm^2), the maximum storage period for Scenario 3 is estimated to be 15 days. Under these conditions:

- For ACC, which are assumed to follow a similar growth pattern to LAB, an increase of 2.0 $\log_{10}$ is predicted. Consequently, ACC levels at the end of this pre-freezing period are estimated to be 3.0, 5.0 and 7.0 $\log_{10}$ CFU/cm^2 for initial contamination levels (N_0) of 1, 3 and 5 $\log_{10}$ CFU/cm^2, respectively. When accounting for the additional 2 $\log_{10}$ increase in LAB predicted during the post-defrosting subsequent storage phase, the final levels at the end of subsequent storage would reach approximately 5.0, 7.0 and 9 $\log_{10}$ CFU/cm^2, respectively.
- The levels of *E. coli* before freezing would remain without changes to the assumed initial contamination levels ($N_0 = 0$, 1 or 2 $\log_{10}$ CFU/cm^2), as no growth is assumed at 3°C for this mesophilic indicator microorganism.
- For *Enterobacteriaceae*, assuming growth behaviour similar to that of *Y. enterocolitica*, an increase of 4.6 $\log_{10}$ is predicted during the 15 days pre-freezing storage phase. Thus, for initial levels of 0, 1.5 and 3 $\log_{10}$ CFU/cm^2, the predicted concentrations before freezing would be 4.6, 6.1 and 7.6 $\log_{10}$ CFU/cm^2, respectively. Considering the additional growth expected during the subsequent post-defrosting storage phase at 4°C, the final *Enterobacteriaceae* levels would reach approximately 7.4, 8.9 and 9 $\log_{10}$ CFU/cm^2, depending on the initial contamination level.

For Scenario 5 (3°/VP15/42d), the pre-freezing storage duration is also primarily constrained by the achievement of the spoilage-associated level by LAB; assuming an intermediate initial level of LAB (3 $\log_{10}$ CFU/cm^2), the maximum storage period for Scenario 5 is estimated to be 16 days. Under these conditions:

- For aerobic colony counts (ACC), which are assumed to follow a similar growth pattern to LAB, an increase of 2.0 $\log_{10}$ is predicted. Consequently, ACC levels at the end of this pre-freezing period are estimated to be 3.0, 5.0 and 7.0 $\log_{10}$ CFU/cm^2 for initial contamination levels (N_0) of 1, 3 and 5 $\log_{10}$ CFU/cm^2, respectively. When accounting for the additional 2 $\log_{10}$ increase in LAB predicted during the subsequent post-defrosting storage phase, the final levels at the end of subsequent storage would reach approximately 5.0, 7.0 and 9 $\log_{10}$ CFU/cm^2, respectively.
- The levels of *E. coli* before freezing would remain without changes to the assumed initial contamination levels ($N_0 = 0$, 1 or 2 $\log_{10}$ CFU/cm^2), as no growth is assumed at 3°C for this mesophilic indicator microorganism.
- For *Enterobacteriaceae*, assuming growth behaviour similar to that of *Y. enterocolitica*, an increase of 5.0 $\log_{10}$ is predicted during the 16 days pre-freezing storage phase. Thus, for initial levels of 0, 1.5 and 3 $\log_{10}$ CFU/cm^2, the predicted concentrations before freezing would be 5.0, 6.5 and 7.9 $\log_{10}$ CFU/cm^2, respectively. Considering the additional growth (2.8 $\log_{10}$) expected during the subsequent post-defrosting storage phase at 4°C, the final *Enterobacteriaceae* levels would reach approximately 7.8, 9.0 and 9.0 $\log_{10}$ CFU/cm^2, depending on the initial contamination level.

3.9.2 | Considerations with respect to identified uncertainties

In order to assess the impact of uncertainties on the outcomes of the assessment and to express the overall uncertainty in relation to the terms of reference, besides the considerations described in Section 3.5.3, 3.6.3 and 3.7.3, the following considerations were taken into account:

- Outcome of modelling:
  The predicted bacterial indicator levels and equivalence times rely on deterministic growth models assuming homogeneous and constant conditions. The lack of variability data, not only on the pH and a_w but also the unknown initial level of contamination, as well as the oscillations of temperature during storage, may lead to under- or over-estimation of growth potential, particularly for mixed microbial populations included in the ACC and Enterobacteriaceae indicators. Some modelling choices may contribute to an over-estimation of the predicted levels of indicators at the two stages: no lag phase, focusing on psychrotolerant/psychrophilic species within the diverse microbial group.
- Reliability of predictive models:
  The level of confidence associated with the predictive microbiology models used varies substantially between microorganisms (see Section 3.5.3). The model for *E. coli* is organism-specific and well-validated, providing high confidence in growth estimates. In contrast, predictions for aerobic colony count (ACC) and *Enterobacteriaceae* are based on simplified or surrogate assumptions, resulting in lower confidence. Furthermore, the model for *Y. enterocolitica* was rated with a low confidence score.
- Main sources of uncertainty:
  - For *Enterobacteriaceae*, growth behaviour was assumed to follow that of *Y. enterocolitica*. However, *Yersinia* is unlikely to be the dominant genus in this group and may represent a faster-growing member, potentially leading to highly

conservative (overestimated) growth predictions. Most of the species belonging to the Enterobacteriaceae family/group are mesophilic like *Salmonella* and *E. coli*. If these two species are taken as proxy representing Enterobacteriaceae, the estimated levels of this microbial indicator before freezing would be much lower, and for Scenarios 3 and 5, the level would have no relevant changes during the post-defrosting storage at 4°C (as no growth is expected to occur at storage below the T_{min}). Even if the storage temperature at 4°C is subjected to oscillations, as usually occurring under commercial conditions, mesophilic Enterobacteriaceae may find growth permitting conditions, but the magnitude of increase is expected to be limited though variable depending on the amplitude of the temperature oscillations.
  - For ACC, it was assumed that the indicator levels are fully represented by the growth of LAB or *Pseudomonas*. In reality, these organisms are often minor components of the total aerobic microbiota in raw meat at early stages. Their initial increase may be delayed by a pseudo-lag phase until they become dominant, meaning that the actual increase in ACC may occur later than predicted.

Overall, the above considerations imply that the predicted increases for microbial indicator groups ACC and Enterobacteriaceae are subject to greater uncertainty compared to *E. coli* and should be interpreted with caution. *E. coli* estimates are supported by more robust and validated models.

4 | CONCLUSIONS

ToR 1.1. To compare the effect on survival and growth of relevant food-borne pathogenic bacteria, indicator organisms and spoilage bacteria in fresh meat of ungulates that has been stored/transported at the following conditions applied between slaughter and freezing:

- **Core temperature of maximum 7°C until 15 days after slaughter, aerobic conditions – Scenario 1 (7°/noVP/15d, reference scenario);**
- **Core temperature of maximum 7°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation – Scenario 2 (7°/VPst/42d);**
- **Core temperature of maximum 3°C until 6 weeks after slaughter, vacuum-packed immediately after stabilisation – Scenario 3 (3°/VPst/42d);**
- **Core temperature of maximum 7°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter – Scenario 4 (7°/VP15/42d);**
- **Core temperature of maximum 3°C until 6 weeks after slaughter, vacuum-packed 15 days after slaughter – Scenario 5 (3°/VP15/42d)**.

- Considering the data available in the scientific literature on the input variables needed to estimate bacterial growth in meat, only meat from bovine, ovine, and porcine animals could be included in the assessment. Considering similarities of meat characteristics among species and of relevant bacteria to be considered, the assessment performed for ovine meat can be extrapolated to caprine meat and the assessment performed for bovine meat can be extrapolated to equine meat. The assessment cannot be extrapolated to other ungulate species.
- The following microorganisms were considered relevant for consideration for this assessment, with respect to the five animal species covered:
  - Pathogenic bacteria: *Salmonella* spp. (all animal species), Shiga toxin-producing *E. coli* (bovine, ovine and caprine species), *L. monocytogenes* (all animal species), *Y. enterocolitica* (porcine species), non-proteolytic *C. botulinum* (all animal species).
  - Spoilage bacteria: pseudomonads, lactic acid bacteria (LAB), psychrotolerant *Clostridia* (all animal species).
  - Indicator microorganisms: aerobic colony count, *Enterobacteriaceae*, *E. coli* (all animal species).

- Predictive microbiology models for growth were selected and evaluated based on their validated performance in meat matrices and ability to account for temperature, pH, water activity (a_w) effects and for packaging under vacuum conditions. Confidence scores were assigned to each predictive microbiology model and were considered in the overall uncertainty analysis.
- The growth potential of relevant pathogenic, spoilage and indicator microorganisms in the evaluated scenarios differed from the reference scenario. The magnitude of differences varied across microorganisms, and in some cases, levels close to or even lower than Scenario 1 (7°/noVP/15d, reference scenario) were predicted. In particular:
  - Scenario 2 (7°/VPst/42d) and Scenario 4 (7°/VP15/42d) consistently resulted in microbial levels above Scenario 1 (reference scenario).
  - Scenario 3 (3°/VPst/42d) and 5 (3°/VP15/42d) generally resulted in bacterial levels close to or below the reference, although higher levels can occur, particularly for *L. monocytogenes* and LAB.
  - Scenario 4 represents the reference situation (Scenario 1) followed by additional storage under vacuum packaging. Delayed vacuum packaging does not prevent further growth of pathogens, LAB or indicator organisms, and this

scenario inevitably leads to higher contamination levels than the reference. For this reason, Scenario 4 was not further considered in the assessment.
  - When considering Baseline II conditions (i.e. conservative conditions of temperature, pH and a_w more favourable to microbial growth), predicted growth was substantially higher for all microorganisms considered. Under such conditions, both the reference and alternative scenarios often reached the maximum population density, which makes direct comparisons between scenarios not informative. Therefore, Baseline II conditions were not considered further for additional predictions in the assessment.

- Considering the results obtained and the uncertainties identified:

  - It is judged to be 95%–99% certain (extremely likely) that Scenario 2 (7°/VPst/42d) and Scenario 4 (7°/VP15/42d) result in more growth of at least some of the bacteria assessed compared to Scenario 1 (7°/noVP/15d, reference scenario), and therefore, they are not microbiologically equivalent to Scenario 1.
  - It is judged to be 66%–90% certain (likely) that Scenario 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d) result in more growth of at least some of the bacteria assessed compared to Scenario 1 (7°/noVP/15d, reference scenario), and therefore, they are not microbiologically equivalent to Scenario 1.

ToR 1.2.1. If differences are identified in the survival and growth of relevant food-borne pathogenic or spoilage bacteria (outcome of ToR 1.1), then to identify refrigeration times/temperatures/use of vacuum packaging scenarios for meat intended to be frozen that would result in a similar load of the relevant bacterial hazards as compared to standard fresh (never frozen) meat.

- Predictive models were used to estimate both the storage times leading to microbiological equivalence between alternative Scenarios 2, 3 and 5 and Scenario 1 (7°/noVP/15d, reference scenario) and the times at which spoilage thresholds (determined by LAB or *Pseudomonas*) would be reached. Scenario 4 was not considered further in the assessment, as explained above.
- The assessment indicates that equivalence times vary substantially depending on temperature and packaging.
- For Scenario 2 (7°/VPst/42d):

  - Equivalence was reached earlier than in other scenarios.
  - Equivalence was determined mainly by *Salmonella*, at approximately 5 days for all animal species. The shorter time compared to Scenario 1 (7°/noVP/15d, reference scenario) is explained by the higher a_w assumed for the storage of meat under vacuum-packaging conditions.
  - For spoilage bacteria and indicator microorganisms, equivalence was typically reached later, approximately at 14 days, with LAB as the limiting bacteria.
  - Time-to-spoilage was determined by LAB and was reached between 7 and 24 days, depending on the level of initial contamination. This means that product deterioration may occur soon after equivalence is achieved for pathogens.

- For Scenario 3 (3°/VPst/42d):

  - *L. monocytogenes* (for bovine and ovine meat) and *Y. enterocolitica* (for porcine meat) required approximately 29–34 days to reach equivalence.
  - LAB were the limiting spoilage/indicator microorganisms, with equivalence reached approximately at 29–30 days.
  - Spoilage by LAB occurred at approximately 14–36 days, depending on the initial contamination level, which in some cases coincided with or preceded the equivalence time for pathogens.

- Scenario 5 (3°/VP15/42d):

  - Equivalence was reached later than in other scenarios.
  - Based on *L. monocytogenes* (for bovine and ovine meat) and *Y. enterocolitica* (for porcine meat), equivalence was reached after approximately 29–36 days.
  - Based on the LAB spoilage bacteria, equivalence was reached after approximately 30 days.
  - Spoilage by LAB was predicted after approximately 16–36 days, depending on the initial contamination, often shorter than the pathogen-based equivalence times, thus defining the practical storage limit in this scenario.

- Considering the results obtained and the uncertainties identified:

  - It is judged to be 66%–90% certain (likely) that Scenario 2 (7°/VPst/42d) allows 5–6 days of storage before microbiological equivalence with Scenario 1 (7°/noVP/15d, reference scenario) is reached.
  - It is judged to be 66%–90% certain (likely) that Scenario 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d) allow 29–30 days of storage before microbiological equivalence with Scenario 1 (7°/noVP/15d, reference scenario) is reached.

- Overall, LAB were often the limiting bacteria defining practical shelf-lives (spoilage times). Note that the scenarios for LAB contamination are strongly dependent on the hygiene level. Fresh meat with higher initial contamination was predicted to reach the spoilage threshold of 7 $\log_{10}$ CFU/cm^2 much earlier, which in practice shortens the usable storage time. Based on the initial levels of spoilage bacteria considered in this assessment, it is concluded that under better hygiene conditions (lower initial load of spoilage bacteria), equivalence time is primarily determined by pathogens, whereas under worse hygiene conditions (higher initial load of spoilage bacteria), reaching the spoilage threshold rather than equivalence time (pathogenic and spoilage bacteria) with Scenario 1 (7°/noVP/15d, reference scenario) defines the practical limit.

TOR 2.1. To compare the effect on survival and growth of relevant food-borne pathogenic bacteria, indicator organisms and spoilage bacteria in defrosting scenarios where freezing occurs at −12**°C or** −18**°C, defrosting at 4°C or 7°C, for short (4–8 h) or long (24–72 h) duration, dynamic or static defrosting applied, meat is vacuum-packed or not, and subsequent storage for 7 days at 4°C or 7°C temperature**

- Sixteen defrosting scenarios were evaluated:
  - No increase was predicted for *Salmonella*, STEC, or non-proteolytic *C. botulinum* under any defrosting scenario. Limited growth of *Y. enterocolitica* and *L. monocytogenes* (0.46 $\log_{10}$ and 0.18 $\log_{10}$ respectively) was predicted only under scenarios involving large meat pieces and defrosting durations longer than 20 h.
  - For spoilage bacteria, no or only very small increases were predicted, with predicted LAB growth below 0.2 $\log_{10}$ and only under scenarios involving large meat pieces and defrosting duration longer than 20 h.
  - Considering the results obtained and the uncertainties identified, it is judged to be 90%–95% certain (very likely) that the defrosting phase under the conditions assessed does not lead to relevant growth of pathogenic and spoilage bacteria.
- Subsequent storage of defrosted meat was assessed under two different temperature scenarios:
  - Post-defrosting storage at 7°C led to substantial predicted growth of both pathogenic and spoilage bacteria, with $\log_{10}$ increases nearly as high as those observed under Scenario 1 (reference scenario). Therefore, storage conditions at this temperature were not further considered in the assessment.
  - Post-defrosting storage at 4°C was predicted to result in increases of *Y. enterocolitica* (2.8 $\log_{10}$), *L. monocytogenes* (1.5 $\log_{10}$), LAB (2.0 $\log_{10}$) and *Pseudomonas* (3.8 $\log_{10}$).

TOR 2.2.1 Based on the outcome of the mandate on the microbiological safety of ungulates meat intended to be frozen and ToR 1 of this mandate, provide scenarios that consider the pre-freezing, defrosting and storage conditions that would result in a similar load of the relevant bacterial hazards as compared to standard fresh (never frozen) meat.

- Equivalence times defined under ToR 1.2.1 were adjusted in order to identify the times at pre-freezing stage that would ensure equivalence among scenarios at the end of post-defrosting storage, considering the additional growth during post-defrosting storage at 4°C:
  - For Scenario 2 (7°/VPst/42d), where an equivalence time of 5 days was determined by *Salmonella*, no adjustment was required since *Salmonella* remained the limiting bacteria. *Salmonella* did not exhibit significant growth either during defrosting or the subsequent storage period.
  - For Scenario 3 (3°/VPst/42d) and Scenario 5 (3°/VP15/42d), the adjusted equivalence times were shorter for both pathogenic and spoilage bacteria. *L. monocytogenes* (for bovine and ovine meat) and *Y. enterocolitica* (for porcine meat) reached equivalence at approximately 18–23 days, while LAB reached equivalence at approximately 13–16 days. Only when initial loads of spoilage bacteria are high (5 $\log_{10}$), time-to-spoilage would be the limiting factor and would require immediate freezing after stabilisation.

ToRs 1.2.2./2.2.2 To indicate which bacteria would be most relevant to monitor (verification) in these scenarios and what bacterial load might be expected just before freezing and at the end of storage post defrosting.

- In this scientific opinion, predicted levels of selected indicator microorganisms are presented, considering:
  - three indicator microorganisms as most relevant for verification: ACC (by using *Pseudomonas* or LAB as proxy), *E. coli*, *Enterobacteriaceae* (by using *Y. enterocolitica* as proxy);
  - three different initial contamination levels, which reflect different possible meat hygiene conditions at post-slaughter chilling;
  - two distinct stages: before-freezing and at the end of post-defrosting storage.
- Predicted levels represent examples under the assumption of initial contamination levels and model parameters, which can take a wide range of values and should be further adjusted based on actual measurements in practical settings.

- Considering the results obtained and additional uncertainties identified, and given the range of initial contamination levels assumed:
  - It is judged to be 66%–90% certain (likely) that the predicted levels for *E. coli* cover plausible levels expected under real conditions;
  - It is judged to be 33%–66% certain (about as likely as not) that the predicted levels for ACC and *Enterobacteriaceae* cover plausible levels expected under real conditions.

ABBREVIATIONS

ACC	aerobic colony count
APC	aerobic plate count
AQ(s)	assessment question(s)
aw	water activity
BIOHAZ	biological hazards
BoNT	botulinum neurotoxins
CFL	Courant–Friedrichs–Lewy
CFU	colony forming units
CPM	cardinal parameters type model
DFD	dark, firm and dry
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
ISO	International Organization for Standardization
LAB	lactic acid bacteria
MPD	maximum population density
MSM	mechanically separated meat
N0	initial contamination level
NG	no growth
NS	no spoilage
PSE	pale, soft and exudative
STEC	shiga toxin-producing Escherichia coli
T_{min}	minimum temperature for growth
ToR(s)	term(s) of reference
TVC	total viable count
VP15	vacuum-packed at 15 days after slaughter.
VPst	vacuum-packed at stabilisation
7°/noVP/15d	storage at 7°C in aerobic conditions until 15 days after slaughter represents Scenario 1
7°/VPst/42d	storage at 7°C, vacuum-packed at stabilisation, until 42 days after slaughter, represents Scenario 2
3°/VPst/42d	storage at 3°C, vacuum-packed at stabilisation, until 42 days after slaughter, represents Scenario 3
7°/VP15/42d	storage at 7°C, vacuum-packed 15 days after slaughter, until 42 days after slaughter, represents Scenario 4
3°/VP15/42d	storage at 3°C, vacuum-packed 15 days after slaughter until 42 days after slaughter represents Scenario 5

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ANNEX A

Protocol

Annex A is available at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX B

Time–temperature plots during cooling of bovine, ovine and porcine meat

Annex B is available at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX C

pH values in meat from different ungulate species

Annex C is available at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX D

Additional aw values in meat gathered from different ungulate species

Annex D is available at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX E

Initial contamination levels of microbial indicator groups on chilled ungulates carcasses

Annex E is available as an HTML file (to be downloaded and then opened) at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX F

Uncertainty table

Annex F is available at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX G

Bacterial growth before freezing, comparison at the end of the scenarios (ToR 1.1) – Pathogens – Interactive tables with complete results

Annex G is available as HTML file at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX H

Bacterial growth before freezing, comparison at the end of the scenarios (ToR 1.1) – Indicator microorganisms and spoilage bacteria – Interactive tables with complete results

Annex H is available as HTML file at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX I

Bacterial growth before freezing, equivalence times (ToR 1.2) – Pathogens – Interactive tables with complete results

Annex I is available as HTML file at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX J

Bacterial growth before freezing, equivalence times (ToR 1.2) – Indicator microorganisms and spoilage bacteria – Interactive tables with complete results

Annex J is available as an HTML file at the following link: https://doi.org/10.5281/zenodo.17747830.

ANNEX K

Data and R codes supporting the scientific opinion

Annex K gathers all data and R codes used for the following purposes:

- Predicting pre-freezing, defrosting and post-defrosting microbial growth;
- Modelling meat surface temperature during defrosting.

All the files with relevant data and R scripts are available within a dedicated compressed folder at the following link: https://doi.org/10.5281/zenodo.17747830.