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Guidance on date marking and related food information: part 2 (food information)

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Abstract

A risk-based approach was used to develop guidance to be followed by food business operators (FBOs) when deciding on food information relating to storage conditions and/or time limits for consumption after opening a food package and thawing of frozen foods. After opening the package, contamination may occur, introducing new pathogens into the food and the intrinsic (e.g. pH and a_w), extrinsic (e.g. temperature and gas atmosphere) and implicit (e.g. interactions with competing background microbiota) factors may change, affecting microbiological food safety. Setting a time limit for consumption after opening the package (secondary shelf-life) is complex in view of the many influencing factors and information gaps. A decision tree (DT) was developed to assist FBOs in deciding whether the time limit for consumption after opening, due to safety reasons, is potentially shorter than the initial 'best before' or 'use by' date of the product in its unopened package. For products where opening the package leads to a change of the type of pathogenic microorganisms present in the food and/or factors increasing their growth compared to the unopened product, a shorter time limit for consumption after opening would be appropriate. Freezing prevents the growth of pathogens, however, most pathogenic microorganisms may survive frozen storage, recover during thawing and then grow and/or produce toxins in the food, if conditions are favourable. Moreover, additional contamination may occur from hands, contact surfaces or contamination from other foods and utensils. Good practices for thawing should, from a food safety point of view, minimise growth of and contamination by pathogens between the food being thawed and other foods and/or contact surfaces, especially when removing the food from the package during thawing. Best practices for thawing foods are presented to support FBOs.

Keywords: date marking, food information, food storage, secondary shelf-life, opened package, thawing, public health

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Waiver: In accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management a waiver was granted to an expert of the Working Group. Pursuant to Article 21(6) of the aforementioned Decision, the concerned expert was allowed to take part in the discussions and in the drafting of the scientific output but was not allowed to take up the role of chair within that time frame. Any competing interests are recorded in the respective minutes of the meetings of the working group of the BIOHAZ Panel.

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Summary

Following a request from the European Commission, the EFSA Panel on Biological Hazards (BIOHAZ) was asked for scientific opinions providing guidance on date marking and related food information in relation to the application by food business operators (FBOs) of Regulation (EU) No 1169/2011 on food information provided to consumers as an integrated part of their food safety management system (FSMS). The opinions developed a risk-based approach to be followed by FBOs when deciding on the type of date marking, setting of shelf-life and the associated food information to be provided in order to ensure food safety.

In particular, the European Food Safety Authority (EFSA) was requested to provide scientific advice in **ToR 1** on the factors that make certain foods highly perishable and therefore, after a short period, likely to constitute an immediate danger to human health. How those factors should be considered by FBOs when deciding whether a 'use by' date or a 'best before' date is required, and for setting the shelf-life and the required storage conditions, was also evaluated. Similarly, in **ToR 2**, the factors that make certain foods become unfit for human consumption, but without constituting an immediate danger to human health, were assessed. **ToR 3** requested advice to avoid an increase of food safety risks, specifically related to storage conditions and/or time limits for consumption after opening the package, while **ToR 4** related to advice the FBO can give to consumers regarding thawing of frozen foods including good practices, storage conditions and/or time limits for consumption after thawing. ToRs 1 and 2 were addressed in a previous opinion (EFSA BIOHAZ Panel, 2020a) while ToRs 3 and 4 are covered in this opinion.

To address ToR 3, the intrinsic, extrinsic and implicit factors, which might change once the package is opened, and affect microbiological food safety, are described. A decision tree (DT) was developed to assist FBOs in the decision whether it is appropriate to indicate the time limit and storage conditions for consumption after opening of the package.

To address ToR 4, guidelines, scientific literature and information from other sources was summarised, critically appraised and adapted to provide updated guidance to FBOs on food information to provide to consumers regarding best practices of thawing, storage conditions and/or time limits for consumption after thawing.

After opening a food package, contamination may occur via air flow, fluid drip or due to consumer handling via hands, utensils, containers, etc., which may introduce new pathogens into the food or increase the concentration of pathogens already present.

Opening of the food package may change the food related conditions affecting the ability of pathogenic microorganisms to grow and/or produce toxins (i.e. extrinsic, intrinsic, or implicit factors). Extrinsic factors (such as the atmosphere composition) are probably the most important factors that may change after opening the package. The protection of vacuum or modified atmosphere packaging (MAP) is lost and a change in the growth behaviour (usually increasing the growth capability/rate) of the pathogens in the food can be expected. The effect of changes in the intrinsic (such as a_w or pH) and implicit (such as competing microbiota) factors on pathogen growth after opening the packages should also be considered.

Setting a time limit for consumption after opening the package (secondary shelf-life) is complex in view of the many influencing factors and information gaps. An additional level of complexity comes from the need to consider consumer behaviour and reasonably foreseeable conditions of use, as described in the part 1 opinion.

Opening the package of a food product may impact both safety and quality. For the purpose of this opinion, it is appropriate to establish a time limit for consumption and storage conditions after opening the package when opening can have an impact on product safety.

A DT, consisting of a sequence of five questions, was developed and supported by various application examples to assist FBOs in the decision whether the time limit for consumption after opening, due to safety reasons, is potentially shorter than the initial 'best before' or 'use by' date of the product in its unopened package.[1] The underlying assumptions for the DT are that: a) after opening the package, contamination of the product with pathogenic microorganisms is always possible and b) the time limit for consumption after opening the package in relation to the initial 'use-by' or 'best before' date depends on whether opening the package changes the type of pathogenic microorganisms in the food (e.g. contamination with vegetative cells not present in the unopened food package with, in general, a wider range of growth capabilities compared to growth and/or toxin

[1] Decision tree is available in French, German, Italian and Spanish under 'Supporting Information'. Note that the decision tree in English is the official version.

production from spores), or the factors affecting growth of pathogenic microorganisms compared to the unopened product.

According to the DT, in case of products for which opening the package leads to a change of the type of pathogenic microorganisms present in the food and/or factors increasing their growth compared to the unopened product, the outcome is that a shorter time limit for consumption after opening the package compared to the initial 'best before' or 'use by' date of the unopened food would be appropriate.

Overall, it is considered that the DT will result in appropriate and consistent outcomes on time limits and storage conditions, within the interpretations of regulations and the assumptions made in its development. None of the identified sources of uncertainty was considered more important than any of the others. Taken together, the uncertainties are considered to result in a DT that may overestimate the risk for some food products.

From a food safety point of view, freezing prevents the growth of pathogens. However, even though the concentration of pathogens may decrease over time, elimination is usually not complete during the freezing period depending on the pathogen and initial concentrations, the duration of the frozen storage and conditions during freezing/thawing.

Pathogenic microorganisms that survive frozen storage can recover during thawing and may grow and/or produce toxins in the food during or after thawing if the pH, water activity and storage temperature support growth. Moreover, during the handling of thawed foods, additional contamination may occur from the hands, contact surfaces (e.g. utensils), or from other foods.

Good practices for thawing should minimise contamination by pathogens between the food being thawed and other foods and/or contact surfaces when the food is removed from packaging during thawing, and limit conditions favourable to their growth.

Advice that the FBO may provide to consumers regarding good practices for thawing of frozen foods, storage conditions and time limits for consumption of thawed foods includes using a mode of thawing that ensures sufficient thawing at a time and temperature combination that avoids growth of pathogens which have survived during freezing, also considering further use, keeping thawed foods in the original package or in a clean container and only using clean utensils and hands when handling the food to avoid contamination.

Furthermore, the use of thawed food in food preparations, or storage of thawed food, should be carried out according to instructions from the FBO. The FBO should consider providing advice regarding time-temperature limits for the storage of thawed foods and advice sufficient heat treatment of the thawed foods to eliminate pathogens before consumption.

Advice from the FBO also includes informing consumers that frozen foods are intended to be heat treated/cooked unless the production process implies that the thawed frozen product is safe and can be consumed without cooking as a ready-to-eat food.

Recommendations include the collection of time-temperature data on reasonably foreseeable storage conditions of foods in the European Union (EU) Member States (MS) and to clarify and provide guidelines on how to use these data in secondary shelf-life decisions and developing appropriate level of protection (ALOP)/food safety objective (FSO) for relevant food-pathogen combinations, since the lack of such data is an obstacle for setting the primary and secondary shelf-life of foods in relation to food safety.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background as provided by the European Commission

Food waste prevention is a priority set out in the EU Action Plan for the Circular Economy adopted by the European Commission in December 2015.[2] As part of that Action Plan, the Commission has been called upon to examine ways to improve the use of date marking by actors in the food chain and its understanding by consumers. 'Date marking' is used as an umbrella term to refer both to the 'best before' and 'use by' dates. It is a prerequisite that initiatives aiming to reduce food waste should never compromise food safety.

A Commission study published in February 2018[3] estimated that up to 10% of the 88 million tonnes of food waste generated annually in the EU is linked to date marking. With the support of the sub-group on date marking and food waste prevention[4] of the EU Platform on Food Losses and Food Waste,[5] an immediate priority is the development of EU guidance based on the existing EU requirements in order to ensure more consistent date marking and related food information practices. The study also concluded that the date marking is particularly relevant for food waste prevention for the categories dairy products, fruit juices, chilled meat and fish.

It is important that food business operators (FBO) follow a risk-based approach when deciding on the type of date marking (i.e. 'use by' date versus 'best before' date), setting of shelf-life and the related food information that should be provided on the labelling in order to ensure food safety. Such risk-based approach should be an integrated part of the Food Safety Management System (FSMS) that all FBO are obliged to develop and implement under the current EU food safety legislation, taking into consideration previous scientific opinions of the European Food Safety Authority (EFSA) and Commission guidance.

Especially, clarity is needed on the differentiation between foods that at the end of shelf-life might constitute 'an immediate danger to human health'/become 'injurious to health' due to growth of pathogenic microorganisms, and foods that at the end of shelf-life might become 'unfit for human consumption' due to growth of spoilage non-pathogenic.[6]

Therefore, in order to support FBO and national authorities in implementing correct and consistent practices, there is a need for the scientific advice of EFSA.

1.1.2. Terms of Reference as provided by European Commission

In accordance with Article 29 of Regulation (EC) No 178/2002, the European Commission asks EFSA for scientific opinions providing guidance on date marking and related food information in view of the application by FBO of Regulation (EU) No 1169/2011 on food information to consumers as an integrated part of their FSMS.

The opinions should develop a risk-based approach to be followed by FBO when deciding on the type of date marking (i.e. 'use by' date versus 'best before' date), setting of shelf-life and the related food information that should be provided on the labelling in order to ensure food safety.

In particular, EFSA is requested to provide scientific advice on:

ToR 1) The factors that, from a microbiological point of view, make certain foods highly perishable and therefore likely after a short period to constitute an immediate danger to human health, and on how those factors should be considered by FBO when deciding whether a 'use by' date is required and setting the shelf-life and the required storage conditions, particularly on:

a) The relevant microbiological hazards that should be taken into account by FBO in determining whether a food, from a microbiological point of view, is likely to constitute an immediate danger to human health;
b) The types of foods where it is more likely to find those pathogenic microorganisms;

[2] http://ec.europa.eu/environment/circular-economy/index_en.htm
[3] https://publications.europa.eu/en/publication-detail/-/publication/e7be006f-0d55-11e8-966a-01aa75ed71a1/language-en
[4] https://ec.europa.eu/food/safety/food_waste/eu_actions/date_marking_en
[5] https://ec.europa.eu/food/safety/food_waste/eu_actions/eu-platform_en
[6] Article 24(1) of Regulation (EU) No 1169/2011 and Article 14(2) to (5) of Regulation (EC) No 178/2002.

c) The intrinsic/extrinsic factors that might influence the growth of those pathogenic microorganisms and consequently have an impact on: 1) the decision whether a 'use by' is required, 2) the shelf-life (the period up until when a food is not likely to constitute an immediate danger to human health), either linked to the composition of a food (e.g. pH, a_w, presence of food additives) or to the production process and/or the way a food is marketed (e.g. production processes like pasteurisation, type of packaging), and 3) the storage conditions throughout the food chain and the intended use of the food;
d) How the factors identified above influence the decision whether a 'use by' date is required, the setting of shelf-life and the required storage conditions.

ToR 2) The factors that, from a microbiological point of view and limited to foods intended to be stored at controlled temperatures, make certain foods become unfit for human consumption, but still without constituting an immediate danger to human health, and on how those factors should be considered by FBO when deciding whether a 'best before' date is appropriate and setting the shelf-life and the required storage conditions, particularly on:

a) The intrinsic/extrinsic factors that might influence the growth of spoilage non-pathogenic microorganisms and consequently have an impact on: 1) the shelf-life (the period up until when a food is not likely to become unfit for human consumption); either linked to the composition of a food (e.g. pH, a_w, presence of food additives) or linked to the production process and/or the way a food is marketed (e.g. production processes like pasteurisation, type of packaging), and 2) the storage conditions throughout the food chain and the intended use of the food);
b) How the factors identified above influence the setting of shelf-life and the required storage conditions;
c) The indicative time limits to be applied at EU level to facilitate marketing or donation of foods past the 'best before' date, provided that before the end of that period those foods shall not become unfit for human consumption. Certain Member States (MS) have developed national guidance on this.[7]

EFSA is also requested to provide guidance to be considered by FBO when deciding on the food information to be provided to consumers regarding the shelf-life and the required storage conditions, particularly on:

ToR 3) Storage conditions and/or time limit for consumption after opening the package in order <u>to avoid increase of food safety risks</u>, particularly on:

a) The characteristics of a food and the intrinsic/extrinsic factors which might change once the package is opened, and specifically on which of those factors that should be taken into consideration when providing such information
b) The factors to be considered in deciding whether it is appropriate, and consequently mandatory, to indicate the storage conditions and/or time limit for consumption after opening the package according to Article 25(2) of Regulation (EU) No 1169/2011.

ToR 4) Defrosting of frozen foods including good practices, storage conditions and/or time limit for consumption in order <u>to avoid increase of food safety risks</u>, particularly on:

a) Advice to be given to consumers regarding good practices, storage conditions and/or time limit for consumption to protect consumers from possible health risks.

1.2. Interpretation of the Terms of Reference

The above ToRs were discussed with the requestor of the mandate (European Commission). Some aspects were clarified and interpreted as explained below. Guidance in relation to ToRs 1 and 2 were presented in the part 1 opinion (EFSA BIOHAZ Panel, 2020a), and guidance in relation to ToRs 3 and 4 are presented in this (part 2) opinion. The opinions developed risk-based guidance to be followed by FBOs when deciding on the type of date marking (i.e. 'use by' date vs 'best before' date), on the setting of shelf-life (i.e. time limit for consumption) and on related food information (e.g. storage conditions, time limit for consumption after opening, etc.) to be provided in order to ensure food

[7] Italy - Guide to good practice for charitable organisations, Caritas Italiana, Fondazione Banco Alimentare Onlus, March 2016 (p. 29); Belgium - Circular on the provisions applied to food banks and charity organisations (FR; NL), Belgian food safety agency (Agence fédérale pour la Sécurité de la Chaine Alimentaire), 2017.

safety. This also includes storage conditions and time limits for opened packages, and the same factors, plus good practices for thawing, for frozen foods.

The wording of the terms of reference is based on the legal texts of Regulation (EU) No 1169/2011 and Regulation (EC) No 178/2002. For the purpose of this opinion and in relation to ToR 3 and ToR 4, the wording *to avoid increase of food safety risk* and *to protect consumers from possible health risks* is interpreted as dealing with factors and conditions of relevant foods that may carry pathogenic and/or toxigenic microorganisms, and can support their growth during storage and before consumption and therefore may make the foods injurious to health.

The foods of interest for ToR 3 are non-frozen, both raw and processed, prepacked foods. After opening, the food may be stored in another container or used as an ingredient in a multi-component meal (i.e. during home-made meal preparation) and time limits after opening refer to before consumption or before any further processing of the food in a meal. Only frozen foods are covered in ToR 4.

ToR 3 is interpreted as relating to microbial growth during shelf-life, and the pathogenic microorganisms of interest are bacteria, yeasts, moulds, and their toxins, including biogenic amines/ histamines. Moulds, yeasts and mycotoxins were not considered main hazards in relation to the assessment of increased risks after opening food packages and were excluded from the assessment (see EFSA BIOHAZ Panel, 2020a,b). Pathogenic microorganisms that cannot grow in food, such as food-borne viruses and food-borne parasites are not of relevance for ToR 3. Contamination of food with pathogenic microorganisms after opening the package is considered to be always possible. Thus, hazards of relevance in ToR 3, hereafter referred to as pathogenic microorganisms, are bacteria present in foods after processing and packing when the food leaves the control of the FBO, or introduced after opening the package, and which can potentially grow and/or produce toxin during the shelf-life of the product, under reasonably foreseeable conditions of storage or thawing. In relation to ToR 3 and for the purpose of this opinion, Article 25(2) of Regulation (EU) No 1169/2011 *To enable appropriate storage or use of the food after opening the package, the storage conditions and/or time limit for consumption shall be indicated, where appropriate* and the associated decision on the appropriate and mandatory information are taken to refer only to food safety risks, i.e. negative health effects. Thus, this decision is interpreted as being related only to whether the risk after opening will increase with time or not, i.e. the microbiological pathogens present or potentially introduced after opening of the package can grow and/or produce toxin at an equivalent or higher rate during storage of opened packages. This decision will be dependent on the relevant pathogenic microorganisms and the characteristics of the food.

The shelf life of a food usually refers to how long it can be stored prior to its consumption or use and is, for the purpose of this opinion, interpreted to end when the concentration of a microorganism becomes higher than a predetermined quality or safety threshold level (EFSA BIOHAZ Panel, 2020a,b). As reported and explained in EFSA BIOHAZ Panel (2020a), in the absence of such defined levels, the term 'acceptable level' is used to describe any microorganism level relevant for decisions on date marking taken by the FBO for their product, considering the food characteristics and reasonably foreseeable use. The term is not prescriptive but synonymous with expressions such as 'level of relevance', 'level of concern', 'limit level', 'threshold level', 'microbial limit' or 'level for shelf-life'. For cases in which this threshold could be passed earlier once the container or package has been opened, two different shelf-lives are considered (Nicoli and Calligaris, 2018). A primary shelf-life before opening, represented and expressed by a date marking, and a secondary shelf-life after package opening, represented by a time limit (usually days).

ToR 4 is interpreted as providing guidance to FBOs producing frozen packaged food when deciding on what food information needs to be provided to the consumers, and not as direct guidance or advice from EFSA to consumers. The guidance relates to good practices for the thawing of frozen foods, storage conditions during and after thawing and/or time limits for consumption after thawing to protect consumers from food safety risks. The latter involves the potential survival, growth and toxin production of pathogenic microorganisms during and after thawing and is dependent on the specific pathogens, the characteristics of the food, the reasonably foreseeable conditions of storage, and the intended use (e.g. with or without cooking and cooking instructions). Thus, in ToR 4, aiming to give guidance on good practices for thawed foods to protect consumers from possible risk, food-borne viruses, are also considered to be relevant hazards. The scope is limited to thawing at the domestic level. Commercial settings will involve larger food volumes and different circumstances in relation to competence and control and these activities should be included in the FSMS (including the HACCP plan) of the FBO thawing the foods. Settings involving institutional catering (charitable/food banks/

healthcare facilities), restaurants, etc. are not addressed, although some of the advice may also be relevant to these settings.

2. Data and methodologies

2.1. Literature review

Relevant documents were identified and reviewed based on the knowledge and expertise of the members of the Working Group (WG) and BIOHAZ Panel drafting this scientific opinion. These documents included scientific papers, book chapters, non-peer-review papers (grey literature such as trade journals, news updates and websites), regulations, guidance documents from national and international authorities, scientific opinions and reports known to the experts themselves or retrieved through searches. The reference list of these documents was further screened in order to identify additional relevant publications until reaching a coverage of the subject considered sufficient by the WG.

2.2. Approach to answering ToRs

Guidance on ToR 3a was developed by reviewing the scientific literature and existing guidance.

The approach to develop guidance on ToR 3b, was to develop a decision tree (DT) that can be used by a FBO for a specific food product. The DT is based on the information summarised in Part 1 (EFSA BIOHAZ Panel, 2020a,b) and in this (Part 2) opinion. The DT was developed and evaluated using representative examples (Section 3.2.2).

To address ToR 4, guidelines, scientific literature, and information from other sources was summarised, critically appraised and adapted to provide updated guidance.

2.3. Uncertainty analysis

In applying the EFSA guidance (EFSA Scientific Committee, 2018), special attention was given to discussing whether assessment questions could be defined in relation to the ToRs, to identifying relevant sources of uncertainty, and to evaluating their impact on the assessment question.

The main bulk of the opinion is a review and a summary of the relevant literature found in the identified information sources. The most important assessment question was whether it would be appropriate to include additional information, indicating storage conditions and time limits after opening, for a food product. The answer to this question is based on the outcome of the DT developed in the opinion. The DT is based on data, assumptions and methods. All of these factors may be sources of uncertainty and may contribute uncertainty to the decision on the need for information relating to storage conditions and/or time limits.

To assess the uncertainties about the decision on the need for food information, sources of uncertainties were listed related to the DT itself (within which the relevant questions and structures were included) and assessed based on expert knowledge (Appendix A). The structure of the DT was evaluated, considering whether any relevant questions were missing or whether any questions were included without being relevant, by testing different food examples. The impact (direction and magnitude) of the sources of uncertainty on the decision was also assessed. The impact's direction was expressed as either an underestimation of risk, an overestimation of risk or as inconclusive. Since there are only two alternative outcomes of the decision tree, underestimation relates to a scenario where a food requiring a secondary shelf-life would be classified by the decision tree as not needing this; overestimation relates to a decision on a food not requiring a secondary shelf-life that would erroneously be classified as needing one, and inconclusive when the error could be in either direction. The impact of the uncertainty on the decision (magnitude) was assessed using an ordinal three-level scale from lower to higher importance.

3. Assessment

3.1. Intrinsic, extrinsic and implicit factors which might change once the package is opened and affect microbiological food safety (ToR 3a)

The importance of the FSMS, the influence of food characteristics and storage conditions (i.e. intrinsic, extrinsic and implicit factors), the effect of food processing on the presence and levels of pathogenic microorganisms and their ability to grow and/or produce toxins during the shelf-life period was described in the Part 1 Opinion (EFSA BIOHAZ Panel, 2020a,b). As described, the health risk associated with a food is affected by the food characteristics and the use of the food, including storage and handling (e.g. preparation, cooking, etc.) by the consumer. The impact of these factors on the microbiological safety of the food product is considered by the FBO when deciding on the type of date marking, the shelf-life (date) and on the information provided about storage conditions and intended use of the food. However, since opening the food package may change the conditions affecting the occurrence and ability of pathogenic microorganisms to grow and/or produce toxins, it may be appropriate to provide additional information on storage conditions and time limits for consumption after opening the package. In these cases, the time limit (days) for consumption after opening the package (secondary shelf-life), will relate to an earlier or, in some cases, the same date, but never a later date than the original shelf-life date (primary shelf-life of the unopened product).

The key issue when considering the storage conditions and time limits for opened packages is whether opening of the package will:

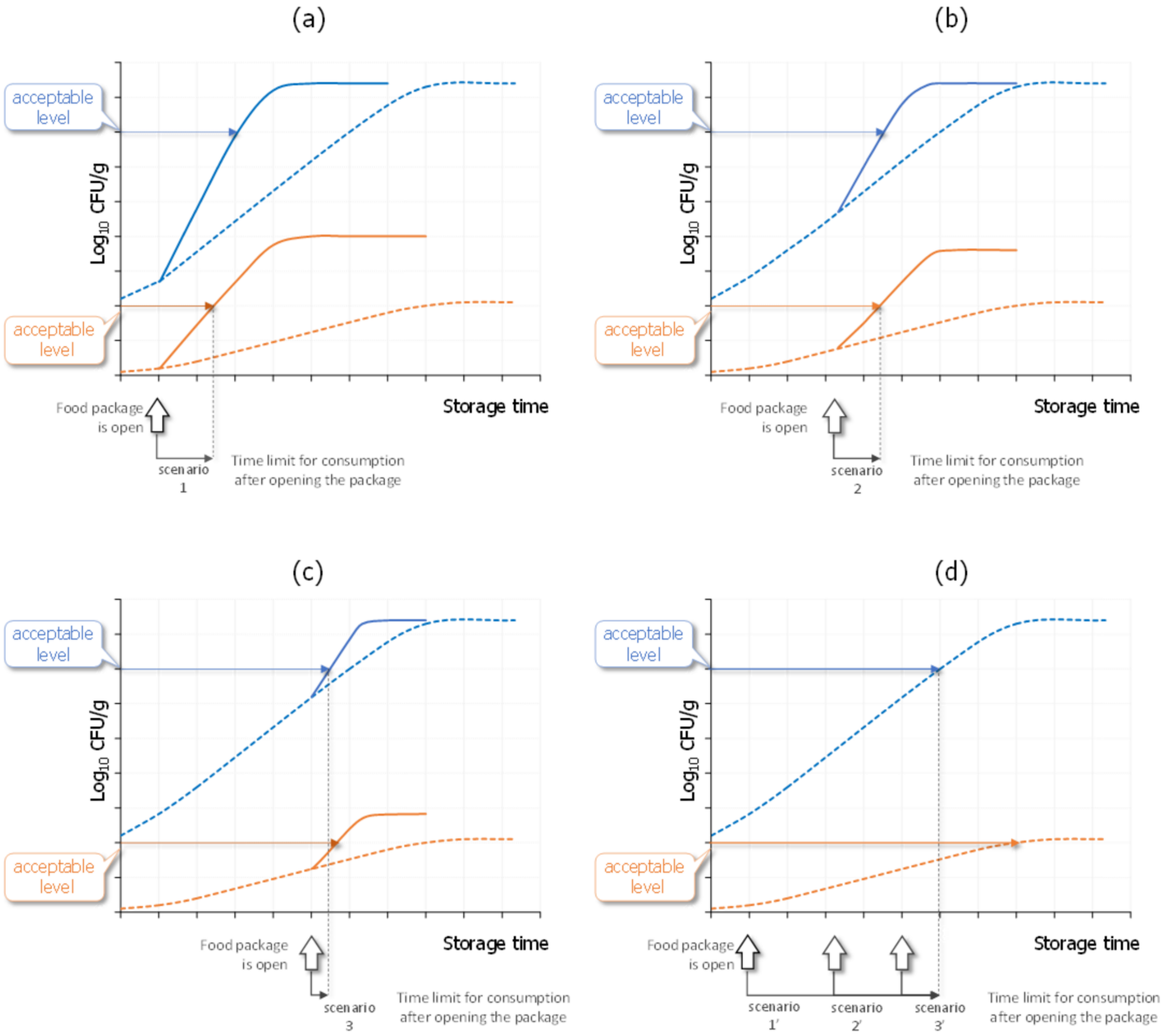
- increase the occurrence and/or initial concentration of pathogenic microorganisms due to, e.g. contamination by the consumer (Section 3.1.1), and/or
- change any of the extrinsic, intrinsic and implicit factors determining potential growth and toxin production of pathogenic microorganisms either present in the food before opening the package or newly introduced due to (re)contamination events after opening the package (EFSA BIOHAZ Panel, 2012) (Section 3.1.2).

3.1.1. Factors influencing the types and initial concentration of pathogenic microorganisms

Factors that may influence the type and initial concentration of pathogenic microorganisms in a food, after the package is opened by consumers, include the time point of opening the package within the product shelf-life (primary shelf-life) and the possible contamination of the food after the package is opened.

3.1.1.1. Time-point within the product shelf-life when the package is opened

Microorganisms present in perishable prepacked foods can grow (increase), survive (remain at constant levels) or die (decrease) during storage depending on the intrinsic, extrinsic and/or implicit factors, which will impact their concentration during the attributed shelf-life (EFSA BIOHAZ Panel, 2020a, b). Therefore, the time point when the food package is opened within the product shelf-life may influence the concentration of microorganisms (either pathogenic or spoilage) present at the time of opening. When the package of a food supporting growth is opened close to the end of the shelf-life date, higher concentrations of bacteria can be expected than when the package is opened at earlier stages of the shelf-life. This situation results in a shortening of the secondary shelf-life in comparison with a food that is opened closer to its production date. Figure 1 illustrates this concept through different scenarios in which the secondary shelf-life (time limit after opening the package) is influenced by the time of opening the package of the food product. In three different scenarios (Figure 1a, b and c), the change of the extrinsic factor (i.e. modified atmosphere packaging), results in an increase of the growth rate of the microorganisms, both of specific spoilage organisms (SSO) and pathogenic microorganisms. In the fourth plot (d), three additional scenarios of opening the package (1′, 2′ and 3′) are presented, in which opening the package does not change extrinsic factors nor the growth rate. In this case, the secondary shelf-life is the same as the primary shelf-life of the unopened package (EFSA BIOHAZ Panel, 2020a,b).



A change of the extrinsic factor (i.e. modified atmosphere packaging) results in an increase of the growth rate (slope of the line), of both specific spoilage organisms (SSO) and pathogenic microorganisms. The three scenarios (a, b and c) with different time-points of opening the package are represented by continuous lines, while dashed lines represent the microbial growth in the packaged food (unopened package). The remaining time before acceptable levels of SSOs or pathogens are exceeded, becomes shorter the later in the primary shelf-life period the package is opened. The fourth plot (d), shows a scenario in which opening the package does not change extrinsic factors nor the microbial growth rate. In this case, the shelf-life is the same as the shelf-life of the unopened package (EFSA BIOHAZ Panel, 2020a). The potential impact between pathogens and SSO are not illustrated in the figure.

Figure 1: Conceptual figure illustrating three scenarios (a, b, c) in which the secondary shelf-life of a food product (time limit after opening the package) is influenced by the time-point of opening the package during the primary shelf-life

Studies on the impact of the time point within the primary product shelf-life on the concentration of *Listeria monocytogenes* present at the time of opening the (vacuum) package and the subsequent behaviour in a variety of ready-to-eat (RTE) cooked meat products were conducted by Lianou et al. (2007a,b) and Byelashov et al. (2008). The results of the challenge tests showed that the concentration of *L. monocytogenes* may increase considerably with the length of the refrigerated vacuum-packed storage time prior to opening. The magnitude of this increase varied depending on the type of product and the presence of antimicrobials (such as organic acids) in their formulation. Table 1 shows the relative change (in terms of $\log_{10}$ increase) of the concentration of *L. monocytogenes* inoculated in prepacked cooked meat products at different times of opening the vacuum package.

Table 1: Impact of the time point within the primary product shelf-life when the package is opened on growth potential of *Listeria monocytogenes* in RTE cooked meat products

Time-point within primary product shelf-life (days at 4°C)	Cooked cured ham (Lianou et al., 2007a)		Cooked uncured turkey breast (Lianou et al., 2007b)		Frankfurters (Byelashov et al., 2008)	
	Without organic acids	With lactate and diacetate[(a)]	Without organic acids	With lactate (1.5%) and diacetate (0.05%)	Without organic acids	With lactate (1.5%) and diacetate (0.1%)
5	–[(b)]	–	1.3	0.1	–	–
10	1.9	0.2	–	–	–	–
15	–	–	4.2	0.9	–	–
20	3.5	0.6	–	–	1.6	0.0
25	–	–	5.7	1.8	–	–
35	5.6	1.0	–	–	–	–
40	–	–	–	–	3.8	0.0
50	–	–	5.6	2.7	–	–
60	5.6	2.9	–	–	4.8	0.0

RTE: ready-to-eat.

Note: Results are expressed as $\log_{10}$ increase of the concentration of *L. monocytogenes* between different time points within the primary product shelf life (product age of vacuum-packed product in days at 4°C) and the initial concentration of packed product, immediately after processing.

(a): Concentration of organic acids was not reported.

(b): Not determined.

The time point, within the product shelf-life, when the package is opened also determines the concentration of background spoilage microorganisms that can interact with newly introduced pathogens during the subsequent storage of the opened package. For instance, Lianou et al. (2007b) observed an increase of the concentration of spoilage microorganisms with the length of storage time before opening the vacuum-packs of cooked uncured turkey breast without organic acids (e.g. 1.7, 2.7, 3.3 and 4.5 $\log_{10}$ CFU/cm^2 after 5, 15, 35 and 50 days of storage at 4°C, respectively). The increase in the concentration of spoilage microorganisms was associated with a decrease in the growth rate of the pathogenic bacteria during subsequent growth in the opened package (aerobic) at 7°C (e.g. 0.51, 0.47, 0.32 and 0.25 $\log_{10}$/day, respectively).

In cooked uncured turkey breast formulated with lactate and diacetate, the concentrations of spoilage bacteria during vacuum-packed storage were much lower (e.g. 1.7, 1.6, 2.0 and 2.9 $\log_{10}$ CFU/cm^2 after 5, 15, 35 and 50 days at 4°C, respectively) and did not influence the growth rate of the pathogen during the subsequent growth in the opened package (aerobic) at 7°C (e.g. 0.15 $\log_{10}$/day on average, irrespective of the time when the package was open). These findings are in agreement with studies performed on cook-in-bag delicatessen meats (formulated with organic acids) (Geornaras et al., 2013), showing that spoilage microorganisms remained below 2 $\log_{10}$ CFU/cm^2 for 180 days at 1.7°C, and that the age of the product prior to opening, slicing, and contaminating with *L. monocytogenes* and re-packing did not affect the behaviour of the pathogen during subsequent storage at 4°C for 13 weeks.

In another example dealing with different types of cheese, a significant inhibition of the growth of inoculated *L. monocytogenes* was observed in samples being close to the end of the shelf-life date, i.e. with high concentrations of background microorganisms (total viable counts), compared with the growth of the pathogen inoculated in samples close to the production date (Kapetanakou et al., 2017).

From these examples, it is clear that the time-point of opening the food package may influence not only the initial concentration of pathogens at the time of opening but also the concentration of spoilage microbiota and the growth potential of pathogens (initially present or introduced after opening) (Figure 1). All these factors may impact on the secondary shelf-life and therefore it is challenging to define a single secondary shelf-life unless simplifying matters and basing the secondary shelf-life on a worst-case scenario. A conditional secondary shelf-life taking into consideration the above factors (e.g. different time-limits depending on the time of opening) may be a more appropriate but more complex alternative.

3.1.1.2. Contamination of the food after the package is opened

When a prepacked food has been opened, the food may be exposed to contamination by pathogenic and spoilage microorganisms. The exposure to contamination after opening the package may introduce new pathogenic microorganisms or increase the concentration of pathogenic microorganisms already present. The routes of contamination may be airflow (in the refrigerator or the surrounding rooms), fluid drip, contaminated kitchen surfaces, kitchen utensils and, in particular, hands when taking pieces of food from the package. In general, handling conditions at the consumer stage do not reach hygienic standards and hygienic awareness usually applied in the food industry and establishments with implemented prerequisite programs (good hygiene practice, GHP) (Haysom and Sharp, 2005; Kennedy et al., 2011; Mihalache et al., 2021). As an example, *L. monocytogenes* can be present in consumers' refrigerators, dishcloths, washing-up brushes and kitchen surfaces and may contaminate foods stored in open packs (Beumer et al., 1996; Dumitrașcu et al., 2020). Consumer hands and utensils may be contaminated by enteric pathogens such as *Salmonella* spp., *E. coli* (Scott, 2000), or consumers may be carriers of *Staphylococcus aureus* (Acco et al., 2003; Uyttendaele et al., 2018). When these pathogens are transferred to the food, growth and/or toxin production may occur depending on the intrinsic, extrinsic and implicit factors (see Section 3.1.2).

Heat-treated shelf-stable foods (with a 'best before' date, e.g. canned vegetables, jam/marmalade, acidified sauces), in which a sterilising treatment have eliminated all spores and vegetative bacteria able to grow in the food, can be stored at ambient temperature while in an unopened package. However, after opening, the food needs to be kept in the refrigerator for the remaining shelf-life if the intrinsic and extrinsic factors of the food support the growth of pathogenic or spoilage microorganisms potentially introduced after opening the package (see Section 3.1.2).

Another example is the growth and toxin production of coagulase positive *S. aureus*, when contamination by food handlers occurs in heat-treated foods such as RTE meals, where the competitive microbiota has been eliminated during processing of the food and the storage temperature is appropriate for the pathogen to grow and produce toxins (e.g. temperature $> 12°C$) (EFSA BIOHAZ Panel, 2012).

Quantitative information on the relative importance of different contamination routes is scarce, but some routes have been investigated using experimental (Kusumaningrum et al., 2003) and/or quantitative microbial risk assessment (QMRA) approaches (Yang et al., 2006). A rate of transfer of pathogens will occur via the various contamination routes to the food (in the opened package). The transfer rate is expressed as the fraction of transfer from the contamination source to the food based on experiments to mimic situations in (home) kitchens. For example, *Salmonella* Enteritidis, *Staphylococcus aureus* and *Campylobacter jejuni* were readily transmitted from wet sponges to stainless-steel surfaces and from these surfaces to cucumber and chicken fillet slices, and transfer rates varied from 20% to 100% contamination of the product (Kusumaningrum et al., 2003). Other examples mimicking situations in the home, include studies quantifying bacterial contamination rates between different pathogens and fresh-cut produce and hands (Jensen et al., 2017), transfer-rates between chicken, cutting boards, hands and knives in the kitchen (Van Asselt et al., 2008), and lettuce and knives (Zilelidou et al., 2015).

3.1.2. Factors influencing the growth behaviour of microorganisms

3.1.2.1. Change of extrinsic factors

In modified atmosphere packaging (MAP) or vacuum-packed food, opening of the packages has a major impact on the gas atmosphere, resulting in a loss of the intended protective effect of the gas composition inside the intact package (e.g. the low O_2 and/or high CO_2 concentration is lost). This situation can result in more favourable conditions for the growth of pathogenic microorganisms already present or introduced due to contamination (see Section 3.1.1.2). For instance, Tsigarida et al. (2000) found no or limited growth of *L. monocytogenes* in meat samples stored in vacuum-packed or MAP (40%CO_2/30% O_2/30%N_2) within oxygen-impermeable film, irrespective of the presence of background microbiota. However, in another study, *L. monocytogenes* grew in meat packed under aerobic conditions (growth rate 0.31 log_{10}/day) but also under vacuum (0.28 log_{10}/day) or MAP (0.13 log_{10}/day) with high permeability material. Therefore, a shift from no growth to a growth rate equivalent to that of aerobic conditions can be expected after opening the package. Other studies, illustrating differences in growth in MAP or vacuum, as compared to air, have been reported for bacteria in frankfurters (Byelashov et al., 2008), RTE meals (Daelman et al., 2013c), and raw salmon (Kuuliala et al., 2019).

The behaviour of *L. monocytogenes* in cold-smoked salmon under different scenarios of storage and opening of packages, were assessed using the Food Spoilage and Safety Predictor[8] (FSSP) model (VKM, 2018). According to the results, the increase of the growth rate of *L. monocytogenes* due to the loss of CO_2 when opening the package at consumer level, would cause a reduction of about 40% in the time needed for the pathogen to reach the maximum acceptable level (i.e. expressed as a 2-log increase, about 6 days in the opened package and 10 days in the unopened package). Predictions were based on intrinsic factors of the smoked salmon considered most likely.

Where active packaging is used, in which antimicrobials are located on or within the food contact material, the greatest impact on pathogen growth can be expected when the food is removed to another container and the active compounds are no longer in contact with the food itself (Yildirim et al., 2018).

Compared to unopened prepacked food stored in the refrigerator, an open packed food, if not consumed at once, can be exposed to high (ambient) temperature for variable or recurrent periods of time when the consumer removes the package from the fridge for handling and preparation before consumption. The impact of abusive temperatures associated with domestic refrigerators of unopened and open (leftover) packages and countertop storage of open packages was stochastically assessed in the QMRA for *L. monocytogenes* in deli meats developed by Yang et al. (2006). The modelling results indicated that inadequate storage associated with refrigeration temperatures showed the greatest contribution to the 10^6-fold increased risk due to food handling within homes.

3.1.2.2. Change of intrinsic factors

A prepacked food has certain intrinsic properties, which determine the growth potential of pathogenic microorganisms during the shelf-life period (EFSA BIOHAZ Panel, 2020a,b). However, when a package is opened, a shift may occur in these properties, although this may not be so evident as for the extrinsic factors (atmosphere and temperature) described in Section 3.1.2.1. The change of intrinsic factors could cause increased or reduced growth, or even lead to a reduction in numbers of some of the microorganisms present.

For example, depending on the equilibrium between the relative humidity of the air in contact with the food (e.g. in the refrigerator) and the a_w of the product, the food may dehydrate and the surface a_w reduce, which may cause a reduction of the growth rate of microorganisms. Alternatively, food may absorb water leading to an increased a_w, thus favouring microbial growth (Devlieghere et al., 2016).

CO_2 gas in MAP packs partially dissolves in the food and may result in a reduction in pH due to the formation of carbonic acid (H_2CO_3) (Devlieghere et al., 1998). However, after opening, the CO_2 decreases rapidly, reversing the reaction, and the dissolved CO_2 is released again, resulting in an increase in the pH of the product.

Furthermore, the pH and/or presence of antimicrobial substances can also be affected due to microbiological proliferation and related metabolism of microorganisms. These effects are further discussed under implicit factors.

3.1.2.3. Change of implicit factors

Any change in intrinsic and extrinsic factors, after opening the package, will have an impact on the interactions between microorganisms and may result in a shift in competitiveness between different microorganisms. In general, changes in the interactions between pathogens and spoilage organisms present in the food, once a food package is opened, is an important implicit factor in the determination of the secondary shelf-life. One group of pathogens or spoilage microorganisms can be of less importance in defining the primary shelf life, but due to the changes that occur when the package is opened, may become more important in the secondary shelf-life. The impact of the change of implicit factors occurring when opening the package is generally less obvious than shifts in the microbial behaviour due to the changes in extrinsic or intrinsic factors.

Changes in extrinsic factors (e.g. atmosphere) can modify the growth rate of spoilage microorganisms and the pathogens to different extents. Under such circumstances, the time at which pathogen growth is inhibited, i.e. when the dominating spoilage microorganism reaches its maximum density, i.e. the Jameson effect (Jameson, 1962), will be different. This phenomenon can be illustrated by combining predictive models for *L. monocytogenes* and lactic acid bacteria (LAB) growth to identify

[8] FSSP v4.0, http://fssp.food.dtu.dk/

the 'risk areas' or 'risk scenarios' where the pathogen reaches the maximum acceptable level (i.e. 100 CFU/g) before the LAB reach their maximum population density (and stops its growth and that of the pathogen) or their spoilage level (and causes the rejection of the product) (Devlieghere et al., 2001; Jofré et al., 2019). This approach was used in this opinion for illustrative purposes to assess the impact of opening the package of a cooked meat product at different temperatures through the simulations provided by the predictive model of Mejlholm and Dalgaard (2013) available within the FSSP tool (Table 2).

In this tool, it is predicted that after opening the package, the loss of CO_2 causes an increase of the microbial growth rate, which is higher for *L. monocytogenes* compared to LAB. Consequently, after opening the package the pathogen will be able to reach the limit (m = M = 100 CFU/g) in the microbiological criterion (Commission Regulation (EC) No 2073/2005) more quickly than when its growth is inhibited due to the interaction with LAB. Compared with the unopened package, this would occur under a slightly wider range of scenarios of temperature and concentration of LAB at the time of opening the package.

Table 2: Results of the simulation of the change of the growth interaction between *L. monocytogenes* and lactic acid bacteria (LAB) when opening the Modified Atmosphere Packaging (MAP) of a cooked meat product[(a)]. The ratio between the time to reach the acceptable level by *L. monocytogenes* and the time LAB reaches their maximum population density is shown in parenthesis. See the footnote for the colour code

Temperature	*L. monocytogenes*			Lactic acid bacteria (LAB)					
	Growth rate (log_{10}/day)	Time (days) to reach the acceptable level (10^2 CFU/g) from		Growth rate (log_{10}/day)	Time (days) to reach the maximum population density of LAB from different concentrations (ratio between the time to reach the acceptable level by *L. monocytogenes* and the time LAB reaches their maximum population density)				
		1 CFU/g	10 CFU/g		10 CFU/g	10^2 CFU/g	10^3CFU/g	10^4CFU/g	10^5CFU/g
MAP (unopened package)									
4°C	0.06	32.0[(b)]		0.32	23.2 (1.4)[(c)]	20.1 (1.6)	17.0 (1.9)	13.9 (2.3)	10.8 (3.0)
			16.0		23.2 (0.7)[(d)]	20.1 (0.8)	17.0 (0.9)[(e)]	13.9 (1.1)	10.8 (1.5)
6°C	0.15	13.0		0.48	15.6 (0.8)	13.5 (1.0)	11.4 (1.1)	9.3 (1.4)	7.3 (1.8)
			6.5		15.6 (0.4)	13.5 (0.5)	11.4 (0.6)	9.3 (0.7)	7.3 (0.9)
8°C	0.27	7.4		0.67	11.2 (0.7)	9.7 (0.8)	8.2 (0.9)	6.7 (1.1)	5.2 (1.4)
			3.7		11.2 (0.3)	9.7 (0.4)	8.2 (0.5)	6.7(0.6)	5.2 (0.7)
10°C	0.38	5.2		0.90	8.4 (0.6)	7.3 (0.7)	6.1 (0.8)	5.0 (1.0)	3.9 (1.3)
			2.6		8.4 (0.3)	7.3 (0.4)	6.1 (0.4)	5.0 (0.5)	3.9 (0.7)
Opened package (loss of CO_2)									
4°C	0.09	22.1		0.36	20.7 (1.1)	18.0 (1.2)	15.2 (1.5)	12.4 (1.8)	9.7 (2.3)
			11.0		20.7 (0.5)	18.0 (0.6)	15.2 (0.7)	12.4 (0.9)	9.7 (1.1)
6°C	0.21	9.7		0.53	14 (0.7)	12.2 (0.8)	10.3 (0.9)	8.4 (1.2)	6.5 (1.5)
			4.8		14 (0.3)	12.2 (0.4)	10.3 (0.5)	8.4 (0.6)	6.5 (0.7)
8°C	0.34	5.9		0.74	10.1 (0.6)	8.8 (0.7)	7.4 (0.8)	6.1 (1.0)	4.7 (1.3)
			3.0		10.1 (0.3)	8.8 (0.3)	7.4 (0.4)	6.1 (0.5)	4.7 (0.6)
10°C	0.47	4.2		0.98	7.6 (0.6)	6.6 (0.6)	5.6 (0.8)	4.6 (0.9)	3.6 (1.2)
			2.1		7.6 (0.3)	6.6 (0.3)	5.6 (0.4)	4.6 (0.5)	3.6 (0.6)

(a): Predictions were performed with the predictive model available on the FSSP v4.0 tool using the input values representing RTE cooked meat products as described in Jofré et al. (2019). Maximum population density of LAB was assumed to be at 2.3×10^8 CFU/g.
(b): Predictions without taking into account the interaction due to the Jameson effect. If LAB reach their maximum population density at an earlier time, *L. monocytogenes* will stop growing and remain below the acceptance level of 100 CFU/g.
(c): Safe scenario (green background): *L. monocytogenes* does not reach the maximum acceptable level as it stops its growth because LAB reach their maximum population density (ratio > 1).
(d): Scenario at risk (red background): *L. monocytogenes* reach the maximum acceptable level before LAB reach their maximum population density (ratio < 1).
(e): Scenario at (intermediate) risk (orange background, ratio close to 1): LAB reach the maximum acceptable level (associated with spoilage) before *L. monocytogenes* reach the maximum acceptable level, however, the pathogen continues growing and reaches the maximum acceptable level before LAB reach their maximum population density.

Changes in the interactions between microorganisms can also occur due to growth of certain groups of microorganisms and their metabolic activity. For instance, metabolic activity by *Pseudomonas* spp. on chicken meat, can be detected following loss of the anaerobic modified atmosphere after opening the package, resulting in autolytic activity (proteolytic denaturation of the meat proteins) and leading to an increase in the pH.

3.1.3. Concluding remarks

- The time point of opening the package within the primary shelf-life can influence the type and concentrations of the microorganisms present in the food (i.e. the closer to the end of shelf-life, the higher the expected concentration of most microorganisms).
- After opening the food package, contamination may occur via air flow, fluid drip or due to consumer handling via hands, utensils, containers, etc., which may introduce new pathogens into the food or increase the concentration of pathogens already present. Quantitative information on the relative importance of the different contamination routes is scarce, a wide range of transfer rates of pathogens to food mimicking situations in the home are reported.
- Opening of the food package may change the food related conditions affecting the ability of pathogenic microorganisms to grow and/or produce toxins (i.e. extrinsic, intrinsic, or implicit factors). Extrinsic factors (such as the atmosphere composition) are probably the most important factors that may change after opening the package. The protection of vacuum or MAP is lost and a change in the growth behaviour (usually increasing the growth capability/rate) of the pathogens in the food can be expected. The effect of changes in the intrinsic (such as a_w or pH) and implicit (such as competing microbiota) factors on pathogen growth after opening the packages should also be considered.
- Setting a time limit for consumption after opening the package (secondary shelf-life) is complex in view of the many influencing factors and information gaps. An additional level of complexity comes from the need to consider consumer behaviour and reasonably foreseeable conditions of use, as described by EFSA BIOHAZ Panel (2020a).

3.2. Guidance on the decision to provide additional information for opened packages (ToR 3 b)

The purpose of this section is to provide guidance to FBOs in determining storage conditions and the time limit after opening the food package.

3.2.1. Development of a decision tree for the time limit for consumption after opening the package

The DT will assist FBOs in deciding whether it is appropriate to indicate the storage conditions and time limit for consumption after opening the package. The underlying assumptions for the DT are that:

- After opening the package, contamination of the product with pathogenic microorganisms is always possible
- The time limit for consumption after opening the package in relation to the initial 'use by' or 'best before' date depends on whether opening the package changes:
  - the type of pathogenic microorganisms in the food (e.g. contamination with vegetative cells not present in the unopened food package with, in general, a wider range of growth capabilities compared to growth and/or toxin production from spores), or
  - the factors affecting growth of pathogenic microorganisms compared to the unopened product.

The DT consists of a sequence of five questions which lead to the decision on whether the secondary shelf-life (i.e. time limit for consumption after opening the package) should be the same (no need for additional information) or shorter (appropriate to indicate storage conditions and/or time limits for consumption) compared to the primary shelf-life ('best before' or 'use by' date of the unopened food (Figure 2)).[9] It should be noted that primary shelf-life is indicated on the label of the unopened foods as a date, while secondary shelf-life is indicated in days (after opening). In the developed DT, the term 'shorter time limit for consumption after opening the package than the initial 'use by' or 'best before' date' means that the secondary shelf-life in days should be shorter than (or in some cases it will turn out the same as) the number of days between the time of opening the package and the 'use by' or 'best before' date. For `use by' date, the term 'shorter' refers only to a shelf-life that is defined by safety and not by quality.

[9] Decision tree is available in French, German, Italian and Spanish under 'Supporting Information'. Note that the decision tree in English is the official version.

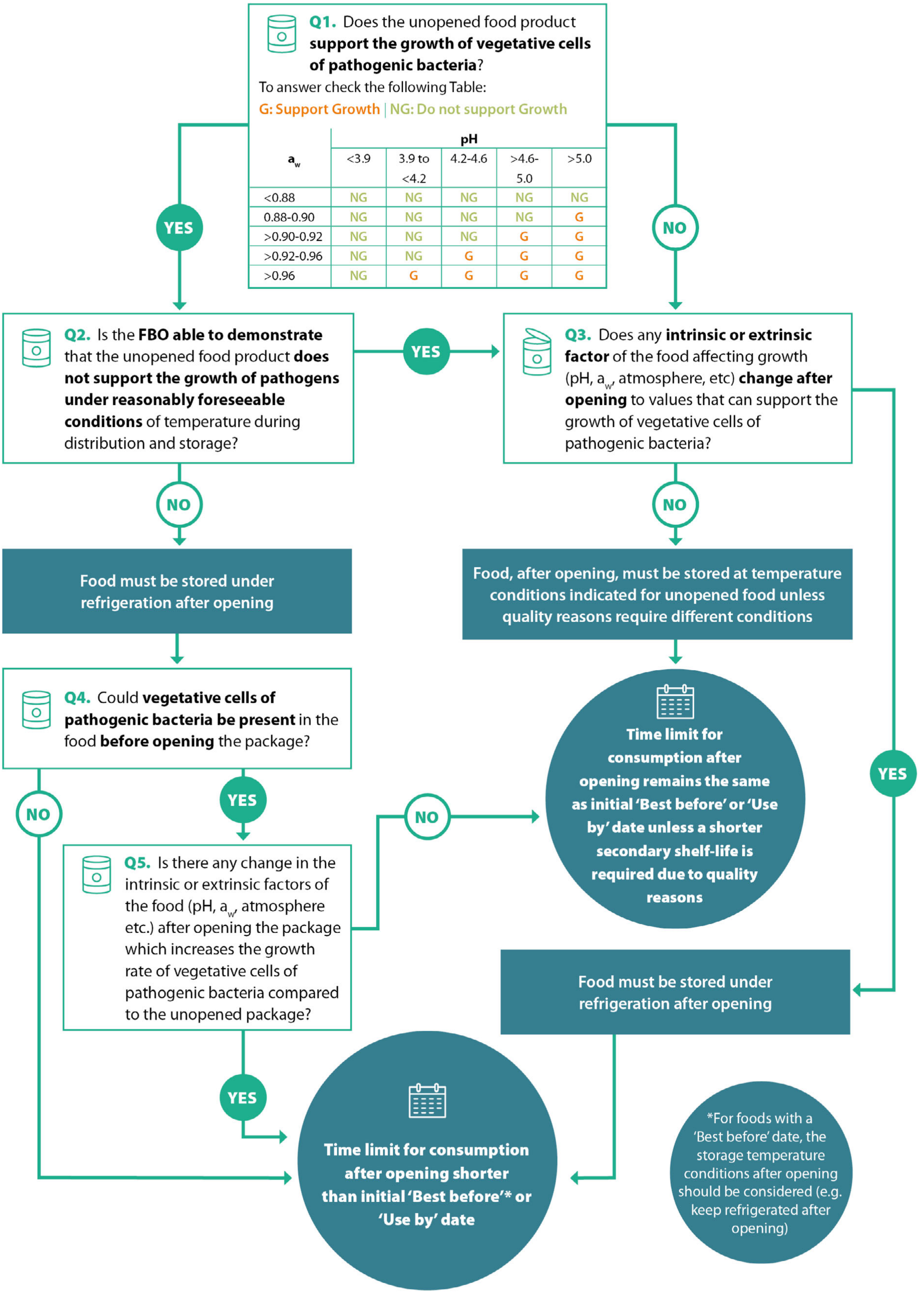


Figure 2: Decision tree for deciding whether additional information about storage conditions and time limit for consumption after opening the package is appropriate

Question 1 (Q1) refers to the ability of the food product, before opening the package, to support the growth of vegetative cells of pathogenic microorganisms, which is evaluated based on the measured pH and a_w through a table provided in the DT. It should be noted that the table refers to optimum growth temperature and optimum conditions for all other factors affecting microbial growth (e.g. absence of preservatives and no MAP or vacuum-packing). Therefore, higher pH and/or a_w in combination with additional hurdles could also inhibit the growth of pathogenic vegetative bacteria, but scientific evidence supporting growth inhibition needs to be provided (Q2). For mixed foods where intrinsic factors such as pH and a_w may change when the ingredients are mixed and/or during subsequent storage, the answers to Q1 and Q2 should be based on the values of intrinsic factors at equilibrium. When convergence to equilibrium is slow, the answers to Q1 and Q2 should be based on the ingredients with the more favourable intrinsic factors for microbial growth.

Food products with a combination of pH and a_w before opening that allow the growth of pathogen's vegetative cells (Q1: Yes) must be stored under refrigeration unless the FBO is able to demonstrate that the product does not support growth of pathogens under reasonably foreseeable conditions of temperature during distribution and storage, due to, for instance, additional hurdles (such as preservatives, packing atmosphere) (Q2: Yes). Demonstration of the latter may require specific studies, e.g. challenge tests focusing on the relevant pathogenic microorganisms based on the food, its characteristics and the storage conditions (see EFSA BIOHAZ Panel, 2020a, Section 3.4.2).

In the case of food products which, based on the characteristics (pH and a_w) before opening the package, do not support growth of vegetative cells of pathogenic microorganisms (Q1: No), the storage time after opening the package is not expected to affect consumer risk as long as the package opening does not result in a change in the intrinsic or extrinsic factors (pH, a_w, atmosphere, etc.) of the food product to values that can support growth of vegetative cells of the pathogenic microorganisms (Q3: No). In this case, the secondary shelf-life after opening is the same as the initial 'best before' date unless a shorter secondary shelf-life is required due to quality reasons. In the case of a food product for which opening the package results in a change in the intrinsic or extrinsic factors (pH, a_w, atmosphere, etc.) to values that can support growth of vegetative cells of the pathogenic microorganisms (Q3: Yes), the product must be stored under refrigeration and the time limit for consumption after opening must be shorter than the initial 'use by' or 'best before' date when the former has been defined based on product's safety.

For food products that support growth of vegetative cells of pathogenic microorganisms before opening, based on the pH and a_w (Q1: Yes) and the FBO is not able to demonstrate that the product does not support growth under reasonably foreseeable conditions of temperature during distribution and storage due to additional hurdles (Q2: No), Q4 refers to the presence of vegetative cells of pathogenic microorganisms in the product before opening the package and Q5 to whether opening the package results in any change in the intrinsic or extrinsic factors (pH, a_w, atmosphere, etc.) of the food product which increases the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package. For example, when vegetative cells of pathogenic bacteria can be present before opening (Q4: Yes), and the food product is packed under aerobic conditions, then opening of the package is not expected to change the type of pathogenic microorganism since vegetative cells with greater growth potential than spores may already be present in the food or the factors affecting their growth (Q5: No). Thus, the time limit for consumption after opening can be the same as the initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons. In the case of a food product in which vegetative cells of pathogenic microorganisms are absent (due to manufacturing/processing steps) (Q4: No), or vegetative cells are present and there is a change in the intrinsic or extrinsic factors of the food after opening the package which increases the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (Q5: Yes), then the time limit for consumption after opening must be shorter than the initial 'use by' or 'best before' date when the former has been defined based on product's safety. This is because in the latter case opening the package is expected to change the type of pathogenic microorganism (e.g. from spores to vegetative cells) present in the food and/or the factors (atmosphere) affecting their growth.

3.2.2. Application examples of the decision tree on the time limit for consumption after opening the package

Table 3 presents some examples of applying the DT to determine the time limit of consumption after opening the package to specific food products. As mentioned previously, the answers to the questions of the DT depend on the processing/packing conditions and the intrinsic and extrinsic factors of the specific food product. This means that the outcome of the DT can be different even for products with the same generic name. Thus, certain assumptions regarding processing/packing conditions, e.g. aseptic conditions or flushing with inert gas, and intrinsic factors were made. In the following paragraphs the application examples are discussed, illustrating how small differences in the formulation/processing/packing conditions and intrinsic/extrinsic factors can affect the outcome of the DT.

Milk and dairy products

UHT milk having a pH > 6.5 and a_w > 0.99 supports the growth of vegetative cells of pathogenic microorganisms while unopened (Q1: Yes) and normally the FBO cannot provide evidence on the opposite considering reasonably foreseeable conditions of temperature during distribution and storage (Q2: No). UHT treatment (> 135°C for 2–5 s) is expected to eliminate spores of food-borne bacterial hazards. The dairy industry often uses aseptic filling units for packing the product and thus, there is no potential for recontamination after the heat treatment and before packing. Based on the above, vegetative cells of pathogenic microorganisms cannot be present in the food before opening the package (Q4: No) and thus the outcome of the DT is that the time limit for consumption after opening the package must be shorter than the initial 'best before' date. In the case of no aseptic packaging, vegetative cells of pathogenic microorganisms can be present in the food before opening the package (Q4: Yes) and there is no change in the intrinsic or extrinsic factors of the UHT milk after opening the package which would, increase the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (Q5: No), then the time limit for consumption after opening can be according to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons.

Yoghurt having a pH > 4.3 and a_w > 0.990 can support the growth of vegetative cells of pathogenic microorganisms (Q1: Yes). If there are other growth inhibitory factors, such as a starter culture that can be used as evidence by the FBO that the food product does not support the growth of pathogens under reasonably foreseeable conditions of temperature during distribution and storage (Q2: Yes) and, assuming that the intrinsic and extrinsic factors (e.g. pH, a_w) of the food product after opening do not change to values that can support growth of vegetative cells of pathogenic microorganisms (Q3: No), the time limit for consumption after opening can be according to the initial 'best before' date unless a shorter secondary shelf-life is required due to quality reasons. If the response to Q2 is No, the FBO has no evidence that the food product does not support growth of pathogens under reasonably foreseeable conditions of temperature during distribution and storage. The output of the DT would be the same (time limit for consumption after opening can be according to initial 'use by' date – when the initial 'use by' date has been defined based on product's safety- unless a shorter secondary shelf-life is required due to quality reasons) since vegetative cells of pathogenic microorganisms can be present in the food before opening the package (Q4: Yes) and opening yogurt package does not normally result in any change in the intrinsic or extrinsic factors (pH, a_w, atmosphere, etc.) of the food product which increases the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (Q5: No).

Meat and meat products

Fresh meat (e.g. fresh pork) having a pH > 5.7 and a_w > 0.99 supports the growth of vegetative cells of pathogenic microorganisms (Q1: Yes) and the FBO does not provide evidence for the opposite considering reasonably foreseeable conditions of temperature during distribution and storage (Q2: No). Vegetative cells of pathogenic microorganisms can be present in the food before opening the package (Q4: Yes) and if there are no changes in intrinsic or extrinsic factors expected after opening the package that increase the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (Q5: No), then the time limit for consumption after opening can be according to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons. On the contrary, for fresh meat products packed under vacuum or MAP opening the package may result in changes of intrinsic and extrinsic factors which increases the growth rate of aerobic pathogens during subsequent storage compared to the unopened package (Q5: Yes), and thus the time limit for consumption after opening the package must be shorter than the initial 'use by' date when the latter has been defined based on product's safety.

A vacuum-packed sliced cooked meat product having a pH = 6.2 and a_w = 0.975 can support the growth of vegetative cells of pathogenic microorganisms (Q1: Yes). When there are no additional hurdles present in the food (e.g. lactate) the FBO does not demonstrate that the food product does not support the growth of pathogens under reasonably foreseeable conditions of temperature during distribution and storage (Q2: No). For products which have not undergone a validated lethal or post-lethality treatment applied in the package, vegetative cells of pathogenic microorganisms can be present in the food product before opening the package, (Q4: Yes) and if there are no changes expected in the intrinsic or extrinsic factors of the food after opening the package which would increase the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (e.g. packed under aerobic conditions) (Q5: No), then the output of the DT is that the time limit for consumption after opening can be according to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons. However, in the case of packing under vacuum or MAP, opening the package results in potentially faster growth of pathogens during subsequent storage compared to the packed product (Q5: Yes), and thus the time limit for consumption after opening the package must be shorter than the initial 'use by' date when the latter has been defined based on product's safety.

For the product described above, although, based on its pH and a_w the answer to Q1 is yes, specific formulations, e.g. with a sufficient amount of lactate to be antimicrobial, can be used by the FBO together with vacuum-packing or MAP to prove that the product does not support the growth of pathogens (Q2: Yes). In the case of no validated lethal or post-lethality treatment applied in the package, pathogen's vegetative cells can be present in the food product before opening the package (Q4: Yes) and since changes in the intrinsic or extrinsic factors of the food are expected after opening the package which increase the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package (i.e. packed under vacuum or MAP)(Q5: Yes) the time limit for consumption after opening the package must be shorter than the initial 'use by' date when the latter has been defined based on product's safety.

Products derived from fruits and vegetables

Fresh fruit juice (e.g. fresh orange juice) having a pH = 3.6 and a_w = 0.995 cannot support the growth of vegetative cells of pathogenic microorganisms (Q1: No) and the intrinsic and extrinsic factors of the food product after opening are not expected to change to values that can support growth of vegetative cells of pathogenic microorganisms (Q3 = No). Thus, growth of pathogens is not expected after opening of the package and the time limit for consumption after opening can be according to initial best-before unless a shorter secondary shelf-life is required due to quality reasons. The above applies also to ***pasteurised fruit juice*** with the same pH = 3.6 and a_w = 0.995.

Other food products

Another example is a ***mixed salad with fresh and canned ingredients,*** which has a combination of pH and a_w, (here pH = 5.5, a_w = 0.94) at equilibrium that supports growth of vegetative cells of pathogenic microorganisms (Q1: Yes) and for which the FBO cannot demonstrate otherwise (Q2: No). For this example, where intrinsic factors such as pH and a_w may evolve/change when the ingredients are mixed and/or during the subsequent storage the answers to Q1 and Q2 should be based on the values of pH and a_w and/or other intrinsic factors at equilibrium. When convergence to equilibrium is slow, the answers to Q1 and Q2 should be based on the ingredients with the more favourable intrinsic factors for microbial growth. As pathogenic vegetative cells can be present in the food before opening the package (Q4: Yes) and changes in the intrinsic or extrinsic factors of the food are not expected (e.g. packed under aerobic conditions) (Q5: No), the time limit for consumption after opening can be according to the initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons. However, if the product is packed under MAP (Q5: Yes), then the time limit for consumption after opening the package must be shorter than the initial 'use by date.

3.2.3. Uncertainty assessment of the decision tree for the need for information about time limit of consumption and storage conditions

Based on discussions and evaluations of examples of food products using the DT, it was considered that all the relevant food safety questions were identified and included. During the development of the DT, the phrasing, relevance and the sequence of questions were discussed. The structure of the tree was deemed logical, and to reflect the relevant events that may take place and influence the outcome of the decision. The key questions in the DT were Q3 and Q5, which aimed to identify food for which a change in growth potential occurs after opening. The responses to these questions were then put in the context if this would change the types of pathogenic microorganism present (Q4) or change the growth behaviour compared to the conditions determining the primary shelf life (Q5). Overall, it is considered that the DT will result in appropriate and consistent outcomes on time limits and storage conditions, within the interpretations of regulations and the assumptions made in its development. None of the identified sources of uncertainty was considered more important than any of the others. Taken together, the uncertainties are considered to result in a DT that may overestimate the risk for some food products.

3.2.4. Concluding remarks

- Opening the package of a food product may impact both safety and quality. For the purpose of this opinion, it is appropriate to establish storage conditions and a time limit for consumption after opening the package when opening can have an impact on product safety.
- A DT, consisting of a sequence of five questions, was developed and supported by various application examples to assist FBOs in the decision whether the time limit for consumption after opening, due to safety reasons, is potentially shorter than the initial 'best before' or 'use by' date of the product in its unopened package.
- The underlying assumptions for the DT are that:
    - After opening the package, contamination of the product with pathogenic microorganisms is always possible
    - The time limit for consumption after opening the package in relation to the initial 'use by' or 'best before' date depends on whether opening the package changes:
        - the type of pathogenic microorganisms in the food (e.g. contamination with vegetative cells not present in the unopened food package with, in general, a wider range of growth capabilities compared to growth and/or toxin production from spores), or
        - the factors affecting growth of pathogenic microorganisms compared to the unopened product.
- According to the DT, in case of products for which opening the package leads to a change of the type of pathogenic microorganisms present in the food and/or factors increasing their growth compared to the unopened product, the outcome is that a shorter time limit for consumption after opening the package compared to the initial 'best before' or 'use by' date of the unopened food would be appropriate.
  The expression 'shorter time limit for consumption after opening' means that the secondary shelf-life in days should be shorter than (or in some cases it may turn out to be the same as) the number of days between the time of opening the package and the 'use by' or 'best before' date. For `use by′ date the term 'shorter' refers only to when shelf-life is defined based on safety criteria, and not based on quality.
- Overall, it is considered that the DT will result in appropriate and consistent outcomes on time limits and storage conditions, within the interpretations of regulations and the assumptions made in its development. None of the identified sources of uncertainty was considered more important than any of the others. Taken together, the uncertainties are considered to result in a DT that may overestimate the risk for some food products.

Table 3: Application examples of the decision tree decision on the labelling of the storage conditions and time limits for consumption after opening food packages

DT questions	Milk and dairy products				Meat and meat products				Products derived from fruits and vegetables		Other food products	
	UHT milk (e.g. pH = 6.6, a_w = 0.995)		**Yoghurt** (e.g. pH = 4.3, a_w = 0.995)		**Fresh meat** (e.g. fresh pork with pH = 5.7, a_w = 0.99)		**Vacuum-packed sliced cooked meat product** (e.g. pH = 6.2, a_w = 0.975)		**Fresh fruit juice** (e.g. fresh orange juice with pH = 3.6, a_w = 0.995)	**Pasteurised fruit juice** (e.g. pasteurised orange juice with pH = 3.6, a_w = 0.995)	**Mixed salad with fresh and canned ingredients** (e.g. leafy salad with canned corn, with a combination of pH = 5.5, a_w = 0.94 for at least one ingredient)	
	Aseptic packing	No aseptic packing	With starter culture inhibiting pathogen growth under refrigeration	With starter culture not inhibiting pathogen growth under refrigeration	Aerobic Packing	MAP or VP	Without lactate Aerobic packing	With lactate MAP or VP			Aerobic packing	MAP
Q1. Does the unopened food product [based on pH and/or a_w] support the growth of vegetative cells of pathogenic bacteria?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes

DT questions	Milk and dairy products				Meat and meat products				Products derived from fruits and vegetables		Other food products	
Q2. Is the FBO able to demonstrate that the unopened food product does not support growth of pathogens under reasonably foreseeable conditions of temperature during distribution and storage?	No	No	Yes	No	No	No	No	Yes	NA	NA	No	No
Q3. Does any intrinsic or extrinsic factor of the food affecting growth (pH, a_w, atmosphere, etc.) change after opening to values that can support the growth of vegetative cells of pathogenic bacteria?	NA	NA	No	NA	NA	NA	NA	Yes	No	No	NA	NA

DT questions	Milk and dairy products				Meat and meat products				Products derived from fruits and vegetables		Other food products	
Q4. Could vegetative cells of pathogenic bacteria be present in the food before opening the package?	No	Yes	NA	Yes	Yes	Yes	Yes	NA	NA	NA	Yes	Yes
Q5. Is there any change in the intrinsic or extrinsic factors of the food (pH, a_w, atmosphere, etc.) after opening the package which increases the growth rate of vegetative cells of pathogenic bacteria compared to the unopened package?	NA	No	NA	No	No	Yes	No	NA	NA	NA	No	Yes

DT questions	Milk and dairy products				Meat and meat products				Products derived from fruits and vegetables		Other food products	
Time limit for consumption after opening shorter than initial 'Best before' or 'Use by' date	Shorter than initial 'best before' date	According to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons	According to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons	According to initial 'best before' unless a shorter secondary shelf-life is required due to quality reasons	According to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons	Shorter than initial 'use by' date	According to initial 'best before' date unless a shorter secondary shelf-life is required due to quality reasons	Shorter than initial 'use by' date	According to initial 'best before' date unless a shorter secondary shelf-life is required due to quality reasons	According to initial 'best before' date unless a shorter secondary shelf-life is required due to quality reasons	According to initial 'use by' date unless a shorter secondary shelf-life is required due to quality reasons	Shorter than initial 'use by' date

DT:= decision tree; FBO: food business operator; NA: not applicable.

3.3. Guidance to food business operators on advice to provide to consumers on thawing of frozen foods including storage conditions and times (ToR 4)

Food-borne outbreaks caused by frozen foods after thawing have occurred and thawing practices and/or a lack of basic hygiene knowledge may have been a contributory factor. An example is the multi-country outbreak of invasive *L. monocytogenes* infections linked to frozen corn (EFSA and ECDC, 2018) where contaminated frozen corn was consumed after thawing and without cooking. It is not clear whether the thawing conditions increased the risk, rather than the initial contamination of the frozen material and the effect of subsequent storage conditions, but this cannot be excluded. Another example is a listeriosis outbreak from ice cream used in milkshake that was stored at abuse temperature which allowed the concentration of *L. monocytogenes* to increase (Chen et al., 2016). After the frozen corn outbreak, the EFSA BIOHAZ Panel (2020b) stated that good practices such as storage of frozen or thawed vegetables in a freezer or refrigerator at the appropriate temperature and following the instructions on labelling for safe preparation are key to obtaining safe frozen or thawed food. Furthermore, risks are reduced or avoided if vegetables are cooked properly after defrosting.

Among the thawing habits of concern are the thawing of perishable food in hot water, or at room temperature overnight, as this leads to storage of food in the 'temperature danger zone', where pathogens may grow, for a period longer than considered acceptable (Byrd-Bredbenner et al., 2013; Tomaszewska et al., 2020).

In this section, critical steps during thawing of frozen foods are discussed (Section 3.3.1), followed by an overview of existing guidance to consumers on good practices for thawing of foods and how to store thawed foods (Section 3.3.2). In Section 3.3.3, advice is provided for FBOs to give to the consumers regarding best thawing practices, storage conditions and retention times for thawed foods.

3.3.1. Critical steps during thawing of frozen foods including storage conditions and times

Freezing and thawing involve processes such as heat transfer, liquid flow and crystal formation. These processes can interfere with the food matrix and the microorganisms present. They also influence the thawing rate. The kinetic aspects of freezing and thawing are well described in food processing text books and studies (e.g. Haugland, 2002; Schlüter, 2003). Formation of ice crystals in the food during freezing and thawing may result in cell membrane damage causing leakage and loss of intracellular hydration water surrounding proteins and macromolecules, leading to drip loss in the food matrix. These physical changes during freezing and thawing are further discussed in Sections 3.3.1.1 and 3.3.1.2. In Section 3.3.1.3, the effect of freezing and thawing on survival and growth of bacteria is described.

3.3.1.1. Heat transfer during freezing and thawing

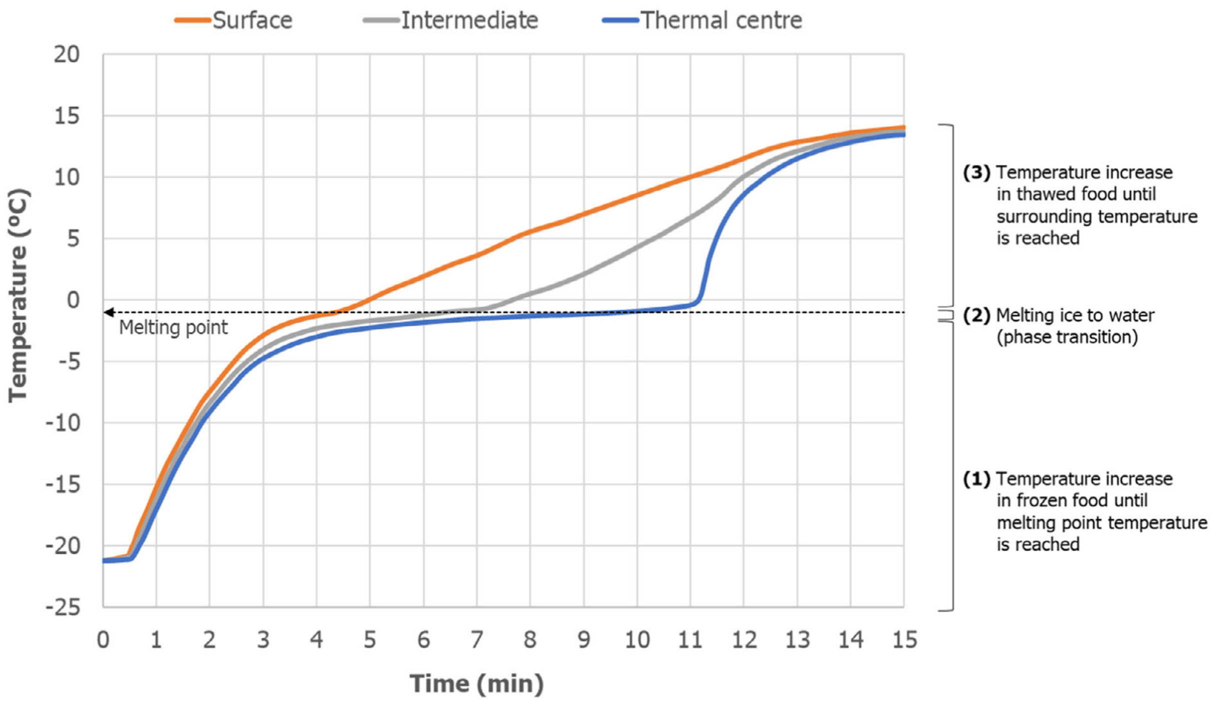
Foods are made up of many different substances, and the freezing temperature is generally below the freezing point of water. Each food will freeze and thaw differently based on the physico-chemical characteristics such as the proportion of water, salts, sugars, proteins or air in the food. The heat transfer rate between the food and the surrounding environment depends on:

- the temperature difference between the frozen food and the surroundings;
- the heat transfer coefficient on the surface between the frozen food and the surroundings, which is related to the air/water flow;
- the surface area of the food.

Thawing may appear to be merely the reversal of freezing but is a more difficult operation than freezing. It requires extra care because thawing creates a region that has a lower thermal conductivity than the food that is still frozen, which impedes the flow of heat. Furthermore, during thawing it is not possible to create as large a temperature difference between the food and the surroundings as during freezing without changing parts of the food due to high temperatures. In addition, temperature-time combinations during thawing may allow bacterial growth (Nesvadba, 2008).

The total amount of energy needed for thawing frozen food depends on the energy needed for the temperature increase and that needed for the phase transition of the water from the frozen to the liquid phase (Kumar et al., 2020). Latent heat is the heat energy that, when added (absorbed) or removed (released), causes a change in the state of a material with no change in temperature (e.g. from ice to water). Relatively large quantities of heat (energy) are needed for the change in state (Pham, 2016). Sensible heat is the heat energy that is added or removed from a material that causes a change in temperature without change of state, e.g. to warm up water (Pham, 2016).

The thawing process is slow, and the surface may reach the ambient temperature long before the core of the food is thawed. The larger the temperature difference between the frozen food and the surroundings, the more rapid the thawing. However, there is a resistance layer around the food, called the stagnant layer, which is subject to steady state conditions. The resistance for heat transfer is larger between food and air than between food and water. Thawing in water is therefore more rapid than thawing in air. However, the stagnant layer can be manipulated, for instance by air blowing under a fan. This will accelerate the thawing, even if the temperature difference at macro scale between the food and the surroundings is the same as without the air movement. During most modes of thawing, the heat transfer occurs over the food surface. The larger the surface area, the more rapid the thawing. Similarly, the smaller the surface to volume ratio, the smaller the relative area for heat transfer with the surroundings, and the slower the thawing. Thawing in a microwave oven is different, as heat generation occurs within the internal part of the food. The heat needed for transforming the water from solid to liquid phase is large (Klinbun and Rattanadecho, 2019), and this is the reason that foods with a high-water content thaw more slowly than foods with a low water content (Kumar et al., 2020). The typical temperature profiles during thawing display different phases, with different durations and rates depending on the location within the food (e.g. thermal centre, surface or an intermediate location) as shown in Figure 3.



Temperature profiles were taken and adapted from Schlüter (2003), corresponding to the thawing process of a 13-mm cylinder of potato in a water bath at atmospheric pressure. Temperature of the thermal centre, the surface and an intermediate location are represented.

Figure 3: Phases of thawing of foods in terms of rates of temperature change

The first phase is a rapid temperature increase in the frozen food (sensible heat absorption). When the temperature approaches the melting point (around −1°C, but varies depending on the food composition), the transition of ice to water begins with very small temperature increases due to the absorption of latent heat (phase 2). Phase 2 is longer in the thermal centre compared to the surface of the food in close contact with the surrounding media. When the ice within the food matrix has melted, the temperature increases more rapidly again, and then the rate of temperature increase gradually slows down as the matrix near the temperature of the surroundings (phase 3). In a large food item (e.g. frozen meat carcass), the phases can occur simultaneously; the surface may reach a higher temperature than zero while there is still ice in the internal parts of the food. The temperature on the surface is then influenced by both the cooling effect of the melting ice in the internal parts and the heat exchange with the surrounding medium (e.g., air or water).

From a food safety point of view, the temperature differences are important because microorganisms in the frozen food, whether they have been introduced before freezing or by recontamination during thawing, may start growing when the temperature is sufficiently high. There may be growth on the surface of the thawing food even if the centre is still frozen.

3.3.1.2. Impact of ice crystals and liquid flow on the food

The liquid flow in frozen and thawed food is linked to the fact that a fraction of water will remain in liquid phase and form an equilibrium with the solid water. Water crystals will be formed in the food during the freezing process. Ice crystallisation in frozen food products and the optimisation of the freezing process has been reviewed by Zhu et al. (2019) and Dalvi-Isfahan et al. (2019). As described in these reviews, rapid freezing to a low temperature leads to small crystals, while slow freezing leads to larger crystals. However, during frozen storage, the crystals may grow in the food. The process is very slow, but a difference in the effect of different freezing temperatures can be observed. At low temperatures, i.e. –25°C and below, the amount of liquid water is small, and the crystals are small. At higher temperatures, i.e. –20°C and warmer, a higher fraction of the water is in the liquid phase and in exchange with the solid phase, with the result that the ice crystals increase in size. Crystals located in the cell membranes can, when they become large enough, may penetrate the membranes of bacteria and/or animal or plant cells present in the food matrix. The result is a reduced fraction of surviving bacteria (see Section 3.3.1.3 for further details), and a liquid loss from the food matrix during thawing. The effect can be seen in high water content foods such as berries, and also in, e.g. frozen fish. Another effect of the water flow within frozen food is that macromolecules are surrounded by hydration water, i.e. water in liquid phase. During freezing, this water is slowly lost, resulting in a drier texture of the food. Under rapid thawing, the liquid and the food components that are in solution are lost as drip loss. During slow thawing, however, part of the drip loss is absorbed into the food, and a more succulent food quality is obtained. The mode of thawing, including the temperature, is therefore important for the sensory quality of food as well as for food safety, and advice for thawing commonly considers not only safety but also sensory quality aspects.

3.3.1.3. Freezing and thawing effects on survival and growth of pathogenic microorganisms

Freezing generally reduces the number of viable microorganisms in food, to about 20–90% of the original community (Geiges, 1996), which means a decrease of up to one $\log_{10}$ unit. Many factors influence the behaviours of individual species of microorganisms during the storage of frozen food, and survival is variable for different groups of microorganisms. The most important factors relating to the microorganisms are the numbers of cells, whether vegetative cells or spores, and the growth phase and species of microorganisms. During freezing and thawing, the rate of freezing, the freezing temperature, the storage time, the food characteristics (e.g. components, pH, a_w, protective substances), the type of packing materials (e.g. thickness, water permeability), the thawing rate and temperature are important factors. Finally, since sublethal damage may occur in the microorganisms in foods that have been frozen and then thawed, pathogenic microorganisms, despite being viable, may not be able to grow in the selective agents commonly used in enrichment or plating media to culture and isolate them (Archer, 2004). This could lead to false conclusions about the effect of freezing since damaged, but possibly infectious, microorganisms may become culturable, if placed in media that facilitates repair (resuscitation). Consequently, the methods used for detection and quantification of the microorganisms are vital (e.g. resuscitation step, culture media, incubation temperature/time) (Geiges, 1996; Archer, 2004; Berry et al., 2008).

The effect of ice crystal formation during freezing is more pronounced in food matrices with a high-water content compared to a high lipid content. Bacteria in lipid-rich food are therefore better protected during freezing. Similarly, some natural food components and additives may act as cryoprotectants, thereby enhancing survival of microorganisms during cold storage (e.g. NicAogáin and O'Byrne, 2016 and references therein).

The freeze–thaw process may be associated with cell destruction or injury through cold-shock, intra- and extracellular ice-crystal formation, increased concentration of soluble solids affecting the stability of proteins within microbial cells, membrane damage, outflow of water from the cell (which is related to increase in extracellular osmotic pressure), and membrane-lipid phase transitions, and possibly also to oxidative stress, and thus, constitutes a major stress for microorganisms (Berry et al., 2008). Repeated freeze-thaw cycles generally lead to greater inactivation and lower microbial concentrations, but pathogens may survive, i.e. be cryotolerant; defined as the ability to survive repeated cycles of freeze thawing (Azizoglu and Kathariou, 2010). Actively growing bacteria are more susceptible to freeze–thaw inactivation than stationary phase cells (e.g. Azizoglu et al., 2009), and the cell history including the previous growth temperature can influence cryotolerance. The impact of previous growth temperature may be species-specific as indicated by studies reporting the effects of prior growth temperatures on cryotolerance in laboratory media for *Yersinia enterocolitica* (Azizoglu and Kathariou, 2010) and *L. monocytogenes* (Azizoglu et al., 2009). Evidence of survival and different responses of pathogenic microorganisms in frozen foods is well-described (e.g. ICMSF, 1996, Leroi et al., 2008), including Norovirus (e.g. Jacxsens et al., 2017), and *Campylobacter* (e.g. Umaraw et al., 2017). The survival of pathogens is well documented in outbreaks related to frozen foods (e.g. Norovirus and frozen berries and fruits (Rispens et al., 2019, Nasheri et al., 2019), *L. monocytogenes* on frozen corn (EFSA and ECDC, 2018 and EFSA BIOHAZ Panel, 2020a,b) and *Li. monocytogenes* in ice cream (Pouillot et al., 2016). To generalise, Gram-negative bacteria are more sensitive to freezing than Gram-positive bacteria. Viruses are still capable of infecting host cells after frozen storage, and bacterial spores are minimally affected by freezing (Berry et al., 2008).

Thus, even though freezing and frozen storage reduce the concentration of pathogenic microorganisms, a fraction, depending on concentration and microorganism, of surviving microorganisms usually remains, and the basic means of control is then to avoid or minimise the growth of pathogenic microorganisms during thawing and subsequent storage by controlling the temperature. The lower temperature limit for growth of pathogenic bacteria in foods is above −1.5°C (EFSA BIOHAZ Panel, 2020a). The extent of growth will be dependent on the characteristics of the food (intrinsic, extrinsic, implicit factors) and especially the time temperature conditions (EFSA BIOHAZ Panel, 2020a).

There is usually a lag phase before growth is observed, during which recovery from sublethal injury and acclimation to the growth environment occurs. The length of this lag phase, and thus the impact on the extent of growth is difficult to predict and is dependent both on environmental factors, e.g. extent of sublethal injury and culture methods (Fratamico and Bagi, 2007; Jasson et al., 2009) and genetic/evolutionary factors (Sleight and Lenski, 2007). Occurrence of a lag phase, which would have been a benefit for the food safety of thawed food, provided there is no recontamination during thawing, can therefore not be taken for granted. Growth rates increase with temperature and the higher temperature near the surface relative to the core temperature of the food during thawing can lead to growth of microorganisms which have survived during freezing or contaminated the food during thawing. Thawing at low temperature is therefore important for food safety (EFSA BIOHAZ Panel, 2020a,b).

The flow of liquid during freezing and thawing may alter both the water activity and pH of the food, at least in the microenvironments of the food and, due to cell leaching, the nutrient availability may increase (e.g. Archer, 2004; Devlieghere et al., 2016), but other mechanisms such as microstructural changes affecting both growth rate and lag phase duration may also play a role (Verheyen et al., 2019). Therefore, growth rates of pathogens in thawed foods may not be equal to growth rates in the same foods stored chilled, but unfrozen, particularly if the food consists of cellular components that are easily ruptured during freezing and thawing. This needs to be considered when estimating the acceptable storage time of food after thawing, as was highlighted by Zoellner et al. (2019), in modelling growth of *L. monocytogenes* in thawed vegetables, by EFSA BIOHAZ Panel (2020b) and by Kataoka et al. (2017) on thawed vegetables and seafood.

Thus, if the thawed food is not going to be used immediately, it needs to be stored refrigerated and only for a limited time in order to minimise the growth of pathogens. Most frozen foods are not RTE and may contain pathogens which can start to grow after thawing. Therefore, thawed foods such as meat, fish, vegetables which may be contaminated with pathogens that have the potential for growth need to be stored in refrigerated conditions and for a limited time and should be cooked before consumption. This was highlighted by EFSA for frozen vegetables (EFSA BIOHAZ Panel, 2020a, b), and by Zoellner et al. (2019) who designed a model 'FFLLoRA' providing frozen food manufacturers with a tool to assess *L. monocytogenes* contamination and growth as a result of consumer behaviour when managing rare and/or minimal contamination events in frozen foods.

Thawing and storage may also influence formation of biogenic amines (BA), e.g. histamine, tyramine, in food. For instance, Buchtova et al. (2019) demonstrated the formation of biogenic amines due to the inclusion of thawed tuna in sushi meal preparations. Biogenic amines are formed during growth of BA producing bacteria in foods containing certain free amino acids (EFSA BIOHAZ Panel, 2011). The decarboxylating enzymes activity decreases at refrigeration temperatures, below 5-10°C. However, enzyme activity remains stable during freezing, and after thawing the enzymes can become reactivated again even though the concentration of the enzyme producing bacteria may be below detection (Berry et al., 2008).

3.3.2. Overview of guidelines for consumers on good practices for thawing frozen foods including storage conditions and times

Guidelines for both businesses and consumers are available. We focus here on guidelines directed at consumers. Much of the consumer advice on thawing foods is available on the internet. Some are developed by chefs, others by competent authorities and by consumer organisations. There are also targeted guidelines for charity organisations for frozen donated foods.

3.3.2.1. Key elements in the guidelines

More than 40 guideline documents for freezing and thawing for consumers and producers from most European countries as well as from Australia, USA and Canada have been reviewed (Appendix B). All guidelines, are based on similar principles:

- Frozen foods can contain pathogens and have to be considered to be products with a latent risk.
- Thawing and subsequent storage must limit the growth and spread of pathogens.
- Thawing is a slow process. For accelerated thawing, the guidelines focus on limiting the time in the 'temperature danger zone') between 8 and 63°C (https://www.food.gov.uk/safety-hygiene/chilling.
- The temperature during and after thawing must be sufficiently low to limit growth of pathogens.
- Frozen food can be cooked directly. This will avoid any period of storage for thawed foods where growth can occur.
- Avoiding cross-contamination from the frozen to other foods, and vice versa.

There are, however, some differences between the guidelines in the way they address these elements. For instance, the guidelines for foods frozen for donation and foods produced for frozen distribution have a different focus. Some countries specify which information should be given on the label, and some specify how frozen food can be used if unintentionally thawed. Foods produced and sold as frozen products directly from a FBO are normally frozen immediately after processing, which means that the growth of pathogens in the food before freezing has been limited (Regulation (EC) No 853/2004). FBOs who sell frozen foods need to consider the thawing conditions and adapt the instructions to the consumers accordingly. Food for donation is often frozen shortly before the end of shelf life for chilled storage, which implies that the concentration of pathogens could be close to the acceptable limit for food safety. Thawing at abuse temperatures can lead to growth of these pathogens to unacceptable concentrations. The correct management of thawing conditions is therefore essential for food safety.

3.3.2.2. Modes of thawing

Regarding modes of thawing, the key elements considered are the temperature and time duration during the thawing (Figure 3). The guidelines specify the thawing temperature and/or the thawing conditions. The list below is not exhaustive, but indicates the most common advice:

- **In refrigerator, i.e. chilled conditions.** This method leads to a slow thawing, and the growth of pathogens in the food is limited. Thawing under chilled conditions is included in all the guidelines reviewed. Some guidelines specify that the temperature should be a maximum of 4°C, others 8°C, while most do not specify the temperature more specifically than the temperature of domestic refrigerators.
- **In water, either running or still, with or without package.** This mode of thawing is included in most guidelines. The heat transfer is more rapid in food-to-water surfaces than in food-to-air surfaces at the same temperature. In still water, the temperature difference between the frozen food and the water reduces over time, but a gradient can be maintained when running water or water changes are applied.

- **In boiling water, hot water (e.g. sous vide), in baking oven or frying pan.** Thawing at temperatures above the lethal temperature for the relevant pathogens in the food ensures both a rapid thawing and the elimination of pathogens in one activity, provided that pathogens are on the surface and/or that lethal temperatures are reached also inside the food. About half of the guidelines include this mode of thawing within their list of recommended procedures.
- **In a microwave oven.** Defrosting in a microwave oven is a rapid method and has the benefit that the heat is transferred to the internal parts of the food, not only the surface. The guidelines that suggest microwave thawing also state that food thawed this way should be used immediately. This also applies for other foods that are thawed at ambient or uncontrolled temperature.

Thawing at ambient temperature is not mentioned among the safe modes of thawing in any of the guidelines. However, large food items, such as turkeys, that in some cases cannot be placed in a refrigerator due to space limitations are included as an example where some guidelines specify that thawing at ambient temperature has to be accepted, even though it is not optimal.

Several guidelines indicate the time it takes to thaw a food item of 500 g, for instance 'The safe guide to thawing chicken',[10] and points out that the size of packages of frozen food should be limited to 500 g, because small volumes can be thawed more quickly.

The guidelines mentioned above are all in agreement with scientific literature on growth kinetics at storage temperatures described in part 1 of the opinion and in papers cited above in this chapter.

3.3.2.3. Hygienic practices and storage conditions of thawed food

Several guidelines indicate which information should be given on the label regarding hygienic practices for the consumer. Most guidelines point out that cross-contamination should be avoided, by storing thawing food at a distance from other foods, that clean utensils should be used, etc.

Guidelines from several countries state that the label should include sentences like 'to be consumed immediately after defrosting', or in other ways include instructions that the food should be refrigerated during thawing and cooked immediately. Only one guideline allows for the storage of thawed food, limited to 3 days after thawing (Domestic Practice, The Food Safety Authority of Ireland).[11] In the case of food donations, where a higher microbial load can be expected at the time of freezing, EFSA advises to use the food within 24 h after thawing (EFSA BIOHAZ Panel, 2017).

3.3.2.4. Thermal processing (cooking) of thawed food

Several guidelines suggest the direct use of frozen food in cooking without previous thawing. Some point out this is the preferred method, with the justification that the storage time in the 'temperature danger zone' is avoided. Other guidelines point out that the thawed food must be heated all the way through, including when mixed with hot foods. The recent guideline for frozen vegetables (Profel, 2020), stresses that the vegetables must be fully cooked after thawing.

It should be noted that cooking frozen food will need a longer time and/or heat transfer intensity to achieve the necessary time–temperature for eliminating pathogens than cooking of thawed or tempered foods. This information is not included in most guidelines. Therefore, if cooking instructions are given by the FBO on the label, it should be clear whether the heating begins from the thawed or frozen state.

3.3.2.5. Refreezing

A few guidelines accept refreezing within a certain time provided no temperature abuse has occurred. Most other guidelines advise not to refreeze thawed food as the temperature control during thawing is challenging at consumer level. Some documents do not give a justification, while others point out that toxins and pathogens may have developed in the food during the thawing period. Some guidelines allow refreezing, but only after the thawed food has been treated. Cooking is often mentioned as a suitable treatment in this regard.

[10] https://www.euro-poultry.com/blog/the-safe-guide-to-thawing-chicken
[11] https://www.fsai.i.e/faq/domestic.html

3.3.3. Advice on good practices for thawing food, including storage conditions and time

The basis for advice for a FBO selling frozen foods to the consumer includes knowledge about the conditions of the food at the time when it is frozen, and an understanding of how the freezing and thawing process will influence safety under reasonably foreseeable consumer conditions. If the product is intended to be stored or used some time after the food is thawed, the reasonably foreseeable conditions at this stage also need to be considered.

The basis for providing advice on good practices for thawing, storage conditions and time limits of consumption of thawed food are the assumptions that food may be contaminated with pathogens (i.e. bacterial pathogens and viruses) before freezing (frozen food is, in general, non-RTE food), that pathogens may survive during freezing, that additional contamination can occur during thawing outside the package, and that some of the bacterial pathogens may be able to grow or produce toxins in parts of the thawed food (growth defined as in Part 1, $>$ 0.5 log increase, EFSA BIOHAZ Panel, 2020a), if the food can support growth and time/temperature conditions allow growth (depending on the intrinsic and extrinsic conditions, refer to section 3.1.2).

In Table 4, advice on good practices for thawing foods, including storage conditions and time after thawing, and best practices for handling frozen foods are summarised.

Table 4: Overview of advice that may be given to consumers regarding good practices of thawing of foods, storage conditions and time limits for consumption of thawed foods by FBOs

Aim of advice	Advice	Reason/Motivation/Why	Examples of frozen foods
Avoid growth of microbiological pathogens during thawing	Thaw in refrigerator	Reduce growth rate, temperature does not exceed the refrigeration temperature	Frozen meat, fish and seafood
	Thaw under cold running water	Reduce growth rate, temperature can increase to the temperature of running water	Frozen vegetables, fruits
	Thaw at ambient temperature	Dry pastry should be thawed at room temperature, unwrapped or in a material where released and condensed water will be collected. Typically, moulds grow rapidly on dry pastry in the parts where moisture from thawing is present.	Frozen dry pastry such as frozen croissants
	Thaw in boiling water or mix with hot foods	No opportunity for growth in boiling water or a hot food	Frozen berries, vegetables
	Prepare the frozen food directly, without thawing	No time for growth	Frozen oven-ready food, pizza, lasagne
	Break bigger packages/pieces in smaller parts (inside the package) before thawing	Accelerated thawing due to smaller pieces (larger surface vs volume)	Block of frozen fish, shrimps, berries
Avoid contamination with microbiological pathogens during thawing	Thaw food in the original package or if not possible, in a clean container to avoid contamination	To avoid contamination during handling or dirty home container or utensils	Prepacked frozen meat, vegetables, fish
	Place food on a tray during thawing	• Avoid drip and contamination from the thawing food to other foods • Avoid condensation water dripping to other foods leading to contamination	Frozen meat (especially larger pieces e.g. steak, chicken carcass), fish and seafood
	Use clean utensils and clean hands when handling food, e.g. when breaking larger pieces of food into smaller pieces	Avoid contamination of foods from kitchen utensils and hands	Frozen shrimps, berries, vegetables

Aim of advice	Advice	Reason/Motivation/Why	Examples of frozen foods
Avoid growth of microbiological pathogens during subsequent storage after thawing	Do not store longer than × days at y °C (appropriate time-temperature combination) before consumption	Avoid growth of pathogens in the thawed food	Frozen vegetables: 24h–48h at refrigeration temperatures (Profel, 2020) Other foods: Consult Part 1 opinion (EFSA BIOHAZ Panel, 2020a) for description of tools and procedures for estimating growth potential for different time-temperature combinations.
	Follow the instructions provided on the label	Avoid temperature abuse during storage of the thawed foods	All packaged frozen foods
	Do not refreeze after thawing	Minimise opportunities for pathogen growth due to contamination and time-temperature combinations allowing growth during storage and subsequent freezing and thawing	All thawed foods
	Take only the needed amount of frozen food out for thawing and keep the rest frozen	Minimising the opportunity for growth and contamination by not having to store food which is not needed	Frozen foods where a portion can be taken, e.g. frozen vegetables, berries, shrimps
	Frozen foods that are intended to be eaten frozen should not be used in dishes stored at refrigerated temperature for a long time period	Frozen food, even frozen food intended to be used as is, may contain pathogens at low levels which can start to grow at refrigeration temperatures. In case frozen ingredients are used in a dish, storage time in the refrigerator has to be limited.	Thawed ice cream used in milk shakes
Reduce potential contamination with microbiological pathogens when using thawed food in the final dish/mixed dishes	Cook the thawed food before consumption	Frozen foods may contain pathogens which may grow after thawing, but cooking can eliminate these pathogens	Frozen meat, seafood, vegetables, etc.
	Cook the thawed food before using in a mixed dish that will not be heat treated	In case of mixed dishes, frozen ingredients cannot be used without a heat treatment to eliminate pathogens	Frozen peas or corn in a mixed salad, cake with frozen berries
Inform consumers that most frozen foods are not RTE and that these need to be sufficiently cooked after thawing	Include clear instructions on the package, website, QR code, etc. how to cook frozen foods	Frozen foods may contain pathogens which may survive freezing and grow after thawing, so sufficient cooking and elimination of pathogens is necessary	Frozen meals such as lasagne, pizza

3.3.4. Concluding remarks

- From a food safety point of view, freezing prevents the growth of pathogens. However, even though the concentration of pathogens may decrease over time, elimination is usually not complete during the freezing period depending on the pathogen and initial concentrations, the duration of the frozen storage and conditions during freezing/thawing.
- Pathogenic microorganisms that survive frozen storage can recover during thawing and may grow and/or produce toxins in the food during or after thawing if the pH, water activity and storage temperature support growth. Moreover, during the handling of thawed foods, additional contamination may occur from the hands, contact surfaces (e.g. utensils), or from other foods.
- Good practices for thawing should minimise contamination by pathogens between the food being thawed and other foods and/or contact surfaces when the food is removed from packaging during thawing, and limit conditions favourable to their growth.
- Advice that the FBO may provide to consumers regarding good practices for thawing of frozen foods, storage conditions and time limits for consumption of thawed foods includes:
  - using a mode of thawing that ensures sufficient thawing at a time and temperature combination that avoids growth of pathogens which have survived during freezing, also considering further use;
  - keeping thawed foods in the original package or, if not possible, in a clean container, and only use clean utensils and hands when handling the food to avoid contamination;
  - using thawed food in food preparations, or storing thawed food according to the instructions from the FBO. The FBO should consider providing advice regarding time-temperature limits for storage of thawed foods and advise sufficient heat treatment of the thawed foods to eliminate pathogens before consumption;
  - informing consumers that frozen foods are intended to be heat treated/cooked unless the production process implies that the frozen thawed product is safe and can be consumed without cooking.

4. Conclusions

The opinions should develop a risk-based approach to be followed by FBOs when deciding on the type of date marking (i.e. 'use by' date versus 'best before' date), setting of shelf-life and the related food information that should be provided on the labelling in order to ensure food safety.

ToR 3 - Provide guidance on storage conditions and/or time limit for consumption after opening the package in order to avoid increase of food safety risks

ToR 3a - *The characteristics of a food and the intrinsic/extrinsic factors which might change once the package is opened, and specifically on which of those factors that should be taken into consideration when providing such information*

- The time point of opening the package within the primary shelf-life can influence the type and concentrations of the microorganisms present in the food (i.e. the closer to the end of shelf-life, the higher the expected concentration of most microorganisms).
- After opening a food package, contamination may occur via air flow, fluid drip or due to consumer handling via hands, utensils, containers, etc., which may introduce new pathogens into the food or increase the concentration of pathogens already present.
- Opening the food package may change the conditions affecting the ability of pathogenic microorganisms to grow and/or produce toxins.
  - Extrinsic factors (such as the atmosphere composition) are probably the most important factors that may change after opening the package. The protection of vacuum or MAP is lost and a change in the growth behaviour (usually increasing the growth capability/rate) of the pathogens in the food can be expected.
  - The effect of changes in the intrinsic (such as a_w or pH) and implicit (such as competing microbiota) factors on pathogen growth after opening the packages should also be considered.
- Setting a time limit for consumption after opening the package (secondary shelf-life) is complex in view of the many influencing factors and information gaps. An additional level of complexity

comes from the need to consider consumer behaviour and reasonably foreseeable conditions of use.

ToR 3b - *The factors to be considered in deciding whether it is appropriate, and consequently mandatory, to indicate the storage conditions and/or time limit for consumption after opening the package according to Article 25(2) of Regulation (EU) No. 1169/2011*

- Opening the package of a food product may impact both safety and quality. For the purpose of this opinion, it is appropriate to establish storage conditions and a time limit for consumption after opening the package when opening can have an impact on product safety.
- A DT, consisting of a sequence of five questions, was developed and supported by various application examples to assist FBOs in the decision whether the time limit for consumption after opening, due to safety reasons, is potentially shorter than the initial 'best before' or 'use by' date of the product in its unopened package.
- The underlying assumptions for the DT are that:
  - After opening the package, contamination of the product with pathogenic microorganisms is always possible
  - The time limit for consumption after opening the package in relation to the initial 'use by' or 'best before' date depends on whether opening the package changes:
    - the type of pathogenic microorganisms in the food (e.g. contamination with vegetative cells not present in the unopened food package with, in general, a wider range of growth capabilities compared to growth and/or toxin production from spores), or
    - the factors affecting growth of pathogenic microorganisms compared to the unopened product.
- According to the DT, in case of products for which opening the package leads to a change of the type of pathogenic microorganisms present in the food and/or factors increasing their growth compared to the unopened product, the outcome is that a shorter time limit for consumption after opening the package compared to the initial 'best before' or 'use by' date of the unopened food would be appropriate.
- Overall, it is considered that the DT will result in appropriate and consistent outcomes on time limits and storage conditions, within the interpretations of regulations and the assumptions made in its development. None of the identified sources of uncertainty was considered more important than any of the others. Taken together, the uncertainties are considered to result in a DT that may overestimate the risk for some food products.

ToR 4 - Provide guidance on defrosting of frozen foods including good practices, storage conditions and/or time limit for consumption in order to avoid increase of food safety risks

ToR 4a - *Advice to be given to consumers regarding good practices, storage conditions and/or time limit for consumption to protect consumers from possible health risks*

- From a food safety point of view, freezing prevents the growth of pathogens. However, even though the concentration of pathogens may decrease over time, elimination is usually not complete during the freezing period depending on the pathogen and initial concentrations, the duration of the frozen storage and conditions during freezing/thawing.
- Pathogenic microorganisms that survive frozen storage can recover during thawing and may grow and/or produce toxins in the food during or after thawing if the pH, water activity and storage temperature support growth. Moreover, during the handling of thawed foods, additional contamination may occur from the hands, contact surfaces (e.g. utensils), or from other foods.
- Good practices for thawing should minimise contamination by pathogens between the food being thawed and other foods and/or contact surfaces when the food is removed from packaging during thawing, and limit conditions favourable to their growth.
- Advice that the FBO may provide to consumers regarding good practices for thawing of frozen foods, storage conditions and time limits for consumption of thawed foods includes:
  - using a mode of thawing that ensures sufficient thawing at a time and temperature combination that avoids growth of pathogens which have survived during freezing, also considering further use;

  - keeping thawed foods in the original package or, if not possible, in a clean container, and only use clean utensils and hands when handling the food to avoid contamination;
  - using thawed food in food preparations, or storing thawed food according to the instructions from the FBO. The FBO should consider providing advice regarding time–temperature limits for storage of thawed foods and advise sufficient heat treatment of the thawed foods to eliminate pathogens before consumption;
  - informing consumers that frozen foods are intended to be heat treated/cooked unless the production process implies that the frozen thawed product is safe and can be consumed without cooking.

5. Recommendations

- To provide training activities and support on the DT, for instance webinars, particularly for small food businesses and laboratories, aiming at contributing to a better understanding of the microbial ecology of food and on procedures required to characterise the relevant factors determining secondary shelf-life of perishable foods. Increasing skills and capabilities will facilitate making more consistent and appropriate decisions on the secondary shelf-life and will make procedures for setting the time limit for consumption more achievable. Similar training and support on the DT and approaches described in the opinion may also be useful for competent authorities.
- To collect time-temperature data on reasonably foreseeable storage conditions of foods in EU MS and to clarify and provide guidelines on how to use these data in secondary shelf-life decisions, i.e. what ranges of the existing variation to include, for instance regarding storage temperatures, and consumer behaviour/intended use.
- To develop appropriate level of protection (ALOP)/food safety objective (FSO) for relevant food-pathogen combinations, since the lack of such data is an obstacle for setting the primary and secondary shelf-life of foods in relation to food safety.
- To address knowledge gaps on the effects of thawing on injury/survival/growth of bacteria in order to develop study designs, including recovery on culture media, and protocols to apply when evaluating how pathogens will behave under these conditions, e.g. challenge test using *Listeria monocytogenes* to simulate its behaviour (current challenge test protocols are on chilled and refrigerated foods; the thawing process is not included).
- To carry out studies, using the challenge test protocols developed, in order to generate evidence-based advice on storage conditions (time and temperature) and food preparation after thawing for foods other than frozen vegetables (for which such criteria have already been published).

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Abbreviations

ALOP	appropriate level of protection
CFU	colony forming unit
DT	decision tree
FBO	food business operator
FSSP	Food Spoilage and Safety Predictor
FSO	food safety objective
FSMS	food safety management system
GHP	good hygiene practice
HACCP	Hazard Analysis and Critical Control Points
LAB	lactic acid bacteria
MAP	modified atmosphere packaging
QMRA	quantitative microbial risk assessment
RTE	ready-to-eat
SSO	specific spoilage organisms
ToR	Term of Reference
UHT	ultra-high temperature

Appendix A – Uncertainty analysis

Table A.1: Sources of uncertainty in the decision tree (DT) affecting the decision on the need for information on shelf-life, storage conditions and/or time limits for consumption after opening the food package

Uncertainties related to	Source or location of the uncertainty	Nature, or cause, of the uncertainty as described by the experts	Impact of the uncertainty on the decision if information on secondary shelf-life is appropriate and on the required storage conditions using the decision tree (direction[1] and magnitude[2])
Decision Tree	Assumption: Steps/questions included in the tree	An important step/question may be missing or an irrelevant one is included	Inconclusive/+
	Assumption: Growth potential based on few factors (pH and a_w) as the main determining factor	The potential to initiate growth may be less than that indicated in the tables in Q1 that rely on only two factors (pH and a_w) at optimum conditions	Overestimation/+ (but can be overcome with the correct use of Q2)
	Assumption: Inactivation at consumer stage not considered	Inactivation of hazards can take place in (non-ready to eat) foods at the consumer level (e.g. when foods are heat-treated).	Overestimation/+
	Structure: The relation between questions	The sequence of the questions may not reflect the relevant events that may take place and influence the outcome of the DT.	Inconclusive/+
Decision Tree	Data for limiting pH and a_w	The limits used in the data tables included in Q1 may not be representative for all relevant biological hazards	Underestimation/+ (Not considered likely/important except in case of emergence of hyper-tolerant strains, since the limiting pH and aw are based on the most tolerant vegetative cells or spores known) Overestimation/+

1: Underestimation, i.e. foods requiring secondary shelf-life would classify as not needing this information, Overestimation, i.e. foods not requiring a secondary shelf-life would classify as needing a secondary shelf-life information; Inconclusive, i.e. could influence in either way.
2: Assessment of the magnitude of the uncertainty using a three-level semi-quantitative scale from low to high importance (+, ++ or +++).

Appendix B – Guidelines on thawing

Australian Institute for Food Safety, 2017, 4 methods for defrosting food safely.pdf

Food redistribution in the EU: Austrian hygiene guidelines for industrial kitchens, healthcare facility kitchens and similar public catering facilities. Translated guideline -: LEITLINIE (europa.eu)

Food redistribution in the EU: translation of Belgium circular letter regarding the provisions applying to food banks and charities. Microsoft Word - 2017-09-14_circ-ob_Banques alimentaires_FR_V4.doc (europa.eu)

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Food Safety Authority of Ireland: Home cooking and storage. Temperature Control | FAQs | The Food Safety Authority of Ireland (fsai.i.e)

Food Safety Authority of Ireland: Businesses donation food to charities. The Food Safety Authority of Ireland (fsai.i.e)

EU_2017_C 361_01 Guidelines food donation, guidelines for freezing is given in chapter 5.4

FAO Draft Revised Cood of Practice Processing Handling Quick Frozen Foods pdf

Freeborn 2019 Thawing and Defrosting Food Safety.pdf

FSA Safe method-defrosting. Pdf

FSA. How to chill, freeze and defrost food safely https://www.food.gov.uk/safety-hygiene/chilling

FSANZ 2016 Food safety practices standard 3_2_2.pdf

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Food Safety Market. Infographic Thawing food safely.pdf

National Center for Home preservation, 2014, Freezing Thawing and Preparing Foods for Serving. Pdf

National Environment Agency, Guidelines on Thawing of Food guidelines-on-thawing-of-food.pdf (nea.gov.sg)

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