Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the establishment of shelf-life of certain ready-to-eat foods sliced or cut and packaged before sale in retail establishments

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Abstract

Royal Decree 126/2015, of 27 February, approving the general rule on food information on foodstuffs presented unpackaged for sale to the final consumer and to mass caterers, those packaged in places of sale at the request of the purchaser, and those packaged by retail trade operators (BOE, 2015), states that it is mandatory to mention, among other information, the date of minimum shelf-life or expiry date, in the case of foods packaged by retail trade operators for immediate sale in the establishment or establishments owned by them. The indication of dates on packaged foods is also regulated by Regulation (EU) No. 1169/2011 on the provision of food information to consumers (EU, 2011). The shelf-life

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of such products may vary depending on the type of food and method of preservation.

Accordingly, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has assessed the shelf-life, in relation to the possible presence of *Listeria monocytogenes*, of certain products (cheeses, cooked meat products, including *pâtés*, and cured meat products) which are divided up or sliced and packaged (vacuum packed or not) before sale, in retail establishments, in order to ensure consumer safety. In addition, for vacuum-packed foods, the potential risk due to the possible presence of *Clostridium botulinum*, as well as other possible biological hazards, has been taken into account.

For this purpose, factors associated with the presence and growth of biological hazards (water activity (a_w), pH, ripening time) have been established for each of the product categories considered and published predictive microbiology models have been used to establish shelf-life. Based on the available information, it is concluded that to control the risk of *L. monocytogenes*, the acceptable shelf-life for cooked meat products (such as cooked ham or cooked shoulder) stored at 4 °C with a_w levels of 0.995 or higher and pH of 6.71 or higher (worst case conditions assessed) is maximum 5 days for both air-packed and reduced oxygen atmospheres. For *pâtés* that are divided up, air-packed and stored at 4 °C, with a_w values of 0.988 or more and pH values of 6.5 or more is maximum 6 days. And in the case of divided up or sliced cheeses, with a maturation period of at least 2 weeks and less than 2 months, stored at 4 ºC, the admissible shelf-life is a maximum of 10 days, both for products conserved in the air and in reduced oxygen atmospheres. Products with a_w and pH values that limit the growth of *L. monocytogenes* (such as cured ham, *chorizo*, *salchichón* or cheeses matured for more than 2 months) can be stored at room temperature. The estimated shelf-life for these products would be related to parameters not directly associated with the growth of *L. monocytogenes*.

The manufacturer may establish alternative shelf-lifetimes to those reflected in the report if he is able to demonstrate to the competent health authority that the product has a combination of factors and/or packaging and storage conditions that ensure that the concentration of *L. monocytogenes* is less than 100 CFU/g at the time of consumption.

The risk associated with the presence of *Clostridium botulinum* in vacuum-packed products and enteric viruses has also been assessed and concluded that it would not affect the stated recommendations.

Finally, it is recommended that establishments ensure adequate hygienic and sanitary conditions during the slicing, cutting and packaging of food to prevent contamination by food-borne pathogenic organisms that may pose a risk to consumer health.

Key words

Sliced, cut, shelf-life, *Listeria monocytogenes*, *Clostridium botulinum*, virus.

Suggested citation

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1. Introduction

In retail food establishments it is a very common practice to carry out the fractionation or slicing of ready-to-eat products, such as meat products (sausage, cooked ham, sausage, etc.) and cheeses, which are subsequently delivered to the end consumer packaged in refrigerated showcases or at room temperature, and with a label indicating an expiry date or best-before date which, on occasion, is the same as that indicated in the product before handling it.

Royal Decree 126/2015, of 27 February, approving the general rule on food information on foodstuffs presented unpackaged for sale to the end consumer and to mass caterers, those packaged in places of sale at the request of the purchaser, and those packaged by retail trade operators (BOE, 2015), states that it is mandatory to state, among other information, the date of minimum shelf-life or expiry date, in the case of foods packaged by retail trade operators for immediate sale in the establishment(s) owned by them.

Indicating dates on packaged foods is also regulated by Regulation (EU) No. 1169/2011 on the provision of food information to consumers (EU, 2011). This Regulation defines the date of minimum durability as the date until which the food retains its specific properties when stored correctly. In the case of microbiologically very perishable foodstuffs and which, therefore, may pose an immediate danger to human health, after a short period of time, the date of minimum durability shall be changed to the expiry date. After its "expiry date", food shall not be considered safe within the meaning of paragraphs 2 to 5 of Article 14 of Regulation (EC) No. 178/2002 which sets out the general principles and requirements of food law, establishing the European Food Safety Authority and setting out procedures in matters of food safety (EU, 2002).

Accordingly, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) is requested to report on the shelf-life, in relation to *Listeria monocytogenes,* of certain products (cheeses, cooked meat products (including *pâtés*) and cured meat products) which are split or sliced and packaged (vacuum packed or not) before sale in a retail establishment. In particular, it is requested to assess how the shelf-life of the food, established by the processing company, is affected when sliced or divided up and packaged in the retail trade.

In the case of divided or sliced foods that are vacuum-packed, it is also requested that the Scientific Committee of AESAN evaluate the potential risk due to the presence of *Clostridium botulinum* in these foods, and, in addition, the risk due to enteric viruses will be studied.

It is also requested that the conditions under which these divided and packaged foodstuffs must be kept until the time of sale (storage temperature) be determined to ensure their safety.

2. Physicochemical characteristics of the food to be considered and recommended storage temperature

The following foods, representative of divided and/or sliced foods which are packaged (vacuum packed or not) before sale in retail establishments, will be assessed: cheeses, cooked meat products (including *pâtés*) and cured meat products. It will also set the storage temperature up to the time of sale.

These foods have the following physical-chemical characteristics, which determine whether or not the proliferation of microorganisms, is possible and, therefore, their shelf-life.

2.1 Cheeses

According to the definition provided by the standard of *Codex Alimentarius* CXS 283-1978 (CA, 2021), cheese is defined as the soft, semi-hard, hard and extra-hard product, matured or not matured, and which can be coated, in which the ratio between whey proteins and casein does not exceed that of milk, obtained by methods of coagulation of milk proteins. The *Codex* classification of the different types of cheeses is based on the percentage of moisture without fat or % of HSMG, distinguishing between extra-hard (<51 %), hard (49-56 %), firm or semi-hard (54-69 %) and soft (>69 %). In addition, depending on the percentage of fat (% MG), the standard distinguishes between extra-fat (>60 %), fat (45-60 %), semi-fat (25-45 %), semi-skimmed (10-25 %) and skimmed (<10 %) cheeses.

From the point of view of food safety, the most relevant factors for the categorization of cheeses are the pH values and water activity (a_w), as well as the heat treatment of the source milk (raw or pasteurised) and the ripening time. Other technological factors, such as fermentation or the addition of starter cultures, may affect the development of food-borne pathogens throughout their useful life (Possas et al., 2022). In relation to the physicochemical parameters, these products have a wide range of conditions from a_w, from values around 0.88 for cured cheeses, to values of 0.99 for fresh cheeses. The pH values may range from 5.0 to 6.8.

For the development of this report, the following categorisation will be taken into account, in order to estimate the shelf-life of sliced and divided products:

- Fresh and short-ripened cheeses: those that have not undergone any ripening process or in which the ripening time is less than 2 weeks. Examples: "Queso de Burgos", "Mozzarella", "Feta" or "Quark".
- Intermediate ripening cheeses: those that have a ripening time of between 2 weeks and 2 months. Examples: tender or semi-cured cheeses of pasteurized milk, such as "Gouda", "Emmental", "Gruyère" and "Manchego" semi-cured cheese, among others.
- Cured cheeses: those that have ripened for more than 2 months up to several years. Examples: raw milk cheeses with a ripening time exceeding 60 days, such as cured "Manchego Cheese", "Cheddar", "Parmigiano-Reggiano", "Pecorino Romano" or old cheeses, among others.

Table 1. Moisture (%, v/v), NaCl (%, v/v) and pH values of some types of cheese

Cheeses	Humidity (% v/v)	NaCl (% v/v)	pH
Blue	42	4.5	6.5
Goat's cheese	40	5.6	5.3
Camembert	52	2.5	5.0
Cheddar	37	1.5	5.5
Domiati	55	6.0	4.6
Edam	43	2.0	5.7
Emmental	35	0.7	5.6
Feta	53	3.0	4.5
Gouda	41	2.0	5.8
Grana/Parmesan	31	2.6	5.4
Gruyère	33	1.1	5.7
Idiazabal	29	6.9	5.7
Pecorino Romano	23	5.5	5.4
Provolone	42	3.0	5.4
Roncal	29	6.1	5.5
Roquefort	40	3.5	6.4
Tetilla	42	3.6	5.2
Arzúa - Ulloa	46	3.2	5.4
Villalón	56	3.3	6.7

Adapted from: Esteban et al. (1979) and Fox et al. (2004).

2.2 Cooked meat products

This type of products comprises those meat derivatives made with meat, to which blood, fat or giblets can be added, which have been subjected in their manufacture to a heat treatment sufficient to achieve, in their internal part, a partial or total coagulation of their proteins; additionally they can be subjected to smoking and maturation treatments (BOE, 2014).

The most representative product in this category is cooked ham which has a pH close to neutral (~6.0) and a high a_w (>0.9), which allows the proliferation of food-borne pathogens such as *Listeria monocytogenes* (Aymerich et al., 2005).

Table 2. a_w and pH values for various cooked products that are sold or can be sold sliced		
Commercial name	**a_w**	**pH**
Cooked pork ham	0.999	6.28
	0.978	6.57
Extra-cooked pork ham	0.988	6.14
	0.967	6.09
Smoked cooked pork ham	0.974	6.25
Lean cooked pork ham	0.974	6.49
Roasted boneless pork ham	0.971	6.61
Cooked pork shoulder	0.974	6.75
Turkey breast	0.977	6.24
	0.971	6.63
	0.971	6.51
	0.970	6.50
Turkey ham	0.974	6.36
Chicken breast	0.973	6.52

Adapted from: Romero de Avila et al. (2014). Each value represents a minimum of between 4 and 8 samples.

Within this group are *pâtés* (for cutting or spreading), which are defined as meat pastes, pasteurised or sterilised, made from meat or liver, or both, to which can be added giblets and other ingredients, condiments and additives and which have been subjected to a chopping process (BOE, 2014).

This type of products have values of a_w and pH very similar to those presented by the group of cooked hams (Table 3).

Table 3. Values of a_w and pH for different types of *pâtés*		
Commercial name	**a_w**	**pH**
Foie gras	0.971	6.27
Fine herb *pâté*	0.989	6.23
Country-style *pâté*	0.976	6.44
Roquefort *pâté*	0.981	6.40

Adapted from: Fernández-Salguero et al. (1993).

2.3 Cured meat products

According to Royal Decree 474/2014, cured or non-heat-treated meat derivatives are those products made with meats or meats and fat, as well as other food products, which have not undergone any treatment during production nor, have they been subjected to a curing-maturation process,

accompanied or not by fermentation, earing, marinating-pickling or other non-thermal technological process, sufficient to confer their own organoleptic characteristics (BOE, 2014).

Therefore, most cured meat derivatives do not undergo any heat treatment, meaning that they are usually consumed raw (FAO, 2010). Nowadays, you can find a wide variety of products that are marketed as whole pieces, or sliced and packaged (Toldrá, 2015). From a microbiological point of view, they are products that could be considered safe, depending on the pH levels (4.6-5.3) and a_w (≤0.92) due to the fermentation and salting processes.

There are, in the literature, several classifications of raw-cured meat derivatives, which vary from one country to another. The classification proposed by Roca and Incze (1990) is considered in Table 4, taking into account the fermentation and maturation time.

Table 4. Classification of cured meat products

Type of meat derivative	Production time	Final water content (%)	Final value of a_w
Spreadable	3-5 days	34-42	0.95-0.96
Sliceable: • Short maturation • Long maturation	 1-4 weeks 12-14 weeks	 30-40 20-30	 0.92-0.94 0.85-0.86

Source: Roca and Incze (1990).

Other criteria proposed from a microbiological point of view consider the **a_w** of the product, as well as the application of surface treatment. Thus, Lücke (2003) considers a classification based on the following groups:

- Dried meat product (with or without mould, with or without smoking): final a_w: <0.90.
- Semi-dry meat derivative (with or without mould): final a_w: 0.90-0.95.
- Fresh fermented meat derivative spread: final a_w: 0.94-0.97.

Other classification criteria are based on acidity, degree of chopping of ingredients, addition or non-addition of starter cultures, addition of one or more other ingredients, spices and condiments, or moisture/protein ratio. All these factors decisively influence the general characteristics of the products, meaning that they would be valid classification parameters since they involve various manufacturing technologies (Rubio, 2014).

In this way, cured meat derivatives of low acidity can be found, whose final pH ranges between 5.3 and 6.2 (Aymerich et al., 2006), where low-temperature maturation is carried out (10-12 ºC). On the other hand, acidic cured meat derivatives (pH <5.3) are generally subjected to a fermentation temperature around 20-24 ºC although in certain cases it can increase to 37 ºC (Ordoñez and de la Hoz, 2001).

The temperatures considered in this report have been 4 and 8 ºC for refrigerated products and an ambient temperature of 22 ºC for products preserved without refrigeration.

Table 5. Average a_w and pH values of some cured raw meat products		
Commercial name	**a_w**	**pH**
Cecina	0.859	5.92
Chorizo extra	0.872	5.33
Chorizo primera	0.894	5.34
Cured ham	0.909	5.99
Dry cured pork loin	0.883	5.78
Morcón	0.817	5.35
Salami extra	0.887	4.87
Salami primera	0.846	5.11
Salchichón extra	0.879	5.01
Salchichón primera	0.850	5.43
Salchichón segunda	0.801	4.84
Salchichón tercera	0.784	4.83
Sobrasada	0.828	4.72

Adapted from: Fernández-Salguero (1995).

3. Shelf-life of divided or sliced foods in relation to *Listeria monocytogenes*

Listeria monocytogenes is a psychotrophic pathogenic microorganism, gram positive and generally opportunistic, which has been the subject of numerous studies due to its relationship with multiple food toxins worldwide (Buchanan et al., 2017). *L. monocytogenes* is widely distributed in the environment, and can survive or grow under limiting pH, humidity, salinity conditions and in environments with low oxygen availability (Fernández et al., 1997). In addition to being present in soil, air and aquatic environments, it can reside in the intestinal tract of the host without presenting any apparent symptoms (Buchanan et al., 2017). According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1996), the minimum growth values of *L. monocytogenes* range from -2 to 4 ºC in temperature, 4.2-4.3 in pH and 0.90-0.93 in a_w. The lower limit for the growth of *L. monocytogenes* in environments with a high nutrient content and neutral pH is close to 0 ºC (ICMSF, 1996), despite the fact that growth at temperatures of -1.5 ºC has been observed in a cooked and vacuum-packed meat product (Hudson et al., 1994).

The disease transmitted by *L. monocytogenes*, called listeriosis, can occur due to the intake of contaminated foods, especially ready-to-eat (RTE) foods that have been exposed to inadequate post-processing conditions after previous treatment in the industry (Fang et al., 2013). Meat products, as well as cheeses, both classified as RTE foods, have been implicated in several outbreaks of food-borne listeriosis (Nastasijevic et al., 2017) (Martínez-Ríos and Dalgaard, 2018).

Cross-contamination processes in industry have frequently been linked to the presence of *L. monocytogenes* (Pérez-Rodríguez et al., 2010). It should be noted that the transmission of the pathogen can occur throughout the production of RTE foods, as well as through equipment and processing

environments that are contaminated (Gounadaki et al., 2008) (Giaouris et al., 2013). In fact, other published studies have shown that slicing machines can constitute important reservoirs of *L. monocytogenes*, that can be transferred to finished products (Lin et al., 2006) (Vorst et al., 2006) (Borovic et al., 2014). For this reason, contamination of the final product should be avoided since RTE foods do not undergo any listericidal treatment prior to consumption.

In this regard, the approaches for the control of *L. monocytogenes* in food can vary from a zero tolerance (absence in 25 g of sample), to a maximum level of 100 CFU/g if the manufacturer demonstrates that the product does not tolerate the growth of the pathogen throughout its useful life (Farber et al., 2021).

Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (EU, 2005) sets out microbiological criteria for *L. monocytogenes* in RTE foods, determining its market acceptability. Specifically, the food safety criteria apply to both meat products and cheeses, both of which are considered RTE products. The categorisation of RTE foods is based on whether they can tolerate (category 1.2) or not (category 1.3) the growth of *L. monocytogenes* over their shelf-life (Table 6). Foods with a pH ≤4.4 or an a_w of ≤0.92, products with a pH ≤5.0 and a_w ≤0.94, and products with a shelf-life of less than 5 days are generally considered not to tolerate the growth of *L. monocytogenes*, meaning that they would be in category 1.3. Other RTE foods can be included in this category if there is scientific evidence that they do not tolerate the growth of the pathogen.

Table 6. General scheme of food safety criteria for *L. monocytogenes* in RTE foods according to Regulation (EC) No. 2073/2005 (EU, 2005)

Food category	Sampling plan		Límits		Analytical reference method	Phase in which the criterion is applied
	n	c	m	M		
1.1 Ready-to-eat foods for infants and ready-to-eat foods for special medical purposes [1]	10	0	Not detected in 25 g		EN/ISO 11290-1	Products marketed during their shelf-life
1.2 Ready-to-eat foods that may promote the development of *L. monocytogenes*, other than for infants or for special medical purposes	5	0	100 CFU/g[2]		EN/ISO 11290-2[3]	Products marketed during their shelf-life
	5	0	Not detected in 25 g[4]		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator who produced it
1.3 Ready-to-eat foods that cannot promote the development of *L. monocytogenes*, other than those intended for infants or for special medical purposes [1] [5]	5	0	100 CFU/g		EN/ISO 11290-2[3]	Products marketed during their shelf-life

[1] Under normal circumstances, regular testing with regard to this criterion is not required for the following ready-to-eat foodstuffs:
- those that have undergone heat treatment or other effective process to remove *L. monocytogenes*, where recontamination is not possible following this treatment (e.g., heat-treated products in their final packaging),
- fresh fruit and vegetables, whole and unprocessed,
- bread, biscuits and similar products,
- bottled or packaged water, non-alcoholic soft drinks, beer, cider, wine, spirit drinks and similar products,
- sugar, honey and sweets, including cocoa and chocolate products,
- live bivalve molluscs,
- cooking salt.

[2] This criterion applies if the manufacturer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g during its shelf-life. The operator may set intermediate limits during the process that should be low enough to ensure that the limit of 100 CFU/g at the end of the shelf-life is not exceeded.

[3] On a Petri dish of 140 mm in diameter or three Petri dishes of 90 mm in diameter is seeded 1 ml of inoculum.

[4] This criterion applies to products before they have left the immediate control of the food business operator when the latter cannot demonstrate to the satisfaction of the competent authority that the product will not exceed the limit of 100 CFU/g during its shelf-life.

[5] Products with a pH ≤4.4 or an a_w ≤0.92, products with a pH ≤5.0 and a_w ≤0.94, and products with a shelf-life of less than 5 days are automatically considered to belong to this category. Other product categories may also belong to this category, provided that they are scientifically justified.

In the case of meat and cheese products, several authorities at international level classify different categories according to the potential risk of transmission of listeriosis through consumption. For example, in the case of cheeses, the U.S. Food and Drug Administration classifies them based on moisture content (FDA, 2003). In this sense, it is considered that the intake of fresh cheeses can carry an increased risk of listeriosis, compared to the intake of matured cheeses with both short

and prolonged ripening. In meat products, Garrido et al. (2010) did a challenge test on cooked ham contaminated with *L. monocytogenes* at a low inoculum level, below 10 CFU/g, to simulate a retail level contamination. These authors used two storage temperatures, 5 and 9 ºC, reaching 100 CFU/g of *L. monocytogenes* in 3 days and 2 days respectively, which highlights the need to point out that these foods have to be stored at temperatures below 5 ºC if they are to have a shelf-life of at least 5 days.

However, as shown in the *Guidance Document for the verification of shelf-life studies in relation to Listeria monocytogenes in ready-to-eat foods*, prepared by AESAN, it is possible to carry out challenge studies to demonstrate that an RTE food does not tolerate the growth of *L. monocytogenes,* even when it can present theoretical pH values and an a_w compatible with the development of the pathogen (AESAN, 2019).

3.1 Use of predictive microbiology models for the estimation of the safe shelf-life of RTE foods divided or sliced and packaged at point of sale in relation to *L. monocytogenes*

Predictive microbiology is currently one of the most valuable tools for estimating shelf-life safely. In RTE foods, as reflected in Regulation (EC) No. 2073/2005 of 15 November (EU, 2005), the manufacturer may demonstrate, to the satisfaction of the competent authority, the shelf-life of RTE foods based on the use of mathematical models.

Although there are several definitions in the scientific literature, predictive microbiology can be considered a branch of food microbiology that allows to quantify and predict the responses of a population of microorganisms to different factors that affect it (Van Impe et al., 1995). In the case of RTE foods, as described in previous sections, different microbiological criteria can be applied depending on the growth tolerance of the pathogen. Within the products considered in this report, there are some typologies that, by their very nature or formulation, as well as by the conditions of handling, packaging and storage, may be more susceptible to the growth of *L. monocytogenes* throughout the shelf-life. In contrast, formulations of other product categories can be considered to inhibit their growth.

For the estimation of shelf-life, a distinction will be considered between categories of RTE foods that may favour the growth of *L. monocytogenes*, such as cooked meats, *pâtés* and fresh and short-ripened cheeses. In these types of foods, predictive microbiology models will be applied to estimate the time required to reach 100 CFU/g, which will serve as a reference for the calculation of the shelf-life. It is in this RTE food group that the application of predictive models in this section will be focused.

On the other hand, for other categories of RTE foods, such as raw-cured meat products (for example, *serrano* ham) and cheeses with prolonged ripening, it will be assumed that they do not allow the growth of *L. monocytogenes*, the shelf-life being based on the performance of good hygienic practices, as well as on a microbiological control of the raw material, equipment and facilities (Valero et al., 2018) (Possas et al., 2019) (Possas et al., 2022). This fact is corroborated in previous work carried out on cheeses of prolonged ripening (for example, Cheddar, Parmesan), with times greater than

2 months and values of a_w less than 0.92 in which *L. monocytogenes* did not present growth (Ryser and Marth, 1987) (Yousef and Marth, 1990).

3.1.1 Categorisation of products

For the definition of scenarios, the following categories of RTE foods have been considered that may favour the growth of *L. monocytogenes*, as well as packaging conditions (air and reduced oxygen atmosphere or ROP (Reduced Oxygen Packaging)):

1. Cooked meat products: air-packed and ROP.
2. *Pâté*: air-packed.
3. Cheeses: air-packed and ROP.

The classification is based on a risk assessment study of *L. monocytogenes* in RTE foods published by Pérez-Rodríguez et al. (2017), where values of specific growth rates (μ_{ref}, h^{-1}) are collected for the aforementioned categories. The values of μ_{ref} were collected from a review of studies where the growth of *L. monocytogenes* was reported, for each product category and packaging conditions. In this way, it was possible to adjust a statistical distribution of normal type to the collected data of standardised μ_{ref} at a temperature of 5 ºC, in order to reflect the variability in the formulations and process conditions (Table 7). The starting scenarios are based on the results found by Pérez-Rodríguez et al. (2017) for the selected categories.

Table 7. Estimated mean, standard deviation (SD), minimum, and maximum values for the specific growth rate (μ_{ref}, h^{-1}) values at a reference temperature of 5 °C

Category	Average	SD	Minimum	Maximum
Cooked meat (air-packed)	0.022	0.018	0	0.087
Cooked meat (ROP)	0.026	0.019	0	0.087
Pâté (air-packed)	0.026	0.010	0	0.097
Cheeses (air-packed and ROP)	0.010	0.015	0	0.030

As for the packaging conditions, a distinction will be made for cooked meat, being considered an air packaging (i.e. in passive atmosphere) and under reduced oxygen conditions (ROP), which in turn includes vacuum packaging. In the case of *pâté*, there is not enough information on growth under ROP conditions, meaning that the estimate will be assumed only for those products packaged in air. For cheeses, no differences are assumed in the values of μ_{ref} for both packaging conditions, meaning that the estimates are valid for products packaged in air and ROP.

3.1.2 Definition of scenarios and selection of predictive models

The scenarios evaluated for the estimation of shelf-life time considered storage temperature, pH, a_w levels, as well as ripening time, in the case of cheeses.

The initial categorisation performed of the selected RTE products is shown in Table 8.

Table 8. Categorisation of cooked meat products and cheeses based on their pH and a_w and ripening time				
Categories	**T (ºC)**	**pH**	**a_w**	**Maturation time**
Cooked meat (air-packed)	4, 8 and 22	6.1-6.7	0.968-0.995	-
Cooked meats (ROP)	4, 8 and 22	6.1-6.7	0.968-0.995	-
Pâté (air-packed)	4, 8 and 22	6.2-6.4	0.971-0.988	-
Cheeses (air-packed and ROP)	4, 8 and 22	4.5-6.7	<0.920-0.995	2, 5 and 8 weeks

Firstly, based on the values collected in Tables 2 and 3, cooked meat products and *pâtés* were categorised based on pH values and a_w. For this purpose, the 2.5, 50 and 97.5 percentiles were calculated for the set of pH values and a_w so that the categories were subdivided according to their formulation as follows:

- Cooked meat (air-packed and ROP): category 1 (pH ≥6.7; a_w ≥0.995); category 2 (6.7 > pH ≥ 6.5; 0.995 > a_w ≥ 0.974); and category 3 (6.5 > pH ≥ 6.1; 0.974 > a_w ≥ 0.968).
- *Pâté* (air-packed): category 1 (pH ≥6.4; a_w ≥0.988); category 2 (6.4 > pH ≥ 6.3; 0.988 > a_w ≥ 0.979); and category 3 (6.3 > pH ≥ 6.2; 0.979 > a_w ≥ 0.971).
- Cheeses: were classified into three categories based on ripening time: fresh cheeses without ripening or short ripening (<2 weeks) and intermediate ripening cheeses (2 weeks-2 months). Cured cheeses with maturation times greater than 8 weeks are excluded from these categories, assuming that they do not allow the growth of *L. monocytogenes*, as previously shown.

For the estimation of the shelf-life, the calculated values of μ_{ref} 5 ºC were used and, from them, a secondary model was applied that allows estimating the growth of *L. monocytogenes* at other storage temperatures. To this end, the following equation was considered:

$$\mu_{ref}T = \mu_{ref}5\,^{\circ}C \left(\frac{T - T_{Min}}{5\,^{\circ}C - T_{Min}} \right)^2$$

Where μ_{ref}T is the value of the specific rate (h^{-1}) at the study temperature (4, 8 and 22 ºC), μ_{ref} 5 ºC is the specific rate (h^{-1}) at the reference temperature (5 ºC), and T_{min} is the theoretical minimum temperature value calculated for *L. monocytogenes,* which was estimated at -1.18 ºC (FDA, 2003). No extrapolation has been performed below the temperatures studied (up to 2 ºC), since this would increase the uncertainty of the estimates.

3.1.3 Estimation of shelf-life for *L. monocytogenes* in RTE foods

The shelf-life was calculated based on the time required to reach 100 CFU/g of *L. monocytogenes*, referred to as Time-To-Reach (TTR). The results obtained for the cooked meat products with air packaging are shown in Table 9.

Table 9. Increase time values of 2 logarithmic units/g (TTR (days), 100 CFU/g) of *L. monocytogenes* at the different temperatures, pH and a_w conditions selected for air-packed meat products

Cooked meat: air-packed		
T (ºC)	**Conditions**	**TTR 100 CFU/g (days) (scenario)**
4	a_w 0.995; pH 6.71	5.3 (a)
4	a_w 0.974; pH 6.50	11.6 (b)
4	a_w 0.968; pH 6.11	>25 (c)
8	a_w 0.995; pH 6.71	1.7 (d)
8	a_w 0.974; pH 6.50	3.7 (e)
8	a_w 0.968; pH 6.11	24.7 (f)
22	a_w 0.995; pH 6.71	0.3 (g)
22	a_w 0.974; pH 6.50	0.6 (h)
22	a_w 0.968; pH 6.11	3.9 (i)

As can be seen, the least permissive conditions for the growth of *L. monocytogenes* corresponded to a_w values of 0.968 and pH of 6.11 (scenario (c)), where the estimated shelf-life was greater than 25 days in storage at 4 °C. Storing the product at room temperature considerably increases the growth of *L. monocytogenes*, obtaining TTR values of less than 1 day in those formulations with pH >6.5 and a_w >0.974 (scenarios (g) and (h)).

The estimates provided by the models for the case of cooked meat packed in ROP provided lower TTR values, as shown in Table 10. This fact is due to the slightly higher values of μ_{ref} 5 ºC under reduced oxygen conditions, where *L. monocytogenes* can grow faster (Sant'Ana et al., 2013). However, at 4 °C, for scenario (c) (a_w 0.968 and pH 6.11) the estimated shelf-life is greater than 25 days. At room temperature (22 °C), the estimated shelf-life times are very short, 3 days or less (scenarios (g), (h) and (i) of Table 10).

Table 10. Increase time values of 2 logarithmic units/g (TTR (days), 100 CFU/g) of *L. monocytogenes* at the different conditions of temperature, pH and a_w selected for meat products with ROP

Cooked meat: ROP		
T (ºC)	**Conditions**	**TTR 100 CFU/g (d)**
4	a_w 0.995; pH 6.71	4.8 (a)
4	a_w 0.974; pH 6.50	10 (b)
4	a_w 0.968; pH 6.11	>25 (c)
8	a_w 0.995; pH 6.71	1.6 (d)
8	a_w 0.974; pH 6.50	3.2 (e)
8	a_w 0.968; pH 6.11	19.2 (f)
22	a_w 0.995; pH 6.71	0.3 (g)
22	a_w 0.974; pH 6.50	0.5 (h)
22	a_w 0.968; pH 6.11	3.0 (i)

In the *pâté* case, only passive atmosphere packaging was considered due to the paucity of information about vacuum or modified atmosphere conditions. It can be checked how the TTR values are higher at refrigeration temperatures (4 and 8 ºC) compared to those obtained in cooked meat. However, storage at 22 °C also produces a significant increase in growth with a consequent decrease in the TTR value (Table 11).

Table 11. Increase time values of 2 logarithmic units/g (TTR (days), 100 CFU/g) of *L. monocytogenes* at the different conditions of temperature, pH and a_w selected for the air-packed pâté

***Pâté*: air-packed**		
T (ºC)	**Conditions**	**TTR 100 CFU/g (d)**
4	a_w 0.988; pH 6.44	6.5 (a)
4	a_w 0.979; pH 6.34	10.6 (b)
4	a_w 0.971; pH 6.23	>25 (c)
8	a_w 0.988; pH 6.44	2.1 (d)
8	a_w 0.979; pH 6.34	3.4 (e)
8	a_w 0.971; pH 6.23	8.5 (f)
22	a_w 0.988; pH 6.44	0.3 (g)
22	a_w 0.979; pH 6.34	0.6 (h)
22	a_w 0.971; pH 6.23	1.4 (i)

Finally, for the cheeses, the estimated TTR values are shown in Table 12. It can be seen that the growth of *L. monocytogenes* is slower compared to products of meat origin. The types of cheeses with a longer ripening time have a longer shelf-life, being the TTR values >25 days at temperatures of 4 and 8 ºC.

Table 12. Increase time values of 2 logarithmic units/g [TTR (days), 100 CFU/g] of *L. monocytogenes* at the different conditions of temperature, pH and a_w selected for cheeses with air and ROP

Cheeses: air-packed and ROP		
T (°C)	**Conditions**	**TTR 100 CFU/g (d)**
4	Maturation: 2 weeks	9.9 (a)
4	Maturation: 5 weeks	20.9 (b)
4	Maturation: 8 weeks	>25 (c)
8	Maturation: 2 weeks	3.2 (d)
8	Maturation: 5 weeks	6.7 (e)
8	Maturation: 8 weeks	>25 (f)
22	Maturation: 2 weeks	0.5 (g)
22	Maturation: 5 weeks	1.1 (h)
22	Maturation: 8 weeks	10 (i)

The growth kinetics of *L. monocytogenes* in the different RTE foods are shown in Annex II.

3.1.4 Shelf-life recommendations for split or sliced and packaged products

In this report, the TTR values 100 CFU/g of *L. monocytogenes* have been calculated for each of the scenarios and RTE foods evaluated. It should be noted that the values obtained may have some variability due to the process conditions and formulations of the products, since they can sensitively affect the growth of *L. monocytogenes*.

Also, the estimates provided by the models have a certain margin of safety since it has not included the possible existence of a latency phase, so we can consider that it is a conservative approach.

Finally, the estimates provided refer to sliced or split products which have been repackaged after handling at the point of sale taking into account a food safety criterion. Therefore, apart from these estimates, possible losses in the quality of the product over its useful life should be assessed.

1. Cooked meat products:

   Storage at temperatures between 4 and 8 ºC is recommended for all types of products. Storage at room temperature for prolonged periods of time should be avoided as it may considerably increase the risk to the consumer.

   The shelf-life recommendations are provided for air-packed and ROP products together, given the small differences obtained in the estimates. Thus, the shelf-life would be 5 days at 4 ºC for products with high pH (6.7) and a_w (0.995) values (scenario (a) of both air-packed products and ROP, Tables 9 and 10). In those formulations with lower pH values (6.1-6.5) and a_w (0.974) they can be maintained for 10 days at 4 ºC or 3 days at 8 ºC. Finally, products with low a_w (0.968) and pH (6.1) can be maintained for >25 days and 19 days at temperatures of 4 and 8 ºC, respectively.

2. *Pâtés*:

   As with cooked meat products, storage at temperatures between 4 and 8 ºC is recommended for all types of products. In the case of *pâtés*, storage at room temperature of sliced products is not advisable since *L. monocytogenes* reaches 100 CFU/g in less than 36 hours.

   The shelf-life recommendations are provided for air-packed products. It is recommended that products with higher pH levels (6.44) and a_w (0.988) are kept at 4 ºC for a time not exceeding 6 days. Products with formulations of a_w more limiting to microbial growth (0.979) can be kept for 10 days at 4 °C, while those *pâtés* with a_w (0.971) can be stored at 4 °C for 25 days, or at 8 °C for 7 days.

3. Cheeses:

   Depending on the ripening time, cheeses would allow a longer shelf-life than meat-based RTE foods. However, it is recommended that those fresh cheeses (Burgos type) or with short ripening, less than 2 weeks (tender cheeses) are stored at 4 ºC for an approximate time of 8-9 days. Other types of cheeses with intermediate ripening times (5 weeks) such as semi-cured cheeses could be stored at 4 ºC for 15-20 days, or at 8 ºC for 6 days. Finally, those cheeses with 8 weeks of maturation, due to the decrease in the a_w, allow a storage of 25 days at 4 ºC and 8 ºC, while at 22 ºC they can be preserved for 9-10 days.

4. Shelf-life/risk in vacuum-packed foods in relation to the presence of *Clostridium botulinum* and other possible biological hazards

Refrigerated foods ready for consumption are often packaged under vacuum or in modified atmospheres with low oxygen pressure. This type of packaging restricts the growth of aerobic microorganisms and favours the growth of anaerobes. This may result in favourable conditions for the proliferation of *Clostridium botulinum*, with the risk of botulinum toxin production. This risk is higher in high-protein foods, such as raw-cured or cooked meat products, where a reducing environment results. *C. botulinum* proteolytic strains do not grow at temperatures below 10 °C and would not pose a risk if proper refrigeration is maintained. However, non-proteolytic strains (types B, E and F) are able to grow and produce toxin at temperatures of even 3.3 °C (Eklund et al., 1967).

Therefore, the risk that non-proteolytic strains of *C. botulinum* are able to grow and produce toxin has been assessed from models and literature data. Based on a model published by Fernández et al. (2001) in liquid laboratory medium and available in the ComBase tool (2023), which predicts the growth of these strains based on CO_2 concentration, cooling temperature, pH and NaCl, the probability of growth has been established. Thus, at 5 and 8 °C under optimal pH conditions and at low NaCl concentrations, it would grow above a logarithmic order in 280 h and 102 h, respectively. A growth of at least 100 times the initial number in *C. botulinum* is necessary for the formation of botulinum toxin, which is why a lower level of growth has been considered, so that there is no risk of the formation of said toxin.

In two other studies in which a meat medium was used under anaerobic conditions as a substrate (Fernández and Peck, 1997, 1999), to study the effect of prolonged heat treatments, growth at 5 and 8 ºC after 14 and 7 days, respectively, was observed in the non-heating controls. The presence of toxin was corroborated when growth was detected in the tubes using an ELISA technique. Therefore, based on these studies, it is considered that the shelf-life in products in which *C. botulinum* may be present with favourable conditions for its growth should be 11 days if a refrigeration temperature of 5 ºC is guaranteed, and 4 days if a temperature equal to or lower than 8 ºC is guaranteed.

The previous history of the products (possible heat treatment) and the presence or not of lysozyme in the food can vary the prediction. For example, lysozyme is abundant in egg white, which can be added as an antimicrobial (it has been described in some types of cheese) (Scott and Bernard, 1985).

C. botulinum proteolytic strains cannot grow at temperatures below 10 °C. Therefore, they would only pose a risk in those products stored at room temperature and with an anaerobic environment. This assumption would only occur in cured meat products, in which *C. botulinum* cannot be multiplied due to its a_w, the presence of curing salts and pH (Peck and Lund, 2013).

5. Risk assessment associated with viruses

In the case of contamination of viral origin, the hepatitis E virus (HEV) can be present in raw or undercooked pig products and liver, since this animal is its main reservoir (Kupferschmidt, 2016). For HEV, the effectiveness of the cooking and curing processes for the production of cooked or cured meat products is largely unknown. The lack of methods based on cellular cultivations for

determining of the infectivity of this virus has prevented extensive inactivation studies. According to published inactivation studies, an internal meat temperature of 71 °C for 20 minutes is necessary to completely inactivate the infectivity of HEV (Emerson et al., 2005) (Barnaud et al., 2012) (Imagawa et al., 2018). On the other hand, the incidence of this pathogen in cooked or cured meat products is very limited, because to date there is no standardised and validated methodology for its detection, although it will be available in the near future (ISO, 2023).

In the case of viruses, which do not grow on food, the slicing and cutting processes have no effect on their stability if they were present in the cooked/cured product.

Finally, it should be noted that it is common for ready-to-eat foods to be contaminated during preparation by an infected manipulator (AESAN, 2011). Therefore, the people handling these products, due to a lack of hygiene, can spread viruses, such as HEV, hepatitis A virus or norovirus.

6. Hygienic-sanitary conditions

Food safety is mainly ensured through a preventive approach, such as the adoption of good hygiene practices and the application of procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP). At each stage of production, processing and distribution of food, including retail sale, food business operators should take measures to ensure that the supply, handling and processing of raw materials and foodstuffs under their control are carried out in such a way as to comply with general hygiene rules in accordance with Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs (EU, 2004) and, in the case of retail establishments, with Royal Decree 1021/2022 of 13 December regulating certain hygiene requirements for the production and placing on the market of foodstuffs in retail establishments (BOE, 2022). In particular, they should comply with the applicable process hygiene and food safety criteria as set out in Regulation (EC) No. 2073/2005 (EU, 2005).

The shelf-life estimates made herein consider low contamination of the reference pathogens (both *Listeria monocytogenes* and *Clostridium botulinum*). However, if contamination were to occur higher than those considered, the limits of contamination by *L. monocytogenes* could be reached in shorter times than recommended, which would result in an unacceptable risk to the consumer. In the case of *C. botulinum*, it could even favour the synthesis of botulinum toxin.

Therefore, it is necessary to guarantee adequate hygiene-sanitary conditions, especially during the handling of food in the slicing or splitting stages. In this sense, equipment, utensils and installations that are in contact with food products must be kept clean and disinfected, in a good state of repair and must not be a source of contamination, avoiding, in all cases, cross-contamination between raw foods and those ready for consumption. In the case of *L. monocytogenes* it is necessary to take samples of the equipment and production areas, in order to detect their presence. This bacterium has the ability to form biofilms, the elimination of which can be more difficult.

In addition, all personnel responsible for the handling of this type of product must follow good hygiene practices in compliance with the provisions of the establishment's HACCP system. It is especially recommended to use clean protective clothing, as well as the use and change of gloves, accompanied by handwashing and cleaning and disinfection of equipment, utensils and surfaces in contact with food.

Conclusions of the Scientific Committee

Based on the available information and the results obtained for each of the biological hazards associated with ready-to-eat sliced products assessed in this report, the following conclusions can be drawn:

1. In relation to the risk due to the presence of *Listeria monocytogenes,* the shelf-life of split or sliced and packaged products (vacuum-packed or not) for retail sale is closely associated with the physicochemical characteristics of each food group. Therefore, different scenarios have been considered in this report related to pH values and a_w for meat products, and ripening time in the case of cheeses.
2. It is concluded that, based on the scientific information collected and estimates of the predictive models applied in this report, the acceptable shelf-life for cooked meat products (such as cooked ham or cooked shoulder) stored at 4 °C with a_w levels of 0.995 or higher and pH of 6.71 or higher (worst case conditions assessed) is maximum 5 days for both air-packed and oxygen-reduced atmospheres, as the presence of *L. monocytogenes* does not pose a significant microbiological risk.
3. It is concluded that the permissible shelf-life of *pâtés* that are divided up, air-packed and stored at 4 °C, with a_w values of 0.988 or more and pH values of 6.5 or more is maximum 6 days, since the presence of *L. monocytogenes* does not pose a significant microbiological risk.
4. The admissible shelf-life of divided up or sliced cheeses, with a maturation period of at least 2 weeks and less than 2 months, stored at 4 ºC, is a maximum of 10 days both for products conserved in the air and in reduced oxygen atmospheres, since the presence of *L. monocytogenes* does not pose a significant microbiological risk.
5. Products with a_w and pH values that limit the growth of *L. monocytogenes* (such as cured ham, *chorizo*, *salchichón* or cheeses matured for more than 2 months) can be stored at room temperature. In this type of product, the hygiene-sanitary conditions of the product, as well as the handling process at the point of sale are fundamental to minimize the microbiological risk. The estimated shelf-life for these products would be related to parameters not directly associated with the growth of *L. monocytogenes*.
6. Other alternative scenarios for the three product groups that may guide or orientate the establishment of shelf-life for *L. monocytogenes* have been envisaged in this report.
7. The manufacturer may establish alternative shelf-life times to those reflected in the report if it can demonstrate to the competent health authority that the product has a combination of factors and/or packaging and storage conditions that ensure that the concentration of *L. monocytogenes* is less than 100 CFU/g at the time of consumption.
8. The presence of *Clostridium botulinum* (both proteolytic and non-proteolytic strains), with a low prevalence in sliced and split products, would pose a risk only in products preserved under anaerobic conditions, depending on their pH and storage temperature. The estimated shelf-life would be much longer than that established for *L. monocytogenes*, meaning that it does not affect the indicated recommendations.
9. In the case of enteric viruses, which do not replicate in food, the slicing process has no effect on the stability of the same if they were present in the cooked/cured product. However, for

these pathogens, it is necessary, above all, to intercede in the hygiene conditions of the handlers, their equipment and utensils to avoid possible cross-contamination.

10. Finally, it is recommended that establishments ensure adequate hygienic and sanitary conditions during the processing (slicing, cutting and packaging) of food to prevent contamination by food-borne pathogenic organisms that may pose a risk to consumer health.

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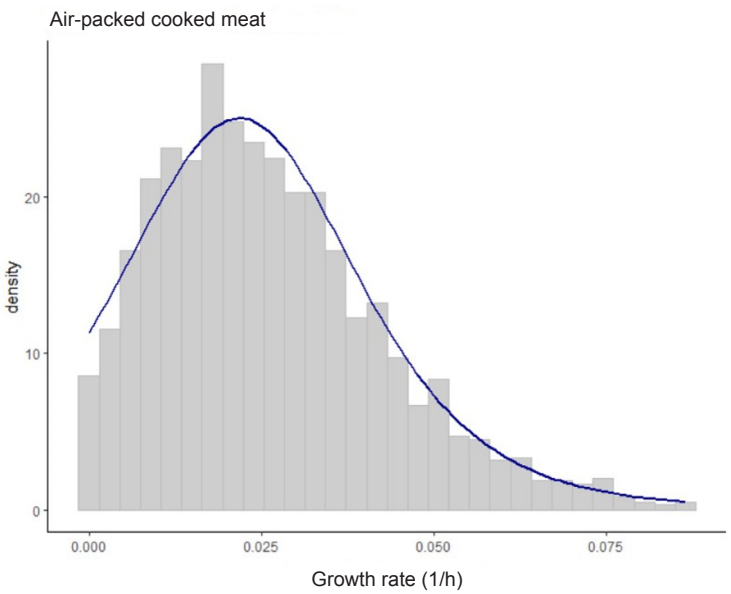
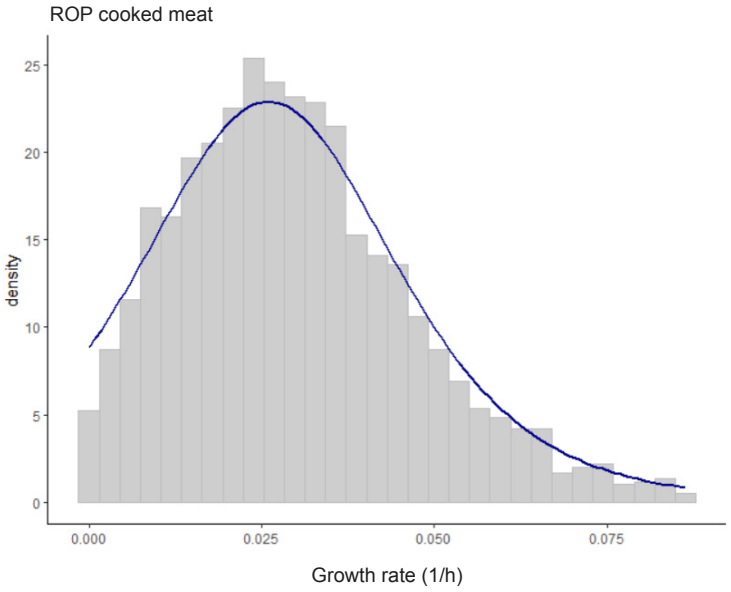
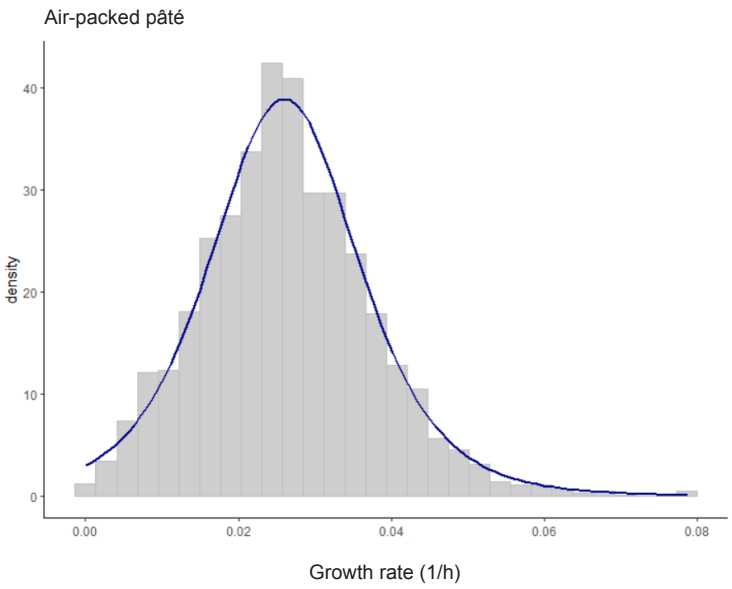
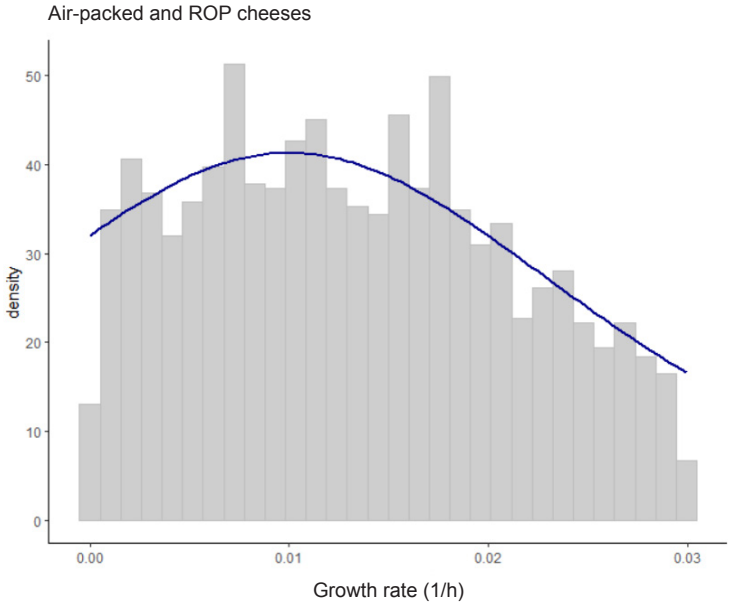
Annex I

Adjusted statistical distribution for values of μ_{ref} 5 ºC for RTE foods that may promote the growth of *L. monocytogenes*



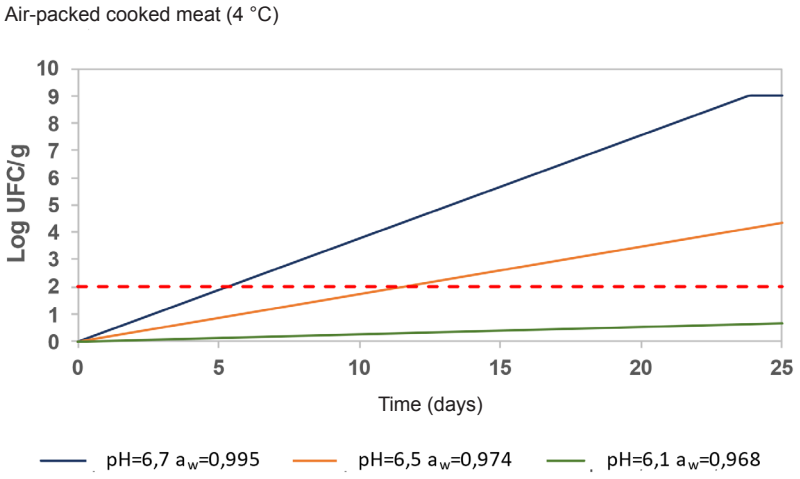


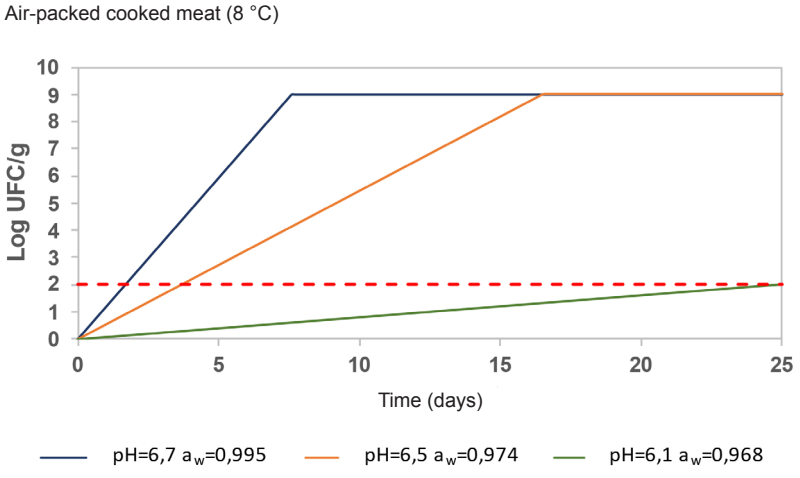

Air-packed pâté
density
0
10
20
30
40
0.00
0.02
0.04
0.06
0.08
Growth rate (1/h)


Air-packed and ROP cheeses
density
0
10
20
30
40
50
0.00
0.01
0.02
0.03
Growth rate (1/h)

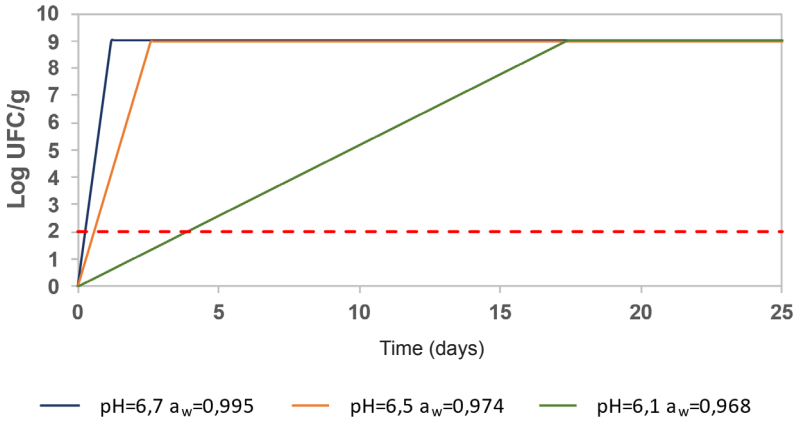
Annex II

Growth kinetics of *L. monocytogenes* in the different RTE foods

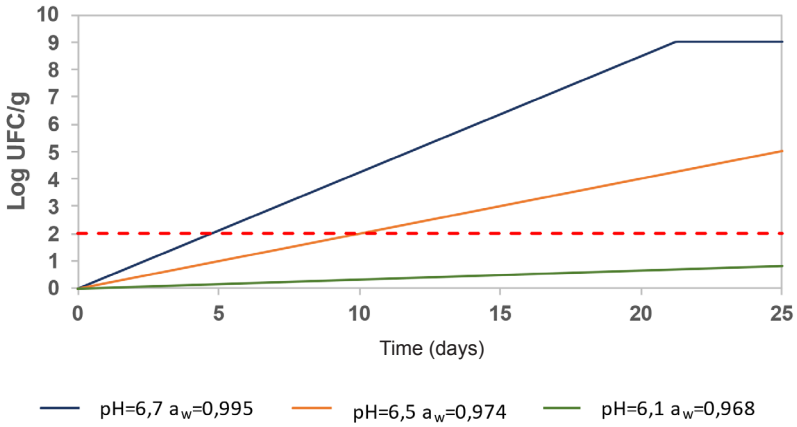




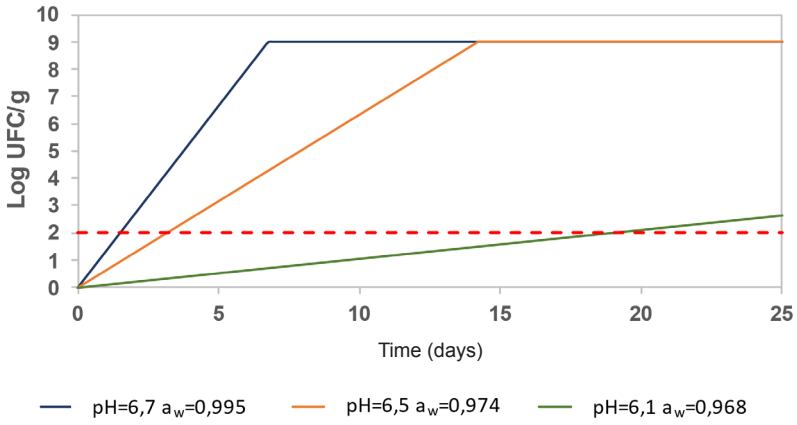
Air-packed cooked meat (22 °C)



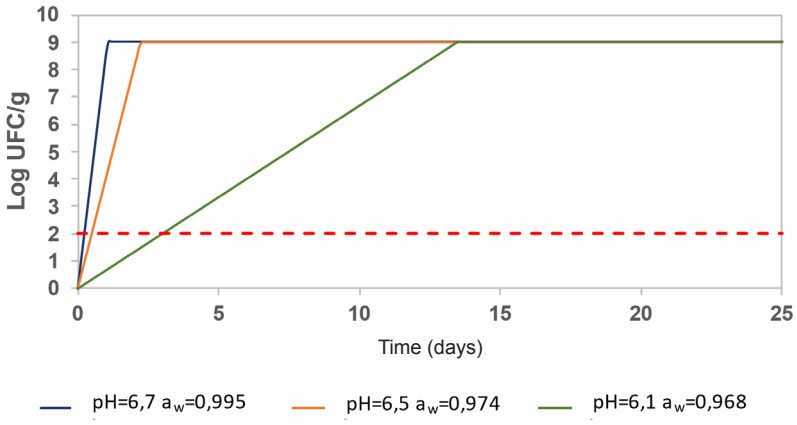
ROP cooked meat (4 °C)

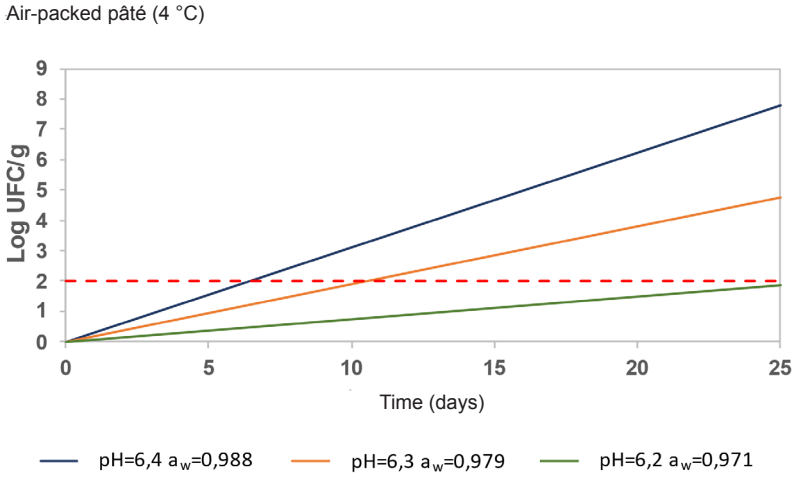


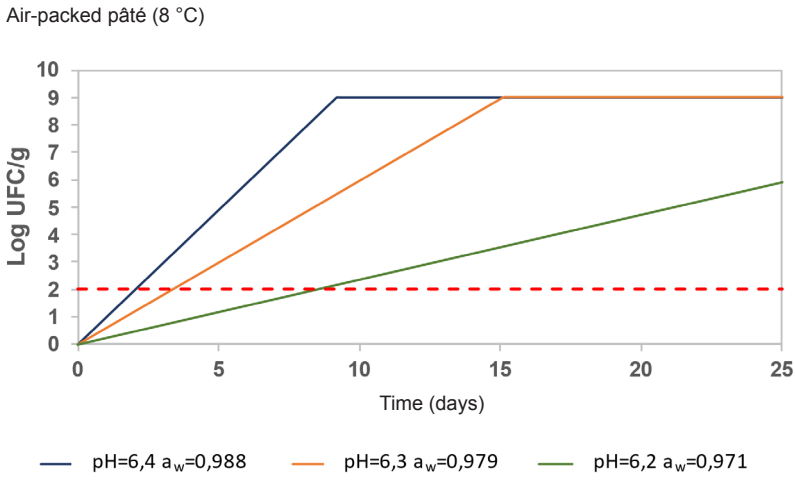
ROP cooked meat (8 °C)



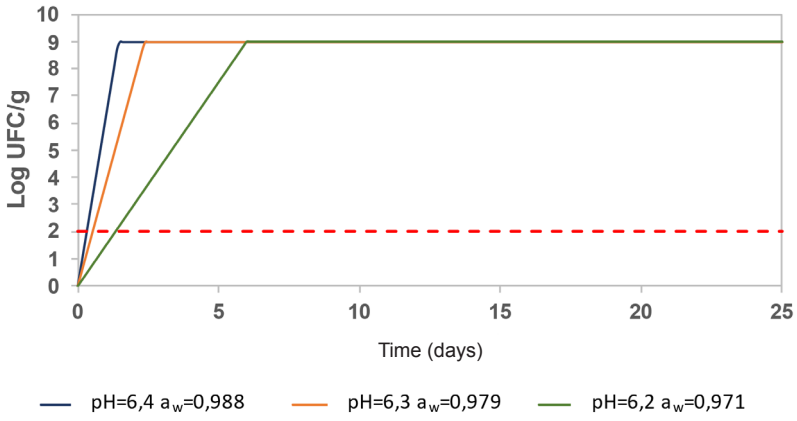
ROP cooked meat (22 °C)



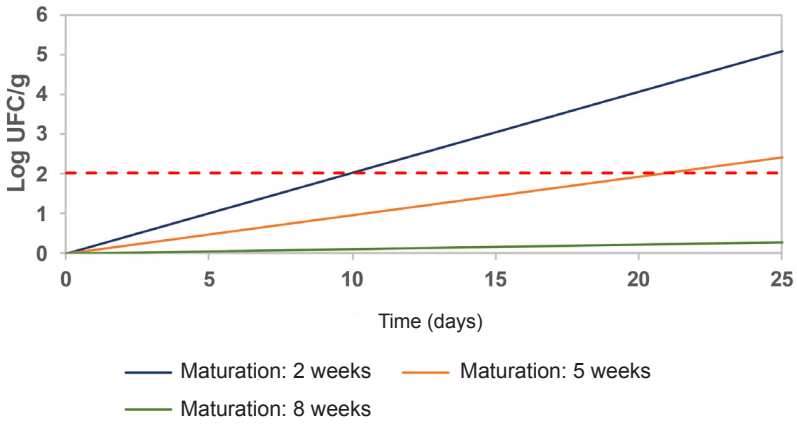

Air-packed pâté (4 °C)
Log UFC/g
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
Time (days)
pH=6,4 a_w=0,988
pH=6,3 a_w=0,979
pH=6,2 a_w=0,971

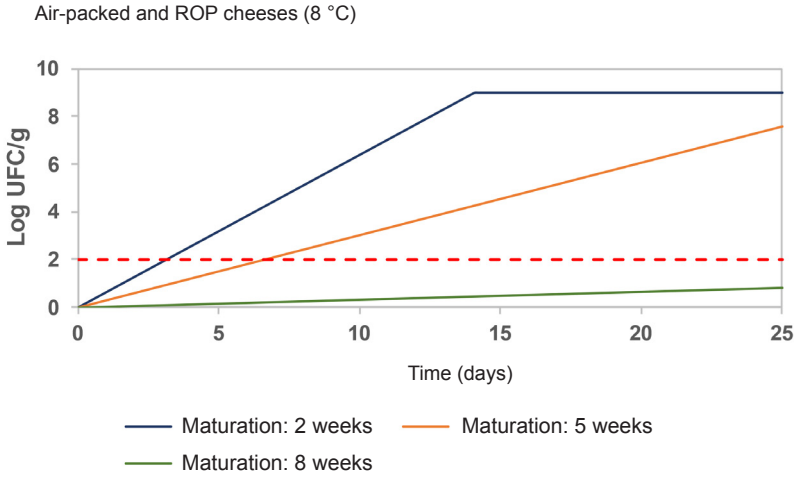

Air-packed pâté (8 °C)
Log UFC/g
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
Time (days)
pH=6,4 a_w=0,988
pH=6,3 a_w=0,979
pH=6,2 a_w=0,971

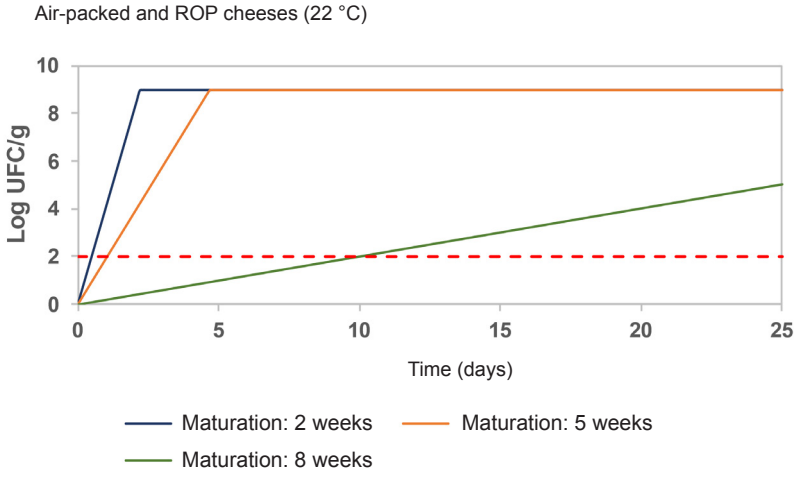
Air-packed pâté (22 °C)



Air-packed and ROP cheeses (4 °C)




Air-packed and ROP cheeses (8 °C)
Log UFC/g
10
8
6
4
2
0
0
5
10
15
20
25
Time (days)
Maturation: 2 weeks
Maturation: 5 weeks
Maturation: 8 weeks


Air-packed and ROP cheeses (22 °C)
Log UFC/g
10
8
6
4
2
0
0
5
10
15
20
25
Time (days)
Maturation: 2 weeks
Maturation: 5 weeks
Maturation: 8 weeks