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A retail prevalence study to investigate microbiological contamination levels in ready-to-eat (RTE) plant-based dairy, meat and fish substitutes

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Abstract

This comprehensive report investigates the microbiological contamination of ready-to-eat (RTE) plant-based dairy, meat and fish substitutes, reflecting the growing market and consumer interest in alternative protein sources. The primary objective was to assess the prevalence and levels of key microbial indicators and pathogens in these products, thereby evaluating their safety and potential public health risks. A systematic sampling strategy was employed, encompassing a diverse range of commercially available RTE plant-based substitutes. Samples underwent rigorous microbiological analysis, including enumeration of total viable counts, Enterobacteriaceae, Listeria monocytogenes, Salmonella spp. and other relevant microorganisms, following standardised laboratory protocols. The results revealed variable levels of microbial contamination across product categories, with certain samples exceeding established safety thresholds for specific pathogens or hygiene indicators. Notably, the occurrence of Listeria monocytogenes and Salmonella spp. was rare but highlighted the necessity for continued vigilance in production and supply chains. The study identified factors influencing contamination, such as product formulation, processing conditions and storage practices, underscoring the importance of robust food safety management systems tailored to plant-based RTE products. In conclusion, while the majority of RTE plant-based substitutes demonstrated satisfactory microbiological quality, isolated instances of elevated contamination levels warrant attention from both industry and regulatory authorities. The findings support the need for ongoing monitoring, targeted risk assessment and the development of specific guidelines to ensure the safety of plant-based RTE foods. This report provides essential data to inform policy, guide industry best practices and protect public health as the sector continues to expand.

Keywords: Dairy and meat alternatives, food safety, microbiological contamination, pathogens, plant-based substitutes, ready-to-eat foods, RTE foods

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FINAL REPORT

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This microbiological prevalence survey was conducted in collaboration with 15 Member States (listed below) and was funded by the European Food Safety Authority (EFSA) Focal Point Tailor-made Activities (TMA). All project partners would like to express their appreciation and thanks to the EFSA Focal Points for their support and to EFSA for funding this project.

Country		Country symbol	Organisation
1	Ireland (Lead organisation)	IE	Food Safety Authority of Ireland (FSAI)
2	Austria	AT	AGES – Austrian Agency for Health and Food Safety (AGES)
3	Belgium	BE	Research group Food Microbiology & Food Preservation, Faculty of Bio-Science Engineering, Ghent University (UGent)
4	Croatia	HR	Croatian Agency for Agriculture and Food (HAPIH), Centre for Food Safety
5	Cyprus	CY	State General Laboratory, Food Microbiology Laboratory
6	Denmark	DK	(1) Research Group for Food Microbiology and Hygiene at the National Food Institute of Denmark part of the Technical University of Denmark (2) The Danish Veterinary and Food Administration (DVFA)
7	Estonia	EE	(1) Food Safety Department, Ministry of Regional Affairs and Agriculture (2) National Centre for Laboratory Research and Risk Assessment (LABRIS)
8	Finland	FI	Risk Assessment Unit and Microbiology Unit, Finnish Food Authority
9	France	FR	ANSES (French agency for food, environmental and occupational health & safety)
10	Germany	DE	German Federal Institute for Risk Assessment (BfR)
11	Latvia	LV	Institute of Food Safety, Animal Health and Environment (BIOR)
12	The Netherlands	NL	Netherlands Food and Consumer Product Safety Authority (NVWA)
13	Slovakia	SK	The State Veterinary and Food Institute
14	Spain	ES	(1) Spanish Food Safety and Nutrition Agency (AESAN) (2) Department of Food Science and Technology, University of Cordoba
15	Sweden	SE	(1) The Swedish Food Agency (Livsmedelsverket) (2) Swedish University of Agricultural Sciences

Abbreviations

Term	Text
AESAN	Spanish Food Safety and Nutrition Agency
AGES	Austrian Agency for Health and Food Safety
ANSES	French agency for food, environmental and occupational health & safety
a_w	water activity
BCES	*B. cereus* spores
BfR	German Federal Institute for Risk Assessment
BIOR	Latvian Institute of Food Safety, Animal Health and Environment
CCP	Critical Control Point
CFU/g or ml	Colony forming units per gram or millilitre
CPS	Coagulase-positive staphylococci
DVFA	The Danish Veterinary and Food Administration
EU	European Union
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organisation of the United Nations
FSAI	Food Safety Authority of Ireland
GHP	Good Hygiene Practices
HACCP	Hazard Analysis and Critical Control Point
HAPIH	Croatian Agency for Agriculture and Food
HRM	Heat-resistant moulds
HRTS	Heat-resistant thermophilic spore formers
LABRIS	Estonian National Centre for Laboratory Research and Risk Assessment
LOD	Limit of detection
MALDI-ToF	Matrix assisted laser desorption ionization-time of flight
NVWA	Netherlands Food and Consumer Product Safety Authority
PRP	Prerequisite requirements
RASFF	Rapid Alert System for Food and Feed
RTE	Ready-to-eat
STEC	Shiga toxin-producing *E. coli*
Spp.	species
SRCS	Sulphite reducing *Clostridium* spores
SRAB	Sulphite-reducing anaerobic bacteria
TAMBC	Total aerobic mesophilic bacteria count
TBX	Tryptone-Bile-X-Glucuronate Agar
TMA	Tailor-Made Activities
TVC	Total viable count
TYMC	Total yeast and mould
UK	United Kingdom
UKHSA	United Kingdom Health Security Agency
WHO	World Health Organisation

Executive summary

Survey objectives

The growing popularity of ready-to-eat (RTE) plant-based dairy and meat substitutes has increased the emphasis on their food safety and the potential emergence of associated risks. Foodborne outbreaks caused by *Listeria monocytogenes, Salmonella* spp., and *Bacillus cereus* have been attributed to RTE plant-based cheese made from nuts (mostly cashew) and chilled plant-based dairy milk substitutes (oat, almond and coconut-based) (Table 1). Previous recalls and outbreaks of foodborne illness linked to RTE plant-based meat and dairy substitutes, highlight that some food business operators producing these types of products may lack the necessary expertise to identify relevant food safety hazards. The manufacturing processes for some of these plant-based products usually differ from the traditional time-temperature methods used for the animal-based products they replace, such as RTE cooked deli meats, pasteurised dairy milk, and cheese. As a result, microbiological hazards may be present in these foods that are not necessarily eliminated by specific control measures, such as heat treatment during production.

Understanding emerging risks associated with these foods is essential for ensuring their microbiological safety and limiting the risk of foodborne illness to public health. While the range of RTE plant-based dairy, meat and fish substitutes available in the EU market is expanding, there remains a lack of comprehensive studies in the existing literature assessing potential new or emerging food safety risks associated with these products. A literature review was conducted as part of the present survey (Section 1) to compile all available information on food safety hazards and risks related to both RTE and non-RTE plant-based dairy, meat and fish substitutes. This product category is experiencing increased consumer demand. However, while consumption of these products continues to grow, the current evidence base regarding potential microbiological food safety risks is limited. The objective of the literature review was to evaluate the potential for new or emerging risks associated with these RTE plant-based products.

Despite the increasing popularity of plant-based foods, notable research gaps remain in the literature, particularly concerning food safety and emerging risks. To address the knowledge gap, this large European survey funded by the EFSA Focal Point TMA initiative was carried out to examine the microbiological quality of RTE plant-based dairy (milk, cream and cheese), meat and fish substitutes, across 15 different countries (Sections 2 to 4). The aim of the survey was to determine the potential for new or emerging microbiological food safety risks that could be linked to the consumption of RTE plant-based dairy, meat and fish substitutes. The evidence gathered by the survey was used to underpin best practice recommendations and risk communication advice regarding the production of RTE plant-based products to food industry stakeholders, regulators and consumers (Sections 5 and 6).

Literature review

A comprehensive literature review was conducted to examine published research on the microbiological contamination of both RTE and non-RTE plant-based dairy (milk, cream, and cheese), meat and fish substitutes. Additionally, food incident notifications, recalls, and outbreak reports involving microbial pathogens in RTE plant-based substitutes from 1st January 2010 to 1st May 2025 were reviewed. Two categories of research were identified. The predominant focus of the selected scientific studies and articles from the food industry was on quality-related aspects, such as product formulation to ensure comparability with corresponding animal-derived products and sensory evaluations of plant-based products (n=43). A smaller subset of the research concentrated on the microbiological safety of plant-based foods or was related to outbreaks or food recalls (n=36), which was relevant to the scope of the review and were included accordingly.

The findings of the literature review showed that plant-based dairy and meat substitutes can be contaminated at many points along the manufacturing process from raw materials to how they are handled and stored in consumers homes. Although most scientific studies reviewed generally showed that the products investigated met microbiological safety standards, there was limited prevalence data available. Three recent surveys conducted in the United Kingdom (UK) (Willis *et al.,* 2023), Belgium (Van Paepeghem *et al.,* 2024), and Switzerland (Barmettler *et al.,* 2025) on plant-based meat substitutes identified the presence of *L. monocytogenes, B. cereus*, and *Staphylococcus aureus* in a small number of samples. There was also an occasional finding of elevated levels of hygiene indicators such as other non-pathogenic *Listeria* spp., *E. coli* and Enterobacteriaceae as detailed in Table 2. This suggests ongoing challenges related to hygiene and environmental control within the sector. The studies reviewed highlight the importance for strengthened monitoring protocols and temperature management practices to effectively prevent pathogen growth and toxin development in these food products.

Several documented outbreaks of *L. monocytogenes* and *Salmonella* spp. attributed to RTE plant-based dairy substitutes show that food safety risks remain for consumers (Table 1). This underscores that RTE plant-based foods, despite their healthy and sustainable image, are just as vulnerable to microbial hazards as their corresponding RTE animal-derived products. This is particularly the case for plant-based foods with water activity (a_w) and pH values that would support the growth of foodborne pathogens. Occasional pathogen detection, variability in microbial counts, and challenge test evidence of rapid pathogen growth in plant-based dairy and meat substitutes (Kain *et al.*, 2024;

Bartula *et al.,* 2023) highlight the need for strict hygiene, robust manufacturing controls, and proper temperature storage to ensure consumer safety.

Physico-chemical characteristics

The physico-chemical characteristics (pH and a_w) of the samples collected for the microbiological survey were analysed to determine if they could support the growth of most foodborne pathogens should they become contaminated. The majority of the RTE plant-based products tested had pH > 4.4 or a_w > 0.92 or did not have a combined pH ≤ 5.0 and a_w ≤ 0.94. As such they would be legally required to comply with the microbiological criteria set down under Food Category 1.2 'RTE foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes' in Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005).

Microbiological quality

The microbiological quality of the products tested in the present survey was generally good (Table 23). Chilled products labelled with a use by or best before date were stored at refrigerated temperatures in the laboratory and tested at the end of the labelled shelf-life. This was in order to assess the worst-case scenario for contamination and growth of *L. monocytogenes* during the shelf-life of the foods, and to increase the likelihood of identifying any other microbial contamination that may have been present. Preliminary work conducted ahead of starting the survey found that most ambient RTE plant-based dairy milk substitutes placed on the market in the countries participating in the survey were labelled with best before dates and often had an extended shelf-life duration of approx. 12 months. Consequently, sampling and testing these long shelf-life products at the end of their designated best before date posed logistical challenges within the survey timeframe (1st March – 30th November 2025). Therefore, it was agreed with EFSA that samples of ambient RTE plant-based dairy milk substitutes labelled with a long shelf-life duration could be collected as soon to the start of the survey period as possible. These were then stored at ambient temperatures to allow some shelf-life to elapse and tested towards the end of the survey period. This approach facilitated testing of ambient RTE dairy milk substitutes as near as possible to their labelled shelf-life expiration date, thereby increasing the likelihood of identifying any microbial contamination issues that may have been present.

Across 1,533 total samples, *L. monocytogenes* was detected in only 1 sample (from a chilled light oat-based dairy milk substitute) at <10 cfu/g (Table 14). The analysis was conducted one day prior to the product's labelled best before expiry date, and no measurable growth was observed. This finding suggests that the particular type of RTE plant-based milk assessed for the survey did not support the growth of *L. monocytogenes* to >100 cfu/g throughout its shelf-life. However, while the level was below the quantification limit for this particular product at the end of shelf-life, challenge tests have shown that the long shelf-life and physico-chemical characteristics of plant-based milks in general may support growth of *L. monocytogenes* to levels >100 cfu/g (Kain *et al*., 2024; Bartula *et al*., 2024). There may be an increased risk of foodborne illness if the product is kept at a higher temperature than recommended. In 2024, a significant listeriosis outbreak of 20 cases (15 hospitalisations and three fatalities) was associated with chilled almond, oat, and coconut plant-based milk products (Public Health Agency of Canada, 2024).

Salmonella spp. was not detected in any of the samples tested (n=1332). Five PCR-positive but culture-negative results for STEC were identified from n=831 samples, however no viable organisms were cultured confirmed and the samples were not designated as unsatisfactory.

Fourteen of 1,113 samples (1.3 %) enumerated for either *B. cereus* or *Bacillus* spp. were found to have levels between 1,000 and 100,000 cfu/g and thus were designated as borderline in accordance with the guideline microbiological criterion for *B. cereus* in Guidance Note 3 (Food Safety Authority of Ireland, 2020). Three samples (0.3 %) had levels in excess of 100,000 cfu/g and were designated as unsatisfactory. Borderline levels were found in plant-based substitutes for meat (n=7), cheese (n=4), fish (n=2) and dairy cream (n=1). Unsatisfactory levels of >100,000 cfu/g were found in meat substitutes made from tofu (n=2) and a cheese substitute (n=1) made from cashews (Table 16 and Table 17). Of 810 samples tested for *C. perfringens* or *Clostridium* spp., n=99 (12.2%) was found to have borderline levels in cheese, meat and fish substitutes. No unsatisfactory results of >10,000 cfu/g were found.

The presence of *Listeria* spp. (non-*monocytogenes*) was assessed in the samples collected as a hygiene indicator in the present survey. *Listeria* spp. was detected in n=15 (0.98%) of 1,533 samples. They were detected in a dairy cream substitute (n=1), in cheese substitutes (n=4) and in meat substitutes (n=10). There were also low levels of *Listeria* spp. (<100 cfu/g) enumerated in a cheese substitute (n=1) and in meat substitutes (n=9). However, one cheese substitute had a high unsatisfactory count of 3,100 cfu/g *Listeria* spp. This product was a camembert alternative with fermented cauliflower, based on vegetable oil and starch. It was purchased from an online retailer and labelled with a best before date (DE_018) (Table 21). While the presence of *Listeria* spp. may not constitute a regulatory non-compliance, it indicates contamination potential and highlights the importance of robust hygiene controls and environmental monitoring, particularly for these RTE plant-based products (FAO/WHO, 2022).

The *E. coli* test was also conducted on the samples to assess their hygiene status. Unsatisfactory results of >100 cfu/g, although not a hazard, can indicate faecal contamination of raw materials, inadequate heat processing or hygiene, poor temperature and time control and/or increased likelihood of the presence of pathogens (Food Safety Authority of Ireland, 2020). There was a low occurrence overall of the hygiene indicator *E. coli.* Of 1,229 samples tested, *E. coli* was enumerated at levels in excess of 100 cfu/g for n=6 (0.49 %) samples which were designated as unsatisfactory (Table 20). No samples were found to have borderline levels of *E. coli* between 20 and 100 cfu/g. Unsatisfactory results (>100 cfu/g) were found in cheese substitutes (n=3) and meat substitutes (n=3). *E. coli* was enumerated in one sample (ES_051) at a high level of 38,000 cfu/g described as a camembert cheese substitute made from cashews (76%) which contained vegan cultures (Table 22). The same sample (ES_051) had 540,000 cfu/g *Bacillus* spp. enumerated in it (Table 17). There was no *L. monocytogenes, Listeria* spp. or *Salmonella* spp. found in the sample, but a foul odour coming from the sample upon opening was noted.

Conclusions and future research

While the microbiological quality of the samples tested in the present survey was good overall, some issues were noted. The survey highlights the presence of foodborne pathogens in plant-based RTE dairy, meat and fish substitutes while not common, can occur sporadically. Appropriate control of the possible risks should be taken into account under the food business operator's good hygiene practices (GHP) and hazard analysis and critical control point (HACCP)-based procedures under its food safety management system. Food business operators need to manage these hazards appropriately through the prerequisite requirements (PRP) of good supplier control of raw materials, adequate hygiene programmes, staff training, and proper maintenance and temperature control of the cold chain (European Commission, 2022). If not, there is a risk of any potential pathogenic contamination growing to levels which may cause serious foodborne illness, similar to what would be expected for animal-derived RTE dairy, meat and fish products. Further research is necessary to improve understanding of pathogen prevalence in raw materials used for RTE plant-based foods and to validate processing procedures.

As indicated by the present survey, the retail market offers a diverse range of RTE plant-based dairy, meat and fish substitutes. Additional investigation is needed to identify key areas for attention, including critical control points (CCP) in the production processes of these emerging plant-based dairy, meat and fish substitutes. Such studies should inform the further development of tailored guidelines for GHP, time/temperature parameters, and product formulation recommendations to ensure microbiological safety during processing and preservation. Additionally, fit-for-purpose predictive models and challenge testing should support shelf-life determination, providing food business operators with science-based guidance for shelf-life allocation.

During this collaborative survey conducted across 15 Member States, it was observed that while some participating countries have established national guidelines including recommended microbiological criteria for monitoring the contamination of RTE plant-based products on the market, this is not uniformly the case across all Member States. The development and implementation of harmonised microbiological criteria for evaluating the microbiological quality of RTE plant-based dairy, meat and fish substitutes would be valuable in promoting consistency in official assessments of the microbiological compliance of these types of products.

Finally, consumers often perceive plant-based foods as safer than their animal-based counterparts. Continued vigilance by regulators and food business operators is warranted to maintain food safety standards and to address isolated findings that could pose a potential public health concern if left unmanaged. It is recommended that food safety authorities, in collaboration with relevant stakeholders, should review and update risk communication for consumers related to storage and use of RTE plant-based products.

RTE plant-based chilled products sampled in the present survey were often labelled with best before dates rather than use by dates. This was found for 77% (n=334/436) of cheese and 63% (n=323/509) meat substitutes. The best before date is about the quality of the food, while the use by date is about safety (EUFIC, 2021). This means that consumers may choose to eat food labelled with a best before date after it has passed if it looks, smells and tastes fine. Under food safety legislation, food business operators must take consumer behaviour into account by assessing the reasonably foreseeable conditions of distribution, storage and use, and the normal consumption of a food product when determining in a food is unsafe (European Commission, 2002; 2005).

Temperature abuse and long shelf-life durations of product labelled with a best before date, which some consumers may choose to eat long after the labelled date has passed, may facilitate the growth of harmful microorganisms to levels that are considered unsafe. It is important that clear risk communication information is provided to consumers regarding the importance of respecting the labelled storage conditions on products and to ensure proper handling of plant-based RTE products - particularly after opening, when refrigerated storage time is limited - to help ensure food safety all the way to the plate.

1 Literature review

1.1 Introduction

There is a growing variety of RTE plant-based dairy and meat substitutes placed on the market in the EU. However, there is a lack of suitable studies in the literature which assess the potential for new or emerging food safety risks that could be linked to the consumption of these products. This issue was discussed at the 29th meeting of the European Food Safety Authority (EFSA) Emerging Risks Exchange Network and at the 23rd meeting of the EFSA Network on Microbiological Risk Assessment (Gkrintzali *et al.*, 2024, European Food Safety Authority, 2023)

The plant-based formulations of dairy and meat substitutes can include a wide array of different ingredients with variations in chemical, nutritional, and microbiological composition. This includes pulses (e.g. soya, pea, faba bean, chickpea, and mung bean), cereals (e.g. rice, oat, wheat, and quinoa), nuts (e.g. almond, cashew, and coconut), and oilseeds (e.g. canola and sunflower). However, the reformulation of traditional products with new plant ingredients or alterations of processing conditions for the known ingredients requires careful consideration with respect to the microbiological safety and stability of the developed recipes (Kyrylenko *et al*., 2023).

The rise in popularity of RTE plant-based dairy and meat substitutes has driven an increased focus on their food safety and if any emerging risks may be associated with them. The processing of some RTE plant-based substitutes can be different from the traditional time-temperature processes used to produce the animal-based products which they are replacing (e.g. cooked deli meats, pasteurised dairy liquid milk and cheese). This could lead to microbiological hazards being present in these food types that are not removed by a specific critical control step, such as a validated time-temperature treatment during their manufacture.

Consumers are increasingly turning to these substitutes for ethical reasons, environmental sustainability, claimed health benefits and allergen concerns. While plant-based foods may offer some sustainability benefits, they also present distinct microbiological risks compared to animal derived products. Ensuring food safety requires stringent processing controls, effective handling practices, and consumer awareness regarding storage and preparation.

The first aim of this literature review was to collate all available information on the food safety hazards/risks for RTE and non-RTE plant-based dairy and meat substitutes. This is a product category that is growing in consumer demand but for which the evidence-base as regards the potential risk in the current literature is lacking. At the same time, consumption of these products is increasing.

A second aim of the literature review was to provide the scientific basis for carrying out the EFSA Focal Point Tailor-Made Activities (TMA) funded project '*A retail prevalence survey to investigate microbiological contamination levels in RTE plant-based dairy and meat.'* While the present survey was initially funded to investigate the microbiological quality of RTE plant-based dairy milk, cheese and meat substitutes, in agreement with EFSA, the scope was increased at the beginning of the prevalence survey (1st March 2025) to include RTE dairy cream and fish substitutes. This was because some Member States reported seeing these types of products placed on the market in addition to plant-based dairy milk, cheese and meat substitutes.

The prevalence survey was carried out to assess the potential for new or emerging risks that could be linked to the consumption of RTE plant-based dairy, meat and fish substitutes, and to raise awareness regarding these risks for consumers. Understanding contamination patterns and emerging risks associated with these foods is essential for ensuring their microbiological safety and limiting the risk to public health. The objective of the TMA is to contribute valuable data to the scientific evidence-base that can be used to support EU Member State risk managers to evaluate the need (or not) to adapt current risk management measures for animal-based products in order to manage the potential risk for RTE plant-based dairy, meat and fish substitutes.

1.2 Scope

This literature review analysed existing research on the microbiological contamination levels in RTE and non-RTE plant-based dairy, meat and fish substitutes. Additionally, food incident notifications, recalls, and outbreak reports between 1st January 2010 and 1st May 2025 concerning any microbiological pathogens in RTE plant-based dairy, meat and fish substitutes were collated and reviewed to determine their relevance to the scope of this literature review.

1.3 Data and methodology

To assess the microbiological contamination levels in RTE plant-based dairy, meat and fish substitutes, a literature search was conducted in four electronic databases: Google Scholar, ScienceDirect, PubMed, and Web of Science. The following search terms were used solely or in combination with each other:

- 'plant-based'
- 'ready-to-eat'
- 'RTE'

- 'non-ready-to-eat'
- 'vegan'
- 'vegetarian'
- 'dairy-free'
- 'meat-free'
- 'alternative'
- 'substitute'

The date range was limited from 1st January 2010 to 1st May 2025. Articles were considered relevant if they were related to the prevalence of microbiological contamination in RTE and non-RTE plant-based dairy (milk, cream, cheese), meat or fish substitutes. Additionally, food incident notifications, recalls and outbreak reports between 1st January 2010 and 1st May 2025 concerning any microbiological pathogens in RTE and non-RTE plant-based dairy, meat or fish substitutes were collated and reviewed to determine their relevance to the scope of this literature review. During the process of gathering relevant studies, two types of research were identified. The majority of scientific studies or food industry articles gathered focused on quality aspects such as product formulation to ensure similarity to equivalent animal-derived products and sensory evaluations of plant-based products (n=43). A smaller number of the research gathered concentrated on the microbiological safety of plant-based foods or related to foodborne outbreaks and recalls (n=36) which were relevant to the scope and included in the literature review (Figure 1).

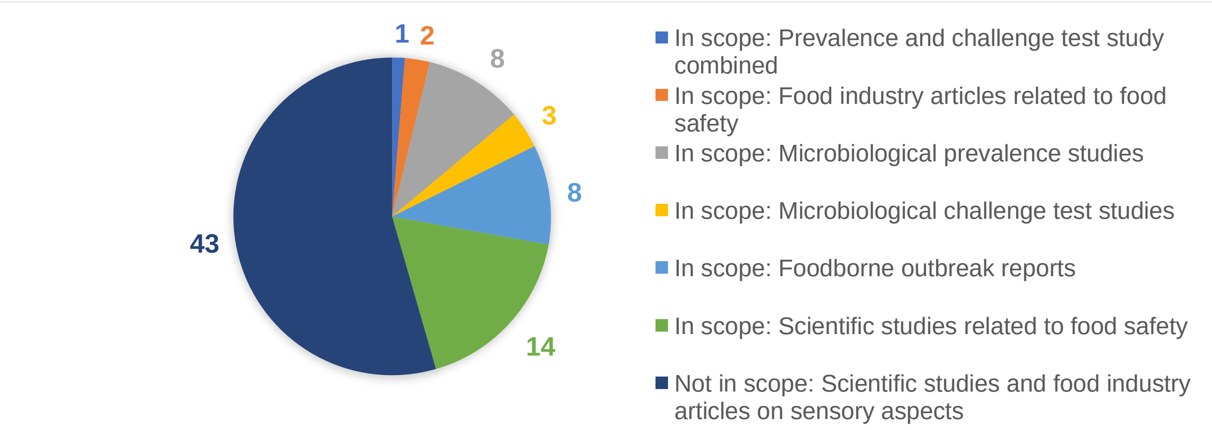


Figure 1 The number of articles and studies in scope and not in scope for this literature review

1.4 Results

1.4.1 Microbiological risk factors in plant-based foods

Plant-based meat substitutes are generally composed of enhanced and reassembled proteins extracted from sources such as legumes (e.g. soybeans, peas, and chickpeas), grains (e.g. wheat), pseudocereals (e.g. quinoa), and seeds (e.g. flaxseed, and chia seed) (Langyan *et al*., 2022, McClements *et al*., 2019). The choice of raw materials and specific processing steps, such as extrusion or cooking, significantly influence the occurrence, survival, and growth of spoilage and pathogenic microorganisms (Kyrylenko *et al*., 2023). Studies indicate that microbiological spoilage profiles vary depending on ingredients, processing methods, and storage conditions (Roch *et al*., 2024, Tóth *et al*., 2021).

Plant-based meat substitutes including RTE plant-based meat substitutes are created so that they mimic the protein, fat, and moisture content of conventional meat. They maintain neutral pH levels, creating a favourable environment for spoilage and pathogenic microorganisms. Also, as consumer adoption of plant-based proteins grows, concerns regarding allergens, processing contaminants, and anti-nutritional compounds also rise (Willis *et al*., 2024). In addition to bacterial contamination, viral transmission through food handlers and mycotoxin presence in plant-based ingredients also present potential health risks, as heat treatment may not reliably eliminate these hazards (Banach *et al*., 2023).

RTE plant-based dairy substitutes, including soya, almond, oat, rice, and coconut-based products, are often produced by soaking, grinding, and fermentation (Paul *et al*., 2020, McClements *et al*., 2019). Plant-based dairy substitutes including those made from nuts and oats have been linked to foodborne outbreaks (Table 1).

A study by Bartula *et al.* (2023) on the growth of foodborne pathogens *L. monocytogenes, Salmonella* and *B. cereus* in plant-based dairy substitutes indicated that these products including coconut, almond, and cashew milk substitutes, support the growth of *Salmonella* and *L. monocytogenes* at higher rates than in bovine milk, posing risks for listeriosis and salmonellosis. *L. monocytogenes* remains a significant safety concern within the RTE food sector. The pathogen can grow at refrigerated temperatures, presenting challenges for ensuring the safety of RTE products (Butler *et al*., 2023).

While heat treatments such as pasteurisation reduce microbial hazards, spore-forming bacteria such as *B. cereus* and *Clostridium* remain a challenge in RTE plant-based milk due to their ability to form resilient spores (Wells-Bennik *et al*., 2016). In addition, if post-process contamination of plant-based milks occurs after pasteurisation, and these products are subjected to temperature abuse during subsequent storage at manufacturing, distribution, retail, and consumer level, this can enable bacterial spores that survived pasteurisation to germinate and grow (Bylund and Lindqvist, 2025).

The vulnerability of these products to microbial contamination remains an issue. For example, the raw materials from which these food products are made from are susceptible to contamination with natural toxins, in particular mycotoxins (Banach *et al*., 2022, Food and Agriculture Organisation, 2022). Also, the presence of pathogenic bacteria in these foods has been documented in the studies outlined within this literature review (Table 2).

There are potential opportunities for microbial contamination of plant-based ingredients before, during, and after harvest, as well as during manufacturing, and in the retail and domestic environments. Furthermore, these products are usually subject to further processing steps such as fermentation during production of the final product, which may introduce additional opportunities for pathogen growth (Willis *et al*., 2024). Therefore, ensuring food safety is of critical importance for plant-based dairy, meat and fish substitutes, given their increasing consumption in recent years.

1.4.2 Foodborne outbreaks from RTE plant-based dairy substitutes

Foodborne outbreaks linked to RTE plant-based dairy substitutes have been associated with *L. monocytogenes*, *Salmonella*, and *B. cereus*. These incidents are summarised in Table 1. There were no foodborne outbreaks associated with RTE plant-based meat or fish substitutes identified in the available literature research.

In 2024, *L. monocytogenes* contamination in various almond, oat, and coconut plant-based milk products of the same brand were attributed to a listeriosis outbreak in Canada (Table 1). It was a significant outbreak with 20 reported cases, 15 of whom were hospitalised with three fatalities (Public Health Agency of Canada, 2024). This demonstrates the risk *L. monocytogenes* contamination in plant-based RTE dairy products can pose to vulnerable groups, causing serious illness.

In 2022, several cheese substitutes of the same brand caused a *L. monocytogenes* outbreak in France, Germany, Belgium, and the Netherlands. The products included a plant-based camembert cheese, goats' cheese, and blue cheese made from ingredients such as almonds, cashews, and coconut milk. It affected five people in France, including four pregnant women, leading to premature deliveries. Three additional cases were also identified in Germany, Belgium, and the Netherlands (Leclercq *et al.,* 2024; Santé publique France, 2023: Rapid System for Food and Feed (RASFF), 2023).

In 2022, an oat-based milk substitute in Finland and Estonia was the source of a *B. cereus* outbreak (Table 1). The company that produced the product received 29 complaints and two people reported having a stomach illness (Ruokavirasto – Finnish Food Authority, 2022). This reinforces the risk *B. cereus* can have in plant-based milk substitutes.

In 2021, a brie-style cashew nut cheese was the source of an outbreak in the United States (US) with the presence of *Salmonella* Chester, Duisburg, Typhimurium, and Urbana detected in the contaminated product (Table 1). The outbreak involved 20 people. Five of the cases were hospitalised (Centers for Disease Control and Prevention, 2021). The age range of cases was from 1 to 72 years, with a median age of 26 years. The cashew nuts used in the production of brie products were identified as the probable source of contamination. An evaluation of the cashew brie manufacturing process indicated that no lethality treatments (such as pasteurisation or irradiation) were applied prior to cashew processing. The absence of such safety interventions in raw ingredients can increase the risk of contamination in RTE products that are generally regarded as safe by consumers (Centers for Disease Control and Prevention, 2021).

Similarly, in 2017, a fermented cashew cheese spread produced by a restaurant for in-house consumption in Canada caused an outbreak of *S.* Weltevreden. This affected 23 people with one hospitalised (Schmitt *et al.*, 2018). An inspection of the restaurant and review of the food processes identified areas of concern, including the use of rejuvelac as a starter culture for fermentation and inadequate temperature control. Rejuvelac is produced from a wild fermentation that occurs following the soaking of wheat berry or other seeds and may intensify existing hazards present in the seeds (British Columbia Centre for Disease Control, 2017).

In 2014, *S.* Stanley was detected in a cashew cheese in the US. This outbreak resulted in 17 cases and three hospitalised (Centers for Disease Control and Prevention, 2014). The outbreak was linked to six varieties of cashew cheese from the same brand. The age range of cases was from 2 to 83 years, with a median age of 27 years.

Table 1 Foodborne outbreaks from RTE plant-based dairy substitutes

Year	Country	Brand Name	Product Type	Cause of Outbreak	No. of Cases	Hospitalisations	Deaths	Reference(s)
2014	USA	The Cultured Kitchen	Cashew cheese substitutes (Herb, smoked cheddar, pepper jack, habañero cilantro lime, basil pesto, and white cheddar)	*Salmonella* Stanley	17	3	0	Centers for Disease Control and Prevention, (2014)
2017	Canada	Product produced by a restaurant for in-house consumption	Fermented cashew cheese spread	*Salmonella* Weltevreden	23	1	0	Schmitt *et al.* (2018)
2021	USA	Jule's Cashew Brie	Brie-style cashew cheese	*Salmonella* Chester, Duisburg, Typhimurium, and Urbana.	20	5	0	Centers for Disease Control and Prevention (2021)
2022	France, Germany, Belgium, and the Netherlands	Jay & Joy	Cheese substitutes (Camembert, goat cheese, and blue cheese) made from almond, cashew, and coconut.	*L. monocytogenes*	8	4	0	Leclercq *et al.* (2024) Santé publique France (2023) RASFF (2022)
2022	Finland and Estonia	Oatly	Non-refrigerated oat-based milk substitute	*B. cereus*	2	0	0	Ruokavirasto - Finnish Food Authority (2022)
2024	Canada	Silk & Great Value	Refrigerated almond, oat, coconut, chocolate, and cashew milk substitutes	*L. monocytogenes*	20	15	3	Public Health Agency of Canada (2024)

1.4.3 Microbiological prevalence studies on RTE plant-based dairy and meat substitutes

The microbiological prevalence studies on RTE plant-based dairy, meat and fish substitutes are summarised below in Table 2. The existing literature on these studies is limited; however, they underscore important food safety considerations and demonstrate differing microbial profiles affected by product type, processing methods, and storage conditions.

Table 2 Microbiological prevalence studies on RTE plant-based dairy, meat and fish substitutes

Year	Country	Sample type	Sample size	Test parameters	Summary of study results	Reference

2017	Belgium	RTE plant-based sliced meat substitutes and insect-based samples from retail premises (Modified atmosphere packaged (MAP)).	14 plant-based (8 vegetarian meat, 6 vegan meat and 1 insect-based sample)	1. Enumeration of Total Viable Count (TVC)
				2. pH (Table 3)
					• TVC were mostly below the limit of detection (LOD) at <2.0 log_{10} cfu/g.
					  • 2 out of 8 vegetarian samples ranged from 3.1 to 3.8 log_{10} cfu/g.
					  • 3 out of 6 vegan samples ranged from 3.6 to 8.7 log_{10} cfu/g.
					• Total of 426 bacterial isolates.
					  • 21 isolates in vegetarian samples, 224 isolates in vegan samples, and 181 isolates in insect-based samples.
					  • Highest counts in vegan samples with *Lactobacillus sakei* (131 isolates) and *Enterococcus faecium* (40 isolates).
						Geeraerts *et al.* (2017)

2023	United Kingdom	RTE plant-based meat, fish and dairy substitutes from retail, production, and catering premises (Pre-packed and loose).	937 (414 meat substitutes, 246 cheese substitutes, 138 milk and 112 other dairy substitutes, 11 fish substitutes, 16 other including tofu, egg alternatives and vegan desserts)	**Detection of:** 1. *Listeria* spp. 2. *L. monocytogenes.* 3. *Salmonella* spp. **Enumeration of:** 1. *Listeria* spp. 2. *L. monocytogenes.* 3. *B. cereus* 4. β-glucuronidase producing *Escherichia coli* 5. Enterobacteriaceae 6. TVC	• 92% (n=866) satisfactory, 3% (n=27) borderline and 5% (n=43) unsatisfactory due to *E. coli* and Enterobacteriaceae. • *L. monocytogenes* detected in five tofu samples from the same producer (enumerated at ≤20 cfu/g). • *Listeria* spp. detected in four other meat substitute samples (enumerated at ≤20 cfu/g). • *B. cereus* detected at borderline levels in two cheese substitute samples (ranging from 10^3 to <10^5 cfu/g). • No *Salmonella* spp. detected. • *E. coli* enumerated in three cheese substitute samples at >10^2 cfu/g. • Enterobacteriaceae enumerated in 30 cheese, meat, and other dairy substitute samples (10^2 to 10^4 cfu/g) and 38 cheese and meat substitute samples (>10^4 cfu/g). • TVC in 88% of samples was <10^4 cfu/g.	Willis *et al.* (2023)

Year	Country	Sample type	Sample size	Test parameters	Summary of study results	Reference
				pH and water activity (a_w) (Table 3)	• 15% of cheese substitutes, 16% of meat substitutes, 2% of milk substitutes and 5% of other samples had a TVC of >10^4 cfu/g.	
2023	The Netherlands	Plant-based ingredients for dairy substitutes (Pulses: pea, faba bean, chickpea, and mung bean, Cereals/pseudocereals: oat, rice, amaranth, and quinoa, Drupes: coconut, almond and cashew) from production premises throughout Europe, South/North America, and Asia.	88	**Enumeration of:** 1. TVC 2. Total aerobic mesophilic spore count (TMS) 3. Total anaerobic sulphite reducing *Clostridium* spores (SRCS) 4. *B. cereus* spores (BCES) 5. Total heat-resistant thermophilic spore formers (HRTS) 6. Total heat-resistant moulds (HRM)	• TVC ranged from 1.0 to 4.5 $\log_{10}$ cfu/g and highest level of 5.3 $\log_{10}$ cfu/g in drupes. • TMS ranged from ≤4.1 $\log_{10}$ cfu/g. • SRCS were mostly ≤LOD, highest level of 2.5 $\log_{10}$ cfu/g in pulses. • *B. cereus* group spores ranged from ≤LOD to 2.6 $\log_{10}$ cfu/g in pulses. • HRTS were mostly ≥LOD, highest level of 6.3 $\log_{10}$ cfu/g in pulses.	Kyrylenko *et al.* (2023)
2023	Canada	RTE plant-based cheese substitutes (Almond, butter bean, oat, cashew, chickpea, coconut oil, cocoa butter, soybean, palm oil, corn, olive oils, sunflower seed, and navy bean) from retail premises.	556	**Detection of:** 1. *Salmonella* spp. 2. *L. monocytogenes* **Enumeration of:** 1. *S. aureus* 2. *E. coli.*	• 99.2% of the samples were satisfactory • *Salmonella* spp. and *L. monocytogenes* not detected in any sample • *S. aureus* not enumerated in any sample (≤10^4 cfu/g) • *E. coli* enumerated at >10^2 cfu/g in four cashew nut cheese samples	Canadian Food Inspection Agency (2023)

Year	Country	Sample type	Sample size	Test parameters	Summary of study results	Reference
2023	Italy	RTE Plant-based dairy substitutes (Soya, rice, and oat-based) from retail premises.	60	**Detection of:** 1. *L. monocytogenes* 2. *Salmonella* spp. **Enumeration of:** 1. Total aerobic mesophilic bacteria count (TAMBC). 2. CPS 3. Total coliforms 4. Enterobacteriaceae 5. Sulphite-reducing anaerobic bacteria (SRAB). 6. *B. cereus.* 7. Total yeast and mould (TYMC).	• No pathogenic bacteria were detected. • **Soya samples:** • Two samples recorded yeast and mould growth (19 cfu/mL and <4 cfu/mL). • All other samples recorded a count of <1 cfu/mL for each microbiological analysis. • The TAMBC registered a count of <4 cfu/mL on one sample, 5 cfu/mL on one sample, 15 cfu/mL on one sample and 6700 cfu/mL on one sample. • **Rice samples:** • Four samples recorded a yeast and mould count of <4 cfu/mL. • All other samples recorded a count of <1 cfu/mL for each microbiological analysis. • The TAMBC and CPS recorded counts of <4 cfu/mL for one sample. • **Oat samples:** • The TYMC results recorded a count of ≤4 cfu/mL on three samples. • All other samples recorded a count of <1 cfu/mL for each microbiological analysis. • *B. cereus* was identified in one sample with an enumeration of <4 cfu/mL. • The TAMBC and CPS recorded counts of <4 cfu/mL on one sample and two samples, respectively.	Giugliano *et al.* (2023)

Year	Country	Sample type	Sample size	Test parameters	Summary of study results	Reference
2024	Belgium	RTE plant-based sliced meat substitutes, fresh-cut leafy vegetables, and multi-ingredient salad bowls from production premises (pre-packed).	150 (51 vegetarian and vegan deli slices)	**Detection and enumeration of:** *L. monocytogenes*.	From 51 vegetarian and vegan deli slices, *L. monocytogenes* detected in one sample at <10 cfu/g.	Van Paepeghem *et al.* (2024)
2025	Switzerland	RTE and non-RTE plant-based meat substitutes from retail premises.	100	**Detection of:** *Listeria* spp. *L. monocytogenes* *Salmonella* spp. **Enumeration of:** *S. aureus*, *B. cereus* Enterobacteriaceae TVC pH and a_w (Table 3)	• No *Salmonella* spp. or *L. monocytogenes* detected. • *Listeria* spp. detected in seven samples: (*L. innocua* n=6 isolates and *L. seeligeri* n=1 isolate). • *B. cereus* enumerated in five samples at 2 log_{10} cfu/g each. • *S. aureus* enumerated in two samples at 2.70 log_{10} and 2.78 log_{10} cfu/g. • Enterobacteriaceae enumerated in six samples at 2 to 3 log_{10} cfu/g. • TVC ranged from <2 to 7 log_{10} cfu/g.	Barmettler *et al.* (2025)

1.4.3.1 Prevalence of *Listeria* spp. in plant-based dairy and meat substitutes

L. monocytogenes is a pathogenic bacterium which causes a group of diseases which are collectively known as listeriosis. It is of major concern because of its pervasive nature, its ability to survive and grow at refrigeration temperatures (0 - 5 °C), the severity of the disease and the high case fatality rate in vulnerable people (Food Safety Authority of Ireland (FSAI), 2011).

The survey by Barmettler *et al.* (2025) on RTE and non-RTE plant-based meat substitutes from retail premises reported that *L. monocytogenes* was not detected in any of 100 samples, although seven samples tested contained other *Listeria* spp., such as *L. innocua* (n=6) and *L. seeligeri* (n=1) (Table 2), possibly indicating hygiene deficiencies. These samples were plant-based RTE sliced meat and non-RTE burger samples.

A survey was conducted by the UK Health Security Agency (UKHSA) on RTE plant-based substitutes from retail, production, and catering premises (n=937). Of the samples tested, 414 (44%) were meat substitutes, 246 (26%) were vegan cheeses, 138 (15%) were plant-based milks, 112 (12%) were other dairy alternatives, 11 (1%) were fish alternatives, and 16 (2%) were other vegan items, including tofu, egg alternatives, and vegan desserts. The survey found that from 414 meat substitute samples, *L. monocytogenes* was detected in five tofu samples (1.2%) from the same producer at ≤20 cfu/g, and other *Listeria* spp. were present in four other meat substitutes (1%) sampled at retail level. These were: two plant-based burger samples contained *L. welshmeri* and *L. innocua*, respectively, while two plant-based chicken samples both contained *L. seeligeri* at <20 cfu/g (Willis *et al.,* 2024).

L. monocytogenes was not detected by Giugliano *et al.* (2023) in plant-based dairy substitutes (soya, rice, and oat-based) from retail premises (n=60). The Canadian Food Inspection Agency (2023) also did not detect *L. monocytogenes* in RTE plant-based cheese substitutes (n=556).

1.4.3.2 Prevalence of *Salmonella* spp. in plant-based dairy and meat substitutes

While *Salmonella* was not detected in the microbiological prevalence studies on RTE plant-based dairy and meat substitutes detailed in Table 2, the multiple cases of foodborne illness from cashew-based cheese products (Table 1), shows the risk that this pathogen can have in these products.

1.4.3.3 Prevalence of *B. cereus* in plant-based dairy and meat substitutes

A key observation across the microbiological prevalence studies on RTE plant-based dairy and meat substitutes, is the recurring presence of *B. cereus* in plant-based dairy substitutes, a pathogen known for its enterotoxin-producing capabilities, which can lead to foodborne illness. *B. cereus* can form spores, ensuring its survival through all stages of food processing. In addition, time/temperature abuse can enable low levels of *B. cereus* to multiply to dangerous levels in the given product (Food Safety Authority of Ireland (FSAI), 2016).

As shown in Table 2, a survey by Barmettler *et al.* (2025) reported *B. cereus* in 5 out of 100 RTE and non-RTE plant-based meat substitutes tested from retail premises at 2 $\log_{10}$ cfu/g. This highlights the ability of this spore-former to persist despite relatively high manufacturing standards from the producer. Similarly, a survey carried out by the UKHSA enumerated levels of *B. cereus* ranging between 1,000 to <100,000 cfu/g in two plant-based cheese samples from retail. These were designated as borderline using the microbiological criterion limits published in the UKHSA '*Guidelines for assessing the microbiological safety of RTE foods placed on the market*' (Willis *et al.,* 2024). The survey by Giugliano *et al.* (2023) on plant-based dairy substitutes (soya, rice, and oat-based) from retail premises (n=60) detected *B. cereus* in one ultra-high temperature (UHT) treated oat-based sample with an enumeration of <4 cfu/mL.

Furthermore, NIZO food research, identified *B. cereus* as one of the most frequently detected species among 88 plant-based dairy ingredients, especially in oat and pea-based samples (Kyrylenko *et al.,* 2023). *B. cereus* group spores ranged from ≤LOD to 2.6 $\log_{10}$ cfu/g (pulses) and 30% of samples were >1.0 $\log_{10}$ cfu/g (Table 2). From 147 *B. cereus* group isolates, around 9% from various ingredients such as pulses, cereals/pseudocereals and drupes (coconut, almond and cashew) contained the gene required for cereulide production. Cereulide causes emetic illness when the food product is consumed. Cereulide producing strains represent a potential food safety hazard even if no viable organisms are detected. For example, if this heat-stable toxin is produced prior to heat inactivation of the cells (Kyrylenko *et al.,* 2023).

1.4.3.4 Prevalence of Enterobacteriaceae and *E. coli* in plant-based dairy and meat substitutes

Enterobacteriaceae and *E. coli* are commonly used as indicators of hygiene and possible faecal contamination. Hygiene monitoring is crucial in the production of safe plant-based foods. The microbiological prevalence studies reported low-to-moderate levels of Enterobacteriaceae and *E. coli.*

Willis *et al.* (2023) found that 5% of 937 samples were unsatisfactory due to Enterobacteriaceae (>10^4) and β-glucuronidase producing *E. coli* (>10^2 cfu/g). *E. coli* was enumerated in three cheese substitute samples from retail at >10^2 cfu/g. Enterobacteriaceae was enumerated in 30 cheese, meat and other dairy substitute samples from retail, producers, and catering premises at 10^2 to 10^4 cfu/g and 38 cheese and meat substitute samples from retail and producers at >10^4 cfu/g (Table 2). Barmettler *et al.* (2025) reported Enterobacteriaceae in 6 out of 100 RTE and non-RTE plant-based meat substitute samples (2 to 3 $\log_{10}$ cfu/g).

The Canadian Food Inspection Agency (2023) prevalence survey on RTE plant-based cheese substitutes (n=556) found four cashew-based cheese samples with elevated *E. coli* levels (>10^2 cfu/g). The remaining samples tested were found to be satisfactory (*Salmonella* spp. and *L. monocytogenes* not detected, *S. aureus* (≤10^4 cfu/g), *E. coli* (≤10^2 cfu/g)).

1.4.3.5 Prevalence of *Clostridium* spp. in plant-based dairy and meat substitutes

Detection of *Clostridium* spp. was less widespread but still relevant. Kyrylenko *et al.* (2023) identified *C. sporogenes* and *C. tepidum* in 34 isolates from 88 dairy substitute ingredients, with spores present particularly in pea and almond-based samples. Although botulinum toxin genes (boNT-A/B) were not detected, the presence of anaerobic, spore-forming bacteria capable of surviving high-heat processes raises concerns about spoilage and potential toxin production under favourable storage conditions (Table 2).

In 2023, there was a recall of a long-life unsweetened almond milk substitute which was linked to a case of botulism in Australia (New South Wales (NSW) Health, 2023). This product was found to be not correctly labelled with directions to ‘*keep refrigerated.*’ The failure to refrigerate these products can lead to microbial growth and biotoxin contamination which may cause illness if consumed. The person affected was admitted to hospital after experiencing severe symptoms of botulism after drinking the product. The botulism toxin can be extremely dangerous and can cause life-threatening illnesses (NSW Health, 2023).

1.4.3.6 Prevalence of *S. aureus* in plant-based dairy and meat substitutes

Staphylococcus aureus is the most common species of coagulase-positive staphylococci (CPS) to cause illness. Staphylococcal food poisoning is caused by the production of enterotoxins in food. Not all strains of *S. aureus* or species of CPS can produce enterotoxins. The pathogenic potential of an isolate can be confirmed by testing for the presence of the genes for enterotoxins production. There is a risk of sufficient enterotoxin to cause illness being produced when *S. aureus* counts in the food exceed 100,000 cfu/g.

Many healthy people carry *S. aureus* on their skin and in their nose and throat and the microorganisms can be isolated from boils, skin infections and wound infections. Therefore, foods commonly implicated in staphylococcal food poisoning are those that have been contaminated via physical handling and then subjected to time/temperature abuse. For example, sandwiches, ham, potato salads, cream- or custard filled pastries, cold deserts, etc.

As *S. aureus* can produce toxins capable of causing foodborne illness their presence at elevated levels can pose a food safety hazard, possibly resulting in further follow-up actions. FSAI Guidance Note 3 sets a criterion limit of ≥10,000 cfu/g for CPS for RTE food placed on the market (Food Safety Authority of Ireland, 2020). If CPS are detected in food at levels above the limit of 10,000 cfu/g the food should be tested for the presence of enterotoxins. The presence of staphylococcal enterotoxins in food renders the food unsafe and it should be withdrawn or recalled. However, if the presence of staphylococcal enterotoxins is suspected, health actions and interventions should not be delayed pending the confirmed results.

Two published surveys reported investigating the presence of CPS in 556 plant-based cheese substitutes (Canadian Food Inspection Agency, 2023) and 100 meat substitutes (Barmettler *et al.*, 2025) (Table 2). In the Canadian study, *S. aureus* (>10,000 CFU/g) were not found in any of the samples (Canadian Food Inspection Agency, 2023).

The Swiss study detected *S. aureus* in 2 out of 100 (2%) samples (a non-RTE minced hachis and a burger galettes with a total bacterial count of 2.70 $\log_{10}$ and 2.78 $\log_{10}$ CFU/g, respectively). *L. seeligeri* was also identified in the same sample of minced hachis. Both strains were typed as *S. aureus* ST8. They did not harbour genes encoding classical staphylococcus enterotoxins but did harbour genes of the virulence factors staphylococcal enterotoxin-like X (SEIX) and Staphylococcus aureus toxic shock syndrome toxin-1 (TSST-1) (Barmettler *et al.*, 2025).

1.4.3.7 Physico-chemical characteristics in plant-based dairy, meat and fish substitutes

The pH and a_w are critical intrinsic factors that influence the survival and growth of microorganisms in food. The pH range for microbial growth and survival is defined by a minimum and maximum value with an optimum pH for growth and survival. Most microorganisms grow best or optimally at, or near, a neutral pH of 7. Monitoring pH levels during food processing can be an important step in the production of some food products since pH values affect microbial growth. Foods with a low a_w value cannot support microbial growth because microorganisms need water for growth. Pathogenic and spoilage bacteria do not grow in food with an a_w of <0.85, but some yeast and mould can grow at a_w as low as ≤0.60 (Food Safety Authority of Ireland, 2022).

Across the microbiological prevalence studies on RTE plant-based dairy and meat substitutes studies (Table 2), most samples exhibited pH values above 5.0 and a_w levels above 0.94, conditions favourable for microbial growth including growth of pathogens such as *L. monocytogenes* and *B. cereus*. This data is detailed in Table 3.

Table 3 Physico-chemical characteristics of plant-based dairy, meat and fish substitutes in the microbiological surveys reviewed

Sample type	Sample size	pH range	a_w range	Reference
RTE plant-based sliced meat substitutes from retail premises.	14	• 4.86 to 6.11 (Vegetarian samples) • 5.39 to 5.77 (Vegan samples)	• Not tested	Geeraerts *et al.* (2017)
RTE plant-based meat, fish and dairy substitutes from retail, production, and catering premises.	937 (Of these samples, only 772 were tested for pH and 500 were tested for a_w)	• 41 samples <4.0 • 108 samples 4.0 to 4.5 • 623 samples >5.0 • 5 tofu samples with *L. monocytogenes* detected in them had pH values between 5.3 and 6.3 • 2 cheese substitutes with *B. cereus* at borderline levels had pH of 5.2 and 4.4 respectively	• 7 samples <0.90 • 81 samples 0.90 to 0.94 • 412 samples >0.94 • Only 2 tofu samples with *L. monocytogenes* were tested for a_w and both found to be 0.97 • Only 1 cheese substitute with borderline levels of *B. cereus* was tested for a_w and found to be 0.95	Willis *et al.* (2023)
RTE and non-RTE plant-based meat substitutes from retail premises.	100	• 4.6 to 6.9	• 0.87 to 1.0	Barmettler *et al.* (2025)

1.4.4 Microbiological challenge test studies on RTE plant-based dairy and meat substitutes

Microbiological challenge test studies on RTE plant-based dairy and meat substitutes are summarised in Table 4. These studies offer valuable insights into the potential for pathogen growth in RTE plant-based dairy and meat substitutes. This information emphasises the importance of providing consumers with clear handling instructions and maintaining strict manufacturing hygiene practices to ensure public health safety.

Table 4 Microbiological challenge test studies on RTE plant-based dairy and meat substitutes

Year	Country	Sample Type	Sample Size	Test Parameters	Microbial Findings	Reference
2024	Belgium	RTE plant-based sliced meat substitutes, fresh-cut leafy vegetables, and multi-ingredient salad bowls from production premises (pre-packed).	150 (51 vegetarian and vegan deli slices)	Detection and enumeration of *L. monocytogenes* (Table 2) Challenge testing to assess the growth potential of *L. monocytogenes.* pH and a_w (Table 5)	**Challenge Tests:** Growth potential of *L. monocytogenes* was assessed in three vegetarian and vegan deli slices (δ ≥0.50 $\log_{10}$ cfu/g) and no significant growth in any sample (δ <0.50 $\log_{10}$ cfu/g).	Van Paepeghem *et al.* (2024)
2024	Germany	Plant-based milk substitutes (rye, oat, cashew, almond, sunflower, rice, lupin, hemp seed, pea, hazelnut, soya, spelt, coconut, and buckwheat) and one organic whole milk from retail premises.	27	Enumeration of the TVC Challenge testing to assess the growth potential of *B. cereus*, *L. monocytogenes* and *S. enterica*. pH (Table 5)	• Highest TVC after aerobic storage at 32 °C were in oat-based samples with *Staphylococcus pasteuri* (13 cfu/mL) and lupin samples with *Massilia timonae* and *Moraxella osloensis* (15 cfu/mL). • Highest TVC after aerobic storage at 25 °C were in soya, rice, and oat-based samples with *Paenibacillus validus*, *Staphylococcus* spp., and *B. cereus* (2 cfu/mL each). • *B. cereus* group isolates found in three samples, which formed high amounts of enterotoxins and exhibited cytotoxicity towards CaCo-2 cells. • Preliminary tests at 37°C with continuous shaking showed growth of *B. cereus, L. monocytogenes,* and *S. enterica* in all tested samples (maximum bacterial counts of $5^{x}10^{12}$ cfu/mL). • Propagation and persistence tests showed growth of *B. cereus* at lower bacterial counts (10^6 to 10^7 cfu/mL) compared to *L. monocytogenes* and *S. enterica* (10^8 to 10^9 cfu/mL).	Kain *et al.* (2024)
2024	Ireland	Non-refrigerated UHT treated plant-based dairy substitutes (oat and soya-based) from retail premises and pasteurised and refrigerated pea-based substitutes specially produced for this study.	17	Challenge testing to assess the growth potential of *L. monocytogenes* and *L. innocua.*	• *L. monocytogenes* and *L. innocua* detected in all samples, increasing by 2.6 to 3 $\log_{10}$ within 16 hours at room temperature. • Varying concentrations of naturally occurring or added sugar (0 to 3.3%), protein (3.3 to 5%), fat (1.1 to 3.5%) and added fibre (Locust bean gum and sodium alginate, 0 to 1.5%) did not have a statistically significant impact (p >0.05) on the growth rates of *Listeria* spp. in the samples. • The growth of *Listeria* spp. was supported in all milk substitutes at 20 °C regardless of the type.	Bartula *et al.* (2024)

1.4.4.1 Growth of *Listeria* spp. in RTE plant-based dairy and meat substitutes

A microbiological challenge test study conducted by Van Paepeghem *et al.* (2024) on RTE plant-based sliced meat substitutes, fresh-cut leafy vegetable mixes, and multi-ingredient salad bowls from production facilities (n=150) included an analysis of 51 vegetarian and vegan deli sandwich slices (Table 4). *L. monocytogenes* was detected in one plant-based deli sandwich slice sample at <10 cfu/g. The growth potential of *L. monocytogenes* was evaluated in three samples. No significant bacterial growth was observed in any of the deli slice samples suggesting a low risk of bacterial growth during the shelf-life (36 or 45 days). This may be due to the use of additives with antimicrobial properties, such as potassium and sodium acetate, as well as competition for growth with other bacteria such as lactic acid bacteria (LAB), and the application of modified atmosphere packaging with elevated carbon dioxide levels (50% or 30%).

In contrast, Bartula *et al.* (2024) demonstrated significant growth of *L. monocytogenes* and *L. innocua* in all plant-based milk substitutes (n=17), with increases of 2.6 to 3 log_{10} within 16 hours at room temperature, regardless of product composition (sugar, fat, protein, or fibre) (Table 4). Both sugar-containing and sugar-free versions (0 to 3.3%), of oat and soya samples supported *Listeria* growth (2.6 to 2.8 and 3.2 to 3.3 log_{10} cfu/ml within 16 hours). Oat-based samples with varying fat levels (1.1% to 3.5%) all supported *Listeria* growth (2.6 to 2.8 log_{10} cfu/ml within 16 hours and 3.2 log_{10} cfu/ml in 24 hours). Soya-based samples with 3.3 to 5 g of protein supported similar *Listeria* growth (>3 log_{10} cfu/ml within 16 hours). Pea-based samples with added fibre from either locust bean gum or sodium alginate (0 to 1.5%) also supported *Listeria* growth. *Listeria* growth with locust bean gum ranged from 3 to 3.3 log_{10} cfu/ml in 16 hours and 4 to 4.5 log_{10} cfu/ml in 24 hours. *Listeria* growth with sodium alginate ranged from 3 to 3.2 log_{10} cfu/ml within 16 hours.

The study by Bartula *et al.* (2024) emphasises that plant-based substitutes, despite significant differences in their nutritional profiles, all serve as suitable growth media and are susceptible to *L. monocytogenes* contamination. UHT treated plant-based substitutes can be safely stored unopened at room temperature for up to 12 months. However, there is no standardised guideline regarding the optimal storage duration after opening. Product labels typically recommend storing opened items in refrigeration for 5 to 10 days, but compliance with these recommendations among consumers remains uncertain.

Kain *et al.* (2024) also observed *L. monocytogenes* growth in 26 plant-based milk substitutes and one organic whole milk from retail premises. Samples were inoculated with 100 cfu/ml of a four-strain mix of *B. cereus, L. monocytogenes*, and *Salmonella*. *L. monocytogenes* grew to

10^8 to 10^9 cfu/mL at 22 °C after six weeks (Table 4). This indicates that plant-based milk substitutes may allow growth under suitable conditions if they are contaminated with pathogenic bacteria, such as *L. monocytogenes*.

1.4.4.2 Growth of *Salmonella* and *B. cereus* in RTE plant-based dairy and meat substitutes

Kain *et al.* (2024) reported that, under challenge conditions, *Salmonella* increased to between 10^8 to 10^9 cfu/ml (Table 4). Additionally, the researchers identified *B. cereus* group isolates in three plant-based milk samples during challenge testing. All isolates produced high levels of enterotoxins and shown cytotoxic effects on human intestinal cell lines. Under optimal conditions, *B. cereus* populations reached maximum counts of 10^6 to 10^7 cfu/ml.

1.4.4.3 Physico-chemical characteristics in RTE plant-based dairy and meat substitutes

Across the microbiological challenge test studies on RTE plant-based dairy and meat substitutes (Table 4), most samples exhibited pH values above 5.0 and a_w levels above 0.94, conditions favourable for microbial growth including growth of pathogens such as *L. monocytogenes* and *B. cereus*. This data is detailed in Table 5.

Table 5 Physico-chemical characteristics of RTE plant-based dairy and meat substitutes analysed by microbiological challenge test studies

Sample Type	Sample Size	pH Range	a_w Range	Reference
RTE plant-based sliced vegetarian and vegan deli sandwich slice substitutes, from production premises.	51	• pH (day of purchase): 4.7 to 7.3, median of 5.8 • pH (end of shelf life): 4.5 to 7.2, median of 5.6	• a_w (day of purchase): 0.927 to 0.983, median of 0.974 • a_w (end of shelf life): 0.926 to 0.984, median of 0.973	Van Paepeghem *et al.* (2024)
	3 out of 51	• Mean pH (day of purchase): 5.4 ± 0.1 (Vegan) • Mean pH (end of shelf life): 4.7 ± 0.6 (Vegan) • Mean pH (day of purchase): 5.6 ± 0.0 (Vegetarian) • Mean pH (end of shelf life): 4.7 ± 0.1 (Vegetarian)	• Mean a_w (day of purchase): 0.977 ± 0.00 (Vegan) • Mean a_w (day of purchase): 0.973 ± 0.001 (Vegetarian) • Mean a_w (end of shelf life): Not determined	
Plant-based milk substitutes and one organic whole milk product from retail premises.	27	*A representative selection of sample pH after six weeks shown below:* • Oats (10%): 6 to 3 • Soya (8.7%): 7 to 5.3 • Almond (2.3%): 6 to 7.3 • Cashew (6.5%): 6 to 5 • Rice (14%): 5 to 4 • Hemp (3.5%): 7 to 6.7 • Hazelnut (2.8%): 7 to 4 • Coconut (8%): 7 to 4.8 • Organic whole milk: 7 to 6	• Not tested	Kain *et al.* (2024)

1.4.5 Microbiological prevalence and challenge test studies of non-RTE plant-based meat substitutes

Microbiological prevalence and challenge test studies on non-RTE plant-based meat substitutes are summarised in Table 6. These studies provide a comparison of contamination levels between RTE and non-RTE plant-based dairy and meat substitutes.

Table 6 Microbiological prevalence and challenge test studies of non-RTE plant-based meat substitutes

Year	Country	Sample Type	Sample Size	Study Type	Test Parameters	Microbial Findings			Reference
2023	Germany	Non-RTE MAP and frozen plant-based ground meat substitutes from retail premises.	10	Preva-lence study	**Detection of:** 1. *Listeria* spp. 2. Sulphite-reducing *Clostridium* spp. **Enumeration of:** 1. *B. cereus*. 2. CPS 3. Enterobacteriaceae 4. Enterococci 5. LAB. pH and a_w (Table 7)	• Enterobacteriaceae, Enterococci, Presumptive *B. cereus*, *Listeria* spp., and *S. aureus* enumerated at median counts of <0.7 to 1.0 log_{10} cfu/g. • *L. monocytogenes* not detected. • Highest presumptive *B. cereus* level was 3.15 log_{10} cfu/g, 2.70 log_{10} cfu/g for *Listeria* spp. and 3.04 log_{10} cfu/g for *S. aureus*. • *Clostridium* spp. detected in seven samples. • TVC ranged from 0.70 to 8.32 log_{10} cfu/g, mean of 4.33 ± 1.95 log_{10} cfu/g, and median of 3.90 log_{10} cfu/g. • LAB ranged from 0.70 to 7.98 log_{10} cfu/g, mean of 3.75 ± 2.11 log_{10} cfu/g, and median of 3.30 log_{10} cfu/g.			Kabisch *et al.* (2023)
2023	USA	Non-RTE vacuum packaged ground beef, soya-based meat, and pea-based meat substitutes from retail premises.	3	Chall-enge study	Enumeration of the TVC, LAB, yeast and mould, and coliforms. Challenge testing to assess the growth potential of *E. coli* O157: H7, *L. monocytogenes*, and *Salmonella*. pH (Table 7)	**After 4 °C for 7 days:** (log_{10} cfu/g) ***E. coli* O157:H7:** Ground beef: 2.78 ± 0.14 Soya-based: 2.75 ± 0.16 Pea-based: 2.83 ± 0.20 ***Salmonella* spp.:**	**After 22 °C for 24 hours:** (log_{10} cfu/g) ***E. coli* O157:H7:** Ground beef: 5.28 ± 0.10 Soya-based: 3.26 ± 0.32 Pea-based: 4.28 ± 0.14 ***Salmonella* spp.:** Ground beef: 6.29 ± 0.17	**After 32 °C for 24 hours:** (log_{10} cfu/g) ***E. coli* O157:H7:** Ground beef: 8.56 ± 0.04 Soya-based: 5.72 ± 0.10 Pea-based: 5.43 ± 0.08 ***Salmonella* spp.:**	Liu *et al.* (2023)

Year	Country	Sample Type	Sample Size	Study Type	Test Parameters	Microbial Findings			Reference
						Ground beef: 3.20 ± 0.14 Soya-based: 3.46 ± 0.10 Pea-based: 3.23 ± 0.15 ***L. monocytogenes*:** Ground beef: 2.80 ± 0.15 Soya-based: 2.60 ± 0.12 Pea-based: 3.65 ± 0.20	Soya-based: 4.45 ± 0.30 Pea-based: 6.33 ± 0.32 ***L. monocytogenes*:** Ground beef: 5.12 ± 0.29 Soya-based: 3.86 ± 0.19 Pea-based: 4.56 ± 0.36	Ground beef: 7.82 ± 0.50 Soya-based: 7.36 ± 0.10 Pea-based: 7.88 ± 0.46 ***L. monocytogenes*:** Ground beef: 5.69 ± 0.36 Soya-based: 5.08 ± 0.54 Pea-based: 6.30 ± 0.20	
2024	Czechia	Non-RTE plant-based meat substitutes from retail premises (chilled and frozen).	43	Preval-ence study	**Detection of:** *1. Clostridium* spp. **Enumeration of:** 1. Shiga toxin-producing E. coli (STEC) 2. *B. cereus* 3. CPS 4. TVC 5. Enterobacteriaceae 6. Yeasts and moulds 7. LAB 8. *E. coli*	• *E. coli*, STEC, and coagulase-positive *staphylococci* not detected • *Bacillus* spp. enumerated in ~50% samples with a mean count of 2.3 to 2.9 $\log_{10}$ cfu/g. • *B. cereus* enumerated in all plant-based steak samples (n=8), all meatball samples (n=6) and other samples (11 out of 13) at <1.7 $\log_{10}$ cfu/g. Detected in 10 out of 16 plant-based burger samples at <1.7 to 2.5 $\log_{10}$ cfu/g after enrichment. • *Clostridium perfringens* enumerated after enrichment in seven plant-based burger samples, one meatball sample and one other sample at <1.7 $\log_{10}$ cfu/g. • TVC ranged from 1.0 to 7.2 $\log_{10}$ cfu/g • Enterobacteriaceae enumerated in three plant-based burger samples and three other samples with mean of <2.0 $\log_{10}$ cfu/g. • CPS detected in four samples.			Dušková *et al.* (2024)

1.4.5.1 Prevalence and growth of *Listeria* spp. in non-RTE plant-based dairy and meat substitutes

In the survey by Kabisch *et al*. (2023), *L. monocytogenes* was not detected in the 10 non-RTE plant-based ground meat samples investigated. However, *Listeria* spp. (non-*monocytogenes*) was detected at low levels (1.12 ± 0.73 $\log_{10}$ cfu/g), with the highest count reaching 2.70 $\log_{10}$ cfu/g (Table 6). This low-level presence may indicate hygienic lapses or post-processing contamination in the supply chain.

Liu *et al*. (2023) demonstrated the growth potential of *L. monocytogenes* in three non-RTE vacuum packaged ground beef, soya-based meat, and pea-based meat substitutes from retail premises. After 4 °C for seven days, *L. monocytogenes* levels in all three categories ranged from 2.60 to 3.65 $\log_{10}$ cfu/g. After 22 °C for 24 hours, *L. monocytogenes* levels ranged from 3.86 to 5.12 $\log_{10}$ cfu/g. After 32 °C for 24 hours, *L. monocytogenes* ranged from 5.08 to 6.30 $\log_{10}$ cfu/g with pea-based meat having the highest detected level (Table 6). This study emphasises that pathogenic microorganisms have the potential to survive and grow, once incorporated into plant-based meat substitutes. Factors such as storage temperature and the type of plant proteins used can influence the behaviour and growth of *L. monocytogenes* within these products.

1.4.5.2 Prevalence and growth of *Salmonella* in non-RTE plant-based dairy and meat substitutes

A study on the growth potential of *Salmonella* in three non-RTE vacuum packaged ground beef, soya-based meat, and pea-based meat substitutes by Liu *et al*. (2023), showed that *Salmonella* can grow under temperature abuse (Table 6). After 4 °C for seven days, *Salmonella* levels in all three categories ranged from 3.20 to 3.46 $\log_{10}$ cfu/g. After 22 °C for 24 hours, *Salmonella* levels ranged from 4.45 to 6.29 $\log_{10}$ cfu/g. After 32 °C for 24 hours, *Salmonella* ranged from 7.36 to 7.88 $\log_{10}$ cfu/g with pea-based meat having the highest detected level. These findings show how pathogen levels increased substantially from 4°C to 32°C, highlighting the risk of *Salmonella* growth under inadequate storage conditions.

1.4.5.3 Prevalence of *B. cereus* in non-RTE plant-based dairy and meat substitutes

Presumptive *B. cereus* was detected at the highest level in 10 non-RTE plant-based ground meat products at 3.15 $\log_{10}$ cfu/g, as reported by Kabisch *et al*. (2023), who recorded mean counts of 1.39 ± 0.87 $\log_{10}$ cfu/g. In contrast, Dušková *et al*. (2024) found *Bacillus* spp. (mannitol-positive) in around half of all meat substitute samples with mean counts ranging from 2.3 to 2.9 $\log_{10}$ cfu/g. *B. cereus* was detected in all plant-based steak samples (n=8), all plant-based meatball samples (n=6) and other meat substitutes (11 out of 13) at <1.7 $\log_{10}$ cfu/g. *B. cereus* was detected in 10 of 16 plant-based burger samples after enrichment, with counts ranging from <1.7 to 2.5 $\log_{10}$ cfu/g (Table 6).

1.4.5.4 Prevalence of Enterobacteriaceae and growth of *E. coli* in non-RTE plant-based dairy and meat substitutes

Enterobacteriaceae were detected at low levels in non-RTE plant-based ground meat products by Kabisch *et al*. (2023), with median values near or below detection limits (0.7 to 1.0 $\log_{10}$ cfu/g). Similarly, Dušková *et al*. (2024) reported Enterobacteriaceae in plant-based burger and other plant-based meat substitute samples with mean values of <2.0 $\log_{10}$ cfu/g. *E. coli* was found after enrichment in one plant-based burger sample, indicating low but a notable prevalence (Table 6).

Liu *et al*. (2023) showed the growth potential of *E. coli* O157:H7 in non-RTE vacuum packaged ground beef, soya-based meat, and pea-based meat substitutes from retail premises (n=10). After 4°C for seven days, *E. coli* O157:H7 levels in all three categories ranged from 2.75 to 2.83 $\log_{10}$ cfu/g. After 22 °C for 24 hours, *E. coli* O157:H7 ranged from 3.26 to 5.28 $\log_{10}$ cfu/g. After 32 °C for 24 hours, *E. coli* O157:H7 ranged from 5.43 to 8.56 $\log_{10}$ cfu/g with ground beef having the highest level.

1.4.5.5 Prevalence of *Clostridium* spp. in non-RTE plant-based dairy and meat substitutes

Kabisch *et al*. (2023) identified *Clostridium* spp. in 70% of their tested samples (n=10). Dušková et *al*. (2024) reported that *C. perfringens* was not detected by direct plating but detected after enrichment in seven plant-based burger samples, one plant-based meatball sample and one other meat substitute sample at <1.7 $\log_{10}$ cfu/g each (Table 6).

1.4.5.6 Physicochemical characteristics (pH and a_w) in non-RTE plant-based dairy and meat substitutes

In the microbiological prevalence and challenge test studies on non-RTE plant-based meat substitutes (Table 6), samples exhibited pH values above 5.0 and a_w levels above 0.94, conditions favourable for microbial growth including growth of pathogens such as *L. monocytogenes* and *B. cereus*. This data is detailed in Table 7.

Table 7 Physico-chemical characteristics of non-RTE plant-based dairy and meat substitutes in the studies reviewed

Sample Type	Sample Size	Study Type	pH Range	a_w Range	Reference
Non-RTE plant-based ground meat substitutes from retail premises.	10	Microbiological prevalence study	Oats: Mean pH of 6.27 ± 0.097 Pea: Mean pH of 6.39 ± 0.036 Soya: Mean pH of 5.83 ± 0.180	Oats: Mean a_w of 0.978 ± 0.004 Pea: Mean a_w of 0.971 ± 0.002 Soya: Mean a_w of 0.98 ± 0.005	Kabisch *et al.* (2023)
Non-RTE vacuum packaged ground beef, soya-based meat, and pea-based meat substitutes from retail premises.	3	Microbiological challenge test study	**pH after 4 °C for 10 days:** ($\log_{10}$ cfu/g) Ground beef: 5.27 ± 0.12 Soya-based: 6.22 ± 0.03 Pea-based: 6.26 ± 0.11 **pH after 22 °C for 24 hours:** ($\log_{10}$ cfu/g) Ground beef: 5.44 ± 0.02 Soya-based: 6.13 ± 0.04 Pea-based: 7.45 ± 0.01 **pH after 32 °C for 24 hours:** ($\log_{10}$ cfu/g) Ground beef: 5.52 ± 0.06 Soya-based: 5.18 ± 0.01 Pea-based: 5.58 ± 0.10		Liu *et al.* (2023)

1.5 Challenges and future research

Despite the increasing popularity of plant-based foods, notable research gaps remain, particularly concerning food safety and emerging risks. The existing literature on microbiological levels in these foods includes four microbiological prevalence studies on plant-based dairy substitutes, two microbiological prevalence studies on RTE meat substitutes, and two were related to non-RTE meat substitutes. There was a total of two challenge test studies on plant-based dairy substitutes, and one was related to non-RTE meat substitutes.

A particular issue is the risk of *L. monocytogenes* in these RTE plant-based foods, which are often refrigerated and have extended shelf lives. Due to limited data on pathogen presence and growth potential, food manufacturers and regulatory authorities face challenges in assessing listeriosis risks. Previous recalls and outbreaks of foodborne illness linked to RTE plant-based meat and dairy substitutes, highlight that some food business operators producing these types of products may lack the necessary expertise to identify relevant food safety hazards. To reduce the possible risk to public health from microbiological contamination of these products, it is important that robust controls are implemented under a food safety management system. Additionally, it is essential to establish robust procedures for regulatory oversight to ensure that food businesses comply with the applicable food safety legislation.

While research has primarily focused on improving plant-based meat texture, flavour, and appearance based on this literature search, food safety concerns require equal attention. Understanding the microbiological risk factors in plant-based foods is crucial for ensuring safe consumption and market growth (Lin *et al*., 2023). Since plant-based ingredients are available in various forms, such as liquids, powders, or whole-seed derivatives, each with different microbial risks, a better understanding of microbial composition is necessary to enhance risk assessment and food safety management (Kyrylenko *et al*., 2023).

Future research should prioritise multidisciplinary approaches that integrate food technology, microbiology, and regulatory science to develop robust food safety standards. Comprehensive microbial risk assessments, improved preservation techniques, and continued regulatory monitoring will be vital in ensuring the safe expansion of the plant-based food industry.

1.6 Conclusion

There are multiple opportunities for microbial contamination of plant-based ingredients at various stages, including pre-harvest, harvest, processing, and throughout retail, catering, and domestic handling. Currently, there is a limited amount of microbiological prevalence data on RTE plant-based dairy and meat substitutes. For this reason, guidelines on the application of Good Hygiene Practices (GHP) for the control of *L. monocytogenes* in RTE foods throughout the food chain, from primary production to consumption, have been revised in December 2025 by the Codex alimentarius (Joint FAO/WHO Food Standards Programme CODEX Committee on Food Hygiene, 2025). Nonetheless, the growing demand for these products has prompted further research into their microbiological safety.

The findings of the research articles related to microbiological safety and associated risks of RTE plant-based dairy and meat substitutes highlight the significance of maintaining good hygiene practices, proper storage conditions, and strict adherence to manufacturing protocols to limit contamination with microbial hazards, as is the case for producing any type of RTE food in general. A high proportion of the studied plant-based products reported in the literature met microbiological safety standards. However as shown in Table 1, there have been occasional outbreaks of *L. monocytogenes* and *Salmonella* linked to RTE plant-based dairy substitutes. This highlights the potential risks associated with these products if they become contaminated with pathogens. While marketed as healthy and sustainable substitutes, plant-based foods are also susceptible to microbial contamination and should be manufactured under a robust food safety management system.

Although many tested products analysed in the prevalence surveys reviewed and detailed in Table 2 were microbiologically satisfactory, there was the occasional detection of pathogens such as *L. monocytogenes, B. cereus* and *S. aureus* in RTE and non-RTE meat substitutes. Elevated levels of hygiene indicators such as *E. coli* and Enterobacteriaceae were also reported occasionally, mostly in RTE cheese substitutes. Some of the studies reviewed investigated the physico-chemical characteristics of the samples tested. Certain plant-based products such as milk, cheese and meat substitutes had high a_w and/or pH levels, indicating that these products may be at higher risk for microbial growth throughout the shelf-life. The detection of pathogens, even at low levels, indicates potential vulnerabilities that may arise from inconsistent hygiene during manufacturing, cross-contamination, or inadequate temperature control during storage and distribution. Also, the challenge test studies reviewed demonstrated that pathogens can grow in these products to high levels if they are contaminated, posing a high risk to consumers (Table 4).

2 Microbiological prevalence survey

2.1 Aim of the microbiological prevalence survey

The main aims of this survey were to:

1. Determine the microbiological quality of RTE plant-based dairy (milk, cream and cheese), meat and fish substitutes at or as close to the end of the shelf-life duration as possible[1]
2. Generate relevant prevalence data that can be used for future microbial risk assessment of these products,
3. Collaborate with other Member States in order to generate relevant scientific evidence,
4. Raise awareness regarding the possible food safety risks for consumers of RTE plant-based dairy, meat and fish substitutes.

The generated dataset and country reports are available at: https://doi.org/10.5281/zenodo.21398411.

3 Data and methodology

3.1 Test parameters

All project partners were required to test the samples for the following parameters:

- *L. monocytogenes* (enumeration and detection method)
- *Listeria* spp. (enumeration and detection method)

Project partners were required to test the samples for at least four of the following additional parameters depending on the testing capacity and capability of the laboratory:

- pH
- Water activity (a_w)
- *Salmonella* spp. (detection method)
- *E. coli* (enumeration method)
- Shiga toxin-producing *E. coli* (STEC)
- *B. cereus* or *Bacillus* spp. (enumeration method)

[1] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before expiry dates that extended beyond 30th November 2025 were sampled at the start of the survey period and stored to let their shelf-life progress. These samples were then tested at the end of the survey period as close to the expiry date as possible, to increase the likelihood of detecting any microbial contamination that may be present.

- *C. perfringens* or *Clostridium* spp. (enumeration method)

3.2 Analytical test method

The samples were tested using the most recent edition of the relevant ISO reference method for each test parameter listed, or an equivalent alternative analytical method which is validated against the relevant ISO reference method in accordance with the protocol set out in standard EN ISO 16140-2.

3.3 Sample collection

While the present survey was initially funded to investigate the microbiological quality of RTE plant-based dairy milk, cheese and meat substitutes, in agreement with EFSA the scope was increased at the beginning of the survey (1st March 2025) to include RTE dairy cream and fish substitutes. This was because some Member States reported seeing these types of products placed on the market in addition to plant-based dairy milk, cheese and meat substitutes.

Samples of RTE plant-based dairy (milk, cream and cheese), fish and meat substitutes were collected while placed on the market at retail level across 15 Member States and tested over a 9-month period from 1st March to 30th November 2025. The prevalence survey investigated the microbiological quality of pre-packed RTE plant-based dairy, meat and fish substitutes in the EU such as:

- Plant-based milks and creams (e.g. oat, soya, rice, coconut etc.)
- Vegan/vegetarian cheese substitutes (e.g. plant-based alternatives to cheddar, brie, mozzarella, cheese spread etc.)
- Vegan/vegetarian meat substitutes (e.g. plant-based alternatives to deli meat slices, chicken pieces, pepperoni etc.)
- Vegan/vegetarian fish substitutes (e.g. plant-based alternatives to smoked salmon, tuna etc.)
- RTE dairy or meat alternatives made from ingredients such as tofu, tempeh or seitan.

The survey did not investigate the microbiological quality of non-RTE plant-based meat or fish substitutes, or plant-based butter spreads, desserts, yogurts or ice cream as these were not within the scope of the survey.

3.4 Type of establishments

Suitable RTE plant-based dairy, meat and fish substitutes were sampled by project partners from the following establishments:

- Food retailers such as supermarkets, corner shops, convenience stores, petrol stations etc.
- Specialist retailers such as health food shops, pharmacies, artisan retailers, farm shops etc.
- Online retailers.

3.5 Testing stage for samples

3.5.1 Chilled RTE plant-based dairy and meat substitutes

Chilled products labelled with a use by or best before date were stored at refrigerated temperatures in the laboratory and tested at the end of the foods labelled shelf-life if it expired prior to the end of the survey period (30th November 2025). This was in order to assess the worst-case scenario for contamination and growth of *L. monocytogenes* during the shelf-life of the foods, and to increase the likelihood of identifying any other microbial contamination that may have been present.

3.5.2 Ambient RTE plant-based dairy labelled with a best before date

Preliminary work conducted ahead of starting the survey, showed that most ambient RTE plant-based milk substitutes placed on the market in the countries participating in the survey were labelled with best before dates and had an extended shelf-life duration of approx. 12 months. Consequently, sampling and testing these long shelf-life products at the end of their designated best before date posed logistical challenges within the survey timeframe (1st March – 30th November 2025). Therefore, it was agreed with EFSA that samples of ambient RTE plant-based dairy milk substitutes labelled with a long shelf-life duration could be collected as soon to the start of the survey period as possible. These were then stored at ambient temperatures to allow some shelf-life to elapse. They were tested towards the end of the survey period. This approach facilitated testing of ambient RTE dairy milk substitutes as near as possible to their labelled shelf-life expiration date, thereby increasing the likelihood of detecting any microbial contamination issues.

3.6 Transport and storage of samples

3.6.1 Chilled RTE plant-based substitutes

Chilled samples were transported to the laboratory in cool boxes/bags fitted with ice packs. During transport and storage, freezing and thawing was avoided. Samples were transported and stored at or below the storage temperature given on the food product label as applicable. The following refrigeration temperatures were used if this was not explicitly stated on the label:

- Refrigeration temperatures during transport of 5 ± 3ºC (ISO 7218:2024[2])
- Refrigeration temperatures during storage of 5 ± 3ºC (ISO 7218:2024).

3.6.2 Ambient RTE plant-based substitutes

Any plant-based RTE dairy, meat or fish substitutes without specific refrigeration or freezing instructions sampled during the survey were considered ambient products (e.g. ultra-heat-treated shelf-stable RTE plant-based milk substitutes, canned RTE fish substitutes).

Ambient samples were transported to the laboratory in a cool box or crate without cool packs to avoid temperature fluctuations. Unopened samples were transported and stored at or below the storage temperature specified by the manufacturer on the label. The following ambient transport and storage temperatures were used if this was not explicitly stated on the label:

- Ambient temperature during transportation of ≤ 27ºC
- Ambient temperature during storage prior to testing of ≤ 27ºC

3.7 Interpretation of microbiological test results

The microbiological test results generated by this survey were assessed for compliance using the microbiological criteria set out in Table 8.

[2] ISO 7218:2024 Microbiology of the food chain — General requirements and guidance for microbiological examinations https://www.iso.org/standard/79508.html

Table 8 Microbiological criteria limits for RTE plant-based dairy and meat substitutes

Test parameter	Test method	Satisfactory	Borderline	Unsatisfactory	Test designation basis
L. monocytogenes	Enumeration	<100 cfu/g	Not applicable	>100 cfu/g[3]	Food Cat. 1.2 & 1.3 in Commission Regulation (EC) No 2073/2005, as amended
	Detection	Not detected in 25 g	Not applicable	Detected in 25 g[4]	Food Cat. 1.2 in Commission Regulation (EC) No 2073/2005, as amended
Listeria spp.	Enumeration	<100 cfu/g	10 – 100 cfu/g	>100 cfu/g	FSAI Guidance Note 3 Guidelines for the Interpretation of Results of Microbiological Testing of RTE Foods Placed on the Market[5]
	Detection	Not detected in 25 g	Not applicable	Detected in 25 g	
Salmonella spp.	Detection	Not detected in 25 g	Not applicable	Detected in 25 g	
E. coli	Enumeration	<20 cfu/g	20 – 100 cfu/g	>100 cfu/g	
STEC	Detection	Not detected in 25 g	Not applicable	Detected in 25 g	
Bacillus cereus[6]	Enumeration	<1000 cfu/g	1000 – 100000 cfu/g	>100000 cfu/g	
Clostridium perfringens[7]	Enumeration	<10 cfu/g	10 – 10000 cfu/g	>10000 cfu/g	

[3] According to footnote 5 of the Food Safety Criteria in Chapter 1 of Regulation 2073/2005, this criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life.
[4] The designation of this test result as ‘unsatisfactory’ will depend on certain factors. For the purposes of this retail prevalence survey, this test was carried out for information purposes only as until 30th June 2026 there is no legal criterion for the ‘not detected in 25 g’ limit in Commission Regulation (EC) No 2073/2005 to assess compliance in RTE products placed on the market. According to footnote 7 of the Food Safety Criteria in Chapter 1 of Regulation 2073/2005, this criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life. Thus, if the product was tested while it was placed on the market within its labelled shelf-life, and *L. monocytogenes* was detected in the product at <100 cfu/g, the risk should be assessed under Art. 14 of Regulation 178/2002, as amended. Any risk management actions required should be taken under that basis.
[5] France and Austria assessed the test results for *B. cereus* and *C. perfringens* respectively, according to national guidelines (Ministrère De L’Agriculture, De L’Agro-Alimentaire et De La Souverainetè Alimentaire, 2023; Kommunikationsplattform Verbraucher:innengesundheit, 2025). The recommended limits for *B. cereus* group enumeration result in Finland are lower than those presented in this report: borderline 100 cfu/g, and unsatisfactory 1,000 cfu/g (Finnish Food and Drink Industry, 2022).
[6] Using the reference method ISO 7932, the result is reported as ‘presumptive *B. cereus*’. The term ‘presumptive’ is used to acknowledge the fact that it is difficult to distinguish *B. cereus* from other closely related but less commonly encountered *Bacillus* species, such as *B. anthracis, B. thuringiensis, B. weilhenstephanensis* and *B. mycoides.* Occasionally, *Bacillus* spp. other than *B. cereus* can be pathogenic if toxin producing. FSAI Guidance Note 3 only has a published guideline microbiological criterion limit for *B. cereus* in RTE food. It does not have a guideline microbiological criterion limit for any other *Bacillus* spp.
[7] FSAI Guidance Note 3 only has a published guideline microbiological criterion limit for *C. perfringens* in RTE food. It does not have a guideline microbiological criterion limit for any other *Clostridium* spp.

4 Results

4.1 Tested parameters

All project partners (n=15) tested a total of 1,533 single samples for *L. monocytogenes* and *Listeria* spp. The majority of project partners assessed the physiochemical characteristics of pH (n=14) and a_w (n=11) in the samples (Figure 2). The detection for *Salmonella* spp. and STEC was carried out by 13 and eight partners respectively, while enumeration of *E. coli,* was carried out by 12 partners. Seven partners tested samples for *B. cereus* and four for *Bacillus* spp. Five partners tested samples for *Clostridium* spp., while three partners specifically tested for *C. perfringens*.

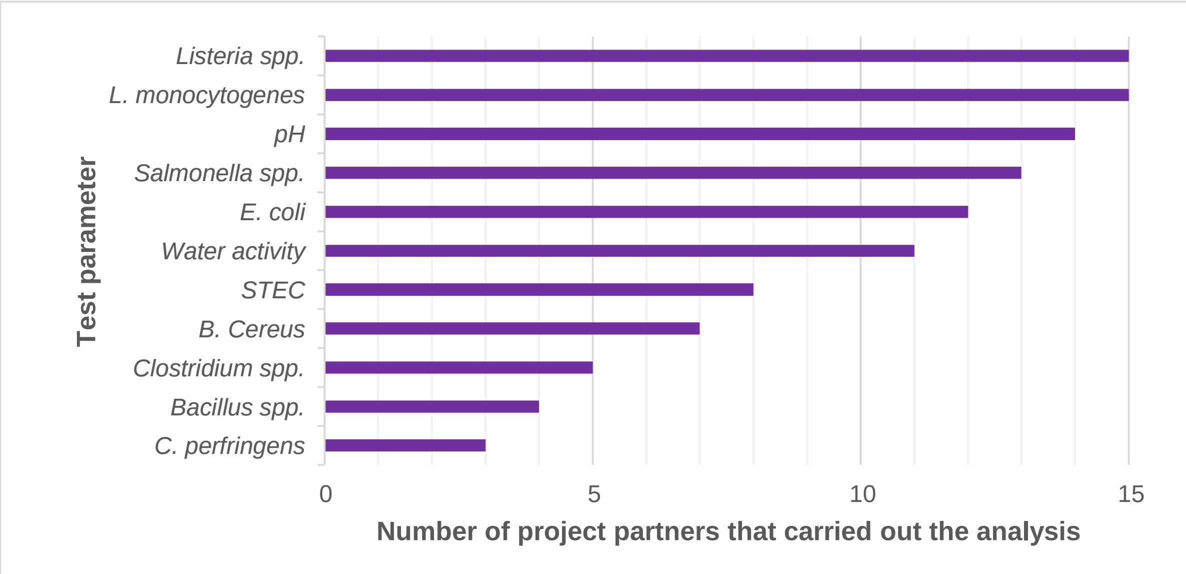


Figure 2 Number of project partners conducting each microbiological test parameter

4.2 Type of establishments

Samples of RTE plant-based dairy, meat and fish substitutes were collected from various establishment types across 15 countries (Table 9). The majority of samples (n=1,254) were collected from traditional retail shops (e.g. supermarkets, corner shops, convenience stores, petrol stations). Plant-based products are often sold in specialist retailers such as health food shops that specifically target a health-conscious consumer. Considering some consumers perceive plant-based products to offer health benefits compared to the same type of animal-derived product, they are willing to spend more money on these products. The present survey ensured that samples were collected from these types of retailers where they existed (n=182) as they often stock specialist brands that are not available in traditional retailers such as supermarkets. However, specialist health-food shops were only present in some countries participating in the survey (n=9). Additionally, there is a growing number of online retailers offering plant-based substitutes for sale. These are often offered for home delivery which increases the potential risk of the cold-chain not being maintained, allowing any microorganisms present in them an opportunity to grow due to temperature abuse for a certain time period. The present survey included these samples where available in the countries participating (n=98).

Table 9 Number of samples of RTE plant-based dairy, meat and fish substitutes collected from various types of establishments across 15 countries

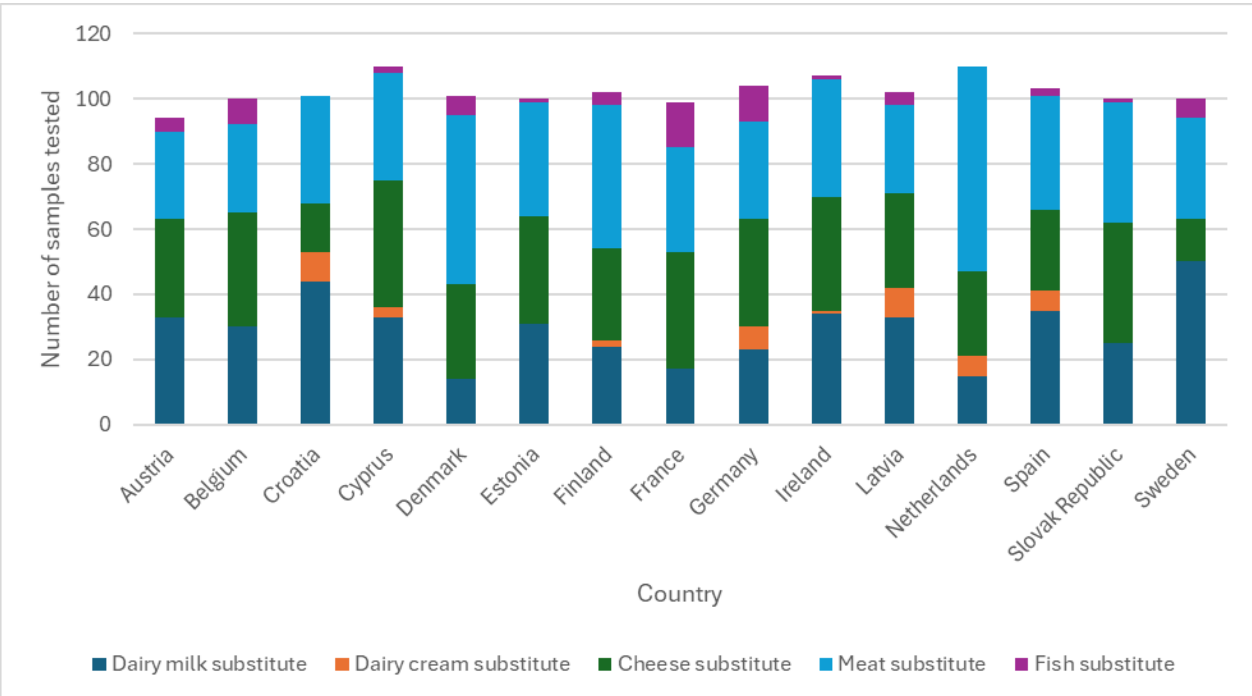
Country	Establishment			Sub-total
	Retail shop[8]	Health food shop[9]	Online retailer[10]	
Austria	76	18	0	94
Belgium	100	0	0	100
Croatia	66	35	0	101
Cyprus	58	36	16	110
Denmark	101	0	0	101
Estonia	96	2	2	100
Finland	102	0	0	102

[8] Includes food retailers such as supermarkets, corner shops, convenience stores, petrol stations, artisan retailers, farm shops etc.
[9] Includes specialist retailers such as health food shops that specifically target a health-conscious consumer or non-prescription health food items sold in pharmacies
[10] Products were purchased from online retailers for delivery

Country	Establishment			Sub-total
	Retail shop[8]	Health food shop[9]	Online retailer[10]	
France[11]	48	6	46	100
Germany	97	0	7	104
Ireland	73	25	9	107
Latvia	76	18	8	102
Netherlands	107	3	0	110
Slovakia	90	0	10	100
Spain	64	39	0	103
Sweden	100	0	0	100
Grand total	**1254**	**182**	**98**	**1534**

4.3 Sample type collected

Each project partner divided the sample allocation into approximately equal thirds: plant-based milks, plant-based cheeses, and plant-based RTE meats, depending on what product types were available in each country. Additionally, samples of plant-based RTE fish substitutes and dairy cream were collected if available (Figure 3).



[11] One sample that was collected was removed from the subsequent analysis. Although identified as a vegetarian product, it was composed mainly of egg white, which did not meet the selection criteria for plant-based substitutes.

Figure 3 Number of each type of RTE plant-based substitute tested within the survey, categorised by country

The majority of RTE plant-based milks collected had a long shelf-life duration at ambient storage temperatures and were labelled with best before dates compared to RTE plant-based cheese, meat and fish (Figure 4). It is interesting to note that while most RTE cheese, meat and fish substitutes were chilled products, the majority (76%, 60% and 77% respectively) were labelled with best before dates rather than use-by dates.

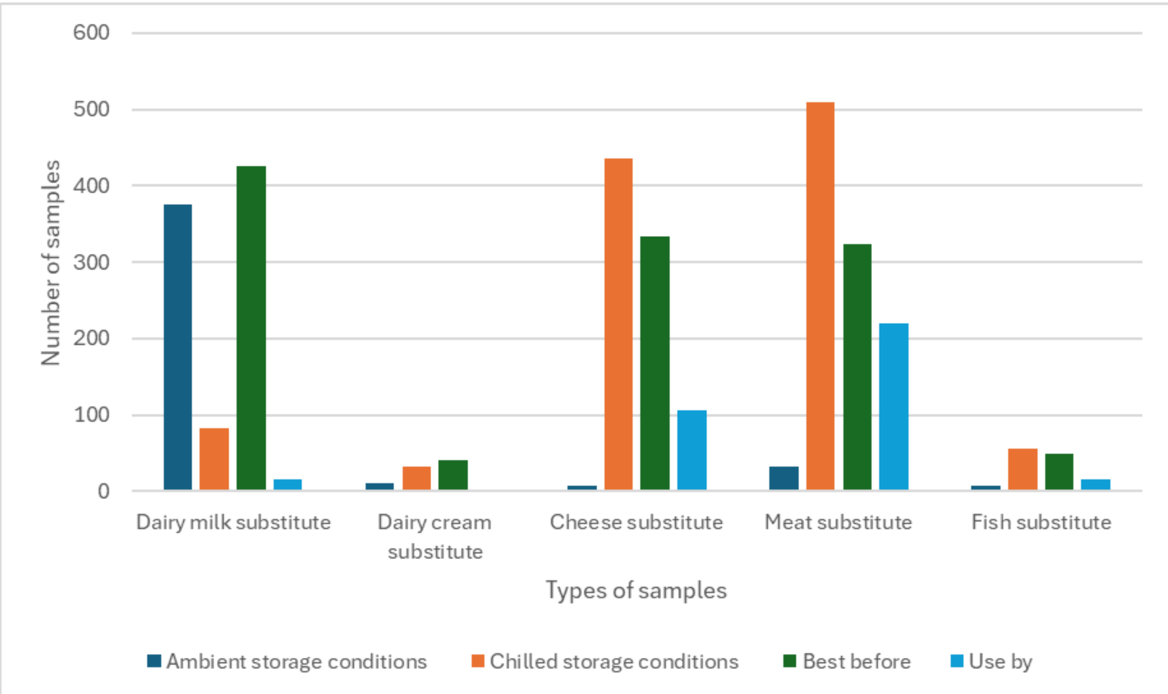


Figure 4 The storage conditions and shelf-life information provided on the label for the tested samples

The plant-based ingredients in the sample types collected varied considerably. There was a huge variety of RTE plant-based dairy, meat and fish substitutes available on the retail market across all 15 countries that took part in the survey. When the plant-based products were classified based on their primary plant-derived ingredients (Table 10), it was observed that various types of plant bases predominate in different product categories.

The majority of dairy milk substitutes tested were primarily made from oats (n=145), followed by soya (n=63) and almond (n=52). Oats are a popular choice for these types of products as they have a neutral flavour, creamy texture, and can be sustainability grown. Coconut was identified in numerous dairy-cream substitutes (n=12) and cheese alternatives (n=190), likely due to its high fat content and its ability to contribute to mouthfeel, thereby mimicking certain sensory characteristics of traditional dairy products.

In the RTE meat substitute category, soy (n=174) and wheat (n=61) were the predominant ingredients. This is consistent with longstanding industry practices, as these ingredients have been widely used as analogues in both RTE and non-RTE meat substitute products. The number of RTE plant-based fish samples available for testing was small (n=64) in comparison to the other product categories. Seaweed (n=13) was the most used main plant-based ingredient in the fish substitute samples. This suggests the fish alternative market is still emerging and relies heavily on blended formulations and marine plants.

Some primary plant-based ingredients appear only in a small number of samples such as jackfruit, mushroom, lentils, olives (meat substitutes), cauliflower, cucumber, walnut (cheese substitutes), carrot, corn (fish substitutes), hemp, hazelnut, macadamia (milk substitutes). Multi-ingredient formulations are widely used in the products sampled, with formulations containing more than two plant-based ingredients identified in 71 milk substitutes, 14 cream substitutes, 123 cheese substitutes, 173 meat substitutes, and 29 fish substitutes. This underscores the importance of blended formulations, particularly for cheese and meat analogues, where achieving the desired texture and functionality presents significant challenges. This is not surprising as most plant-based foods are complex to manufacture and would be considered highly processed foods (Uyttendaele, 2025).

Table 10 Primary plant-based ingredients in the RTE plant-based dairy and meat samples tested

Main plant-based ingredient	Number of sample types tested				
	Dairy milk substitute	Dairy cream substitute	Cheese substitute	Meat substitute	Fish substitute
Almond	52	1	11	0	0
Coconut	22	12	190	13	0
Oats	145	2	5	3	0
Soya/Tofu/Tempeh	63	6	58	174	8
Rice	41	1	0	3	1
Pea/Yellow pea/Bean/Favabean	6	0	3	55	5
Hemp	4	0	1	0	0
Wheat/Spelt/Seitan	6	0	0	61	0
Millet	4	0	0	0	0
Hazelnut	7	0	0	0	0
Tigernut	1	0	0	0	0
Cashew	3	3	36	2	0
Palm oil	1	0	2	0	0
Potato/Potato starch/Starch	2	0	5	0	1
Rapeseed/ Canola oil	5	1	3	24	0
Macadamia nuts	3	0	0	0	0
Buckwheat	1	0	0	0	0
Quinoa	1	0	0	0	0
Rye	3	0	0	0	0
Sunflower	0	3	0	8	1
Chickpeas	0	0	1	1	0
Walnut	0	0	1	0	0
Cauliflower	0	0	2	0	0
Cucumber	0	0	1	0	0
Corn	0	0	1	0	2
Carrot	0	0	0	0	1
Seaweed	0	0	0	0	13
Dietary Fibres	0	0	0	0	3
Olives	0	0	0	2	0
Mycoprotein	0	0	0	11	0
Jackfruit	0	0	0	1	0
Mushroom	0	0	0	5	0
Seeds	0	0	0	1	0
Barley	0	0	0	1	0
Bell pepper	0	0	0	1	0
Vegetable puree	0	0	0	1	0
Lentils	0	0	0	2	0
>2 plant-based ingredients[12]	71	14	123	173	29
Total	**441**	**43**	**443**	**542**	**64**
Grand total samples analysed[13]	**1533**				

[12] Contained more than one main plant-based ingredient.
[13] One sample that was collected by France (FR_058) was removed from the subsequent data analysis presented in this report. Although identified as a vegetarian product, it was composed mainly of egg white, which did not meet the selection criteria for plant-based substitutes.

4.4 Physico-chemical characteristics of the samples collected

Fourteen project partners measured the pH of the samples collected, while 11 partners measured their water activity (a_w). Table 11 displays the combined test results for all samples tested per plant-based category. The test results showed that the pH for plant-based dairy milk, cream and meat substitutes tends to be less acidic than plant-based cheese and fish. The a_w of dairy milk and cream substitutes was the highest at 0.99 ± 0.01, with plant-based cheese slightly lower. The a_w of plant-based meat and fish was the lowest of the sample types tested (0.97 ± 0.2).

The average physico-chemical characteristics measured in the samples tested show that they could support the growth of most foodborne pathogens should they become contaminated (Food Safety Authority of Ireland, 2022). The majority of the RTE plant-based products tested had physico-chemical characteristics of pH > 4.4 or a_w > 0.92 or did not have a combined pH ≤ 5.0 and a_w ≤ 0.94. This means they may be legally required to comply with the microbiological criteria set down under food category 1.2 'RTE foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes' in Commission Regulation (EC) No 2073/2005, as amended, depending on other factors.

However as shown in Table 12 certain products, and in particular plant-based cheese, did have a pH ≤ 4.4. These products did not have the physico-chemical characteristics to support the growth of *L. monocytogenes*. As such, they would be considered as falling under food category 1.3 in Commission Regulation (EC) No 2073/2005, as amended, in accordance with footnote 8 of the Regulation.

The majority of samples (93%) tested for a_w had values that were between 0.941 and ≥0.99 meaning they might support the growth of *L. monocytogenes* in accordance with footnote 8 of the Regulation but growth potential will also be influenced by the products pH value (and other factors). A small number of plant-based cheese, meat and fish samples had a_w values that were <0.920 (n=25, 2.2%). Thus, they would be unable to support the growth of *L. monocytogenes* based solely on their a_w values.

Table 11 Average pH and water activity (a_w) values of the RTE plant-based dairy and meat samples tested at or near the end of the foods labelled shelf-life

RTE Plant-based substitute[14]	pH			a_w		
	Total tested	Average	Standard deviation	Total tested	Average	Standard deviation
Dairy milk and cream	438	6.93	0.69	346	0.99	0.01
Cheese	428	4.73	0.93	338	0.98	0.02
Meat	496	5.60	0.56	376	0.97	0.03
Fish	59	4.96	0.92	53	0.97	0.02
Total samples	**1,421**	**-**	**-**	**1,113**	**-**	**-**

Table 12 Physico-chemical characteristics of RTE plant-based dairy, meat and fish substitutes tested at or near the end of the foods labelled shelf-life[15]

RTE Plant-based substitute	pH results combined for 14 countries					Total
	≤4.4	4.5 to ≤5	5.1 to ≤6	6.1 to ≤7	>7	
Dairy milk and cream	18	13	5	201	201	**438**
Cheese	219	85	79	35	10	**428**
Meat	9	68	329	81	9	**496**
Fish	20	13	20	4	2	**59**
Total (pH)	**266**	**179**	**433**	**321**	**222**	**1,421**
RTE Plant-based substitute	**a_w results combined for 11 countries**					**Total**
	≤0.920	**0.921 to ≤0.940**	**0.941 to ≤0.969**	**0.970 to ≤0.989**	**≥0.990**	
Dairy milk and cream	0	3	66	56	221	**346**
Cheese	3	17	58	161	99	**338**
Meat	19	33	93	178	53	**376**
Fish	3	1	16	27	6	**53**
Total (a_w)	**25**	**54**	**233**	**422**	**379**	**1,113**

[14] The pH was not tested for 102 samples and the a_w was not assessed for 416 samples. The pH and a_w was not determined for 10 and 4 samples, respectively

[15] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

4.5 Prevalence of vegetative foodborne pathogens

The microbiological quality of the products tested was generally good (Table 13). Overall, 1,533 samples of RTE plant-based dairy, meat and cheese were analysed for various parameters depending on the capacity of the participating laboratories to carry out the analysis for certain test parameters. One of the samples collected was excluded from the analysis by France due to its composition. Although it was originally identified as a vegetarian product, it was composed mainly of egg white and thus did not meet the selection criteria for plant-based substitutes.

4.5.1 Prevalence of *L. monocytogenes*

Of the 1,533 samples tested for *L. monocytogenes* using both the detection and enumeration method, *L. monocytogenes* was only detected in 25 g in one sample of a dairy milk substitute described as a '*Chilled light oat-based drink*' that was analysed by Finland (Table 14). However, the pathogen was not enumerable (limit of detection 1 cfu/ml). This gives a *L. monocytogenes* prevalence of 0.07% (n=1/1,533) in the overall number of samples analysed, or a prevalence of 0.2% (n=1/441) in dairy milk and cream substitutes.

The pH or a_w values were not measured for the contaminated product. However, similar oat-based drinks were analysed by other project partners. The analysis of these other products found that 97% (n=127/130) had pH values > 5.0 and a_w values that were >0.940 for 99% (n=111/112) samples. The contaminated product (FI_014) was labelled to be stored at chilled temperatures (max. +8°C) and to consume within 5 days once it was opened. While it is reasonably foreseeable that a consumer could store the unopened product for longer than the labelled shelf-life duration as it was marked with a best before date, the analysis began one day before the best before date, and no measurable growth was present. This absence of measurable growth can support the interpretation that the product does not enable the growth of *L. monocytogenes*.

4.5.2 Prevalence of *Salmonella* spp.

Thirteen project partners tested 1,332 samples collected for *Salmonella* spp. However, no positive detections were found (Table 13).

4.5.3 Prevalence of STEC

Eight project partners tested a total of 831 samples for Shiga toxin-producing *E. coli* (STEC)**.** Of these, Finland found n=4/102 samples (3.9%) PCR positive (presumptive positive STEC) but they were not able to culture viable isolates to confirm its presence in the products. These samples included two packages of pea-based frankfurter sausages from two distinct producers. The other two PCR-positive samples were from beverages: one oat-based and one soy-based with chocolate flavouring (Table 15). These results were regarded as STEC not detected in 25 g in the final report, since STEC was not culture confirmed in these samples (Table 13). There was no pH or a_w characterisation carried out on the samples.

In addition, Germany detected STEC (PCR positive, culture negative) in n=1/104 samples (0.9%). The sample (DE_060) was a plant-based dairy milk substitute made from fermented oats with calcium, ultra-high temperature, retailed at ambient storage temperatures and labelled with a best before date (Table 15). STEC was detected by using real-time PCR for *stx1*, *stx2* and *eae* gene after enrichment in buffered peptone water at 37°C according to ISO/TS13136:2012. The sample showed a weak signal for *stx2* gene after enrichment and for pooled colonies from TBX plate but culture confirmation of a viable STEC isolate was not successful. Physio-chemical characterisation of the product (DE_060) found it to have a pH of 7.5 and a_w of 0.974. Considering the product intended by the manufacturer to be stored at ambient temperatures prior to opening, both of these values would have allowed STEC to grow in the product if the cells were viable (Food Safety Authority of Ireland, 2022).

In total, n=5/831 (0.6 %) samples were PCR positive, culture negative for STEC (n=2 chilled meat substitutes labelled with use by date, n=3 ambient dairy milk substitutes labelled with best before date) (Table 15). While this is concerning, it is notable that none of these STEC isolates could be culture confirmed suggesting that the pathogens were not present in a viable state and thus the samples were not designated as unsatisfactory test results in accordance with Table 8.

Table 13 Prevalence of foodborne pathogens in RTE plant-based dairy, meat and fish substitutes at or near the end of the foods labelled shelf-life[16]

Country	RTE plant-based substitute	Total tested	*L. monocytogenes* Detected in 25 g	*L. monocytogenes* >100 cfu/g	*Salmonella* spp. Detected in 25 g	Shiga toxin-producing *E. coli* (STEC) Detected in 25 g
			Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory
Austria	Dairy milk	33	0	0	0	Not tested
	Dairy cream	0	0	0	0	Not tested
	Cheese	30	0	0	0	Not tested
	Meat	27	0	0	0	Not tested
	Fish	4	0	0	0	Not tested
Belgium	Dairy milk	30	0	0	0	Not tested
	Dairy cream	0	0	0	0	Not tested
	Cheese	35	0	0	0	Not tested
	Meat	27	0	0	0	Not tested
	Fish	8	0	0	0	Not tested
Croatia	Dairy milk	44	0	0	0	0
	Dairy cream	9	0	0	0	0
	Cheese	15	0	0	0	0
	Meat	33	0	0	0	0

[16] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Country	RTE plant-based substitute	Total tested	*L. monocytogenes* Detected in 25 g	*L. monocytogenes* >100 cfu/g	*Salmonella* spp. Detected in 25 g	Shiga toxin-producing *E. coli* (STEC) Detected in 25 g
			Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory
	Fish	0	0	0	0	0
Cyprus	Dairy milk	33	0	0	0	0
	Dairy cream	3	0	0	0	0
	Cheese	39	0	0	0	0
	Meat	33	0	0	0	0
	Fish	2	0	0	0	0
Denmark	Dairy milk	14	0	0	Not tested	0
	Dairy cream	0	0	0	Not tested	0
	Cheese	29	0	0	Not tested	0
	Meat	52	0	0	Not tested	0
	Fish	6	0	0	Not tested	0
Estonia	Dairy milk	31	0	0	0	0
	Dairy cream	0	0	0	0	0
	Cheese	33	0	0	0	0
	Meat	35	0	0	0	0
	Fish	1	0	0	0	0
Finland	Dairy milk	24	1	0	0	0
	Dairy cream	2	0	0	0	0
	Cheese	28	0	0	0	0
	Meat	44	0	0	0	0
	Fish	4	0	0	0	0
France	Dairy milk	17	0	0	0	Not tested
	Dairy cream	0	0	0	0	Not tested
	Cheese	36	0	0	0	Not tested

Country	RTE plant-based substitute	Total tested	*L. monocytogenes* Detected in 25 g	*L. monocytogenes* >100 cfu/g	*Salmonella* spp. Detected in 25 g	Shiga toxin-producing *E. coli* (STEC) Detected in 25 g
			Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory
	Meat	32	0	0	0	Not tested
	Fish	14	0	0	0	Not tested
Germany	Dairy milk	23	0	0	0	0
	Dairy cream	7	0	0	0	0
	Cheese	33	0	0	0	0
	Meat	30	0	0	0	0
	Fish	11	0	0	0	0
Ireland	Dairy milk	34	0	0	0	0
	Dairy cream	1	0	0	0	0
	Cheese	35	0	0	0	0
	Meat	36	0	0	0	0
	Fish	1	0	0	0	0
Latvia	Dairy milk	33	0	0	0	Not tested
	Dairy cream	9	0	0	0	Not tested
	Cheese	29	0	0	0	Not tested
	Meat	27	0	0	0	Not tested
	Fish	4	0	0	0	Not tested
Netherlands	Dairy milk	15	0	0	0	0
	Dairy cream	6	0	0	0	0
	Cheese	26	0	0	0	0
	Meat	63	0	0	0	0
	Fish	0	0	0	0	0
Slovakia	Dairy milk	25	0	0	0	Not tested
	Dairy cream	0	0	0	0	Not tested

Country	RTE plant-based substitute	Total tested	*L. monocytogenes* Detected in 25 g	*L. monocytogenes* >100 cfu/g	*Salmonella* spp. Detected in 25 g	Shiga toxin-producing *E. coli* (STEC) Detected in 25 g
			Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory
	Cheese	37	0	0	0	Not tested
	Meat	37	0	0	0	Not tested
	Fish	1	0	0	0	Not tested
Spain	Dairy milk	35	0	0	0	Not tested
	Dairy cream	6	0	0	0	Not tested
	Cheese	25	0	0	0	Not tested
	Meat	35	0	0	0	Not tested
	Fish	2	0	0	0	Not tested
Sweden	Dairy milk	50	0	0	Not tested	Not tested
	Dairy cream	0	0	0	Not tested	Not tested
	Cheese	13	0	0	Not tested	Not tested
	Meat	31	0	0	Not tested	Not tested
	Fish	6	0	0	Not tested	Not tested
Grand total samples analysed		**1,533**	***L. monocytogenes* Detected in 25 g**	***L. monocytogenes* >100 cfu/g**	***Salmonella* spp. Detected in 25 g**	**Shiga toxin-producing *E. coli* (STEC) Detected in 25 g**
Total number tested for *L. monocytogenes* (detection and enumeration method)		**1,533**	**1**	**0**		
Total number tested for *Salmonella* spp.		**1,332**			**0**	
Total number tested for STEC		**831[17]**				**0**

[17] Two samples were not analysed for STEC in Denmark and The Netherlands, respectively (n=4).

Table 14 Details of RTE plant-based dairy and meat substitutes in which *L. monocytogenes* was detected and/or enumerated at or near the end of the foods labelled shelf-life[18]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*L. monocytogenes* detection method	*L. monocytogenes* enumeration method	pH	a_w
FI_014 Dairy milk substitute	Chilled light oat-based drink	Water, OATS 10%, rape seed oil, acidity regulator (dipotassium phosphate E340(ii)), minerals (calcium carbonate E170, potassium iodide), salt, vitamins (D2, riboflavine E101, B12)	**Before:** Keep chilled, max. +8°C. **After:** Once opened consume within 5 days.	Best before	Supermarket	Detected in 25 ml	< 1 cfu/ml	Not tested	Not tested

[18] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Table 15 Details of RTE plant-based dairy and meat substitutes in which STEC was detected by PCR (presumptive positive) at or near the end of the foods labelled shelf-life[19]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	pH	a_w
FI_002 Meat substitute	Vege frankfurter sausage	Water, pea protein preparation (14%) (pea protein, thickening agents (E407, E425), rapeseed oil), coconut fat, thickening agent (E461), yeast extract, spice extracts	Store below +6°C	Use By Date	Supermarket	Not tested	Not tested
FI_009 Meat substitute	Vege frankfurter sausage chorizo	Water, pea protein 21%, canola oil, stabilizers (methylcellulose, carrageenan, xanthan gum, sodium alginate, konjac, guar gum), potato starch, yeast extract, vinegar powder, dextrose, spices (incl. cayenne pepper, chili), garlic, natural flavorings, salt.	Store below +°6	Use By Date	Supermarket	Not tested	Not tested

[19] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	pH	a_w
FI_063 Dairy milk substitute	Oat drink	OAT base (water, OAT 10,5%), rapeseed oil, calcium, iodized salt, vitamins (riboflavin, D3, B12)	**Before:** Store at room temperature **After:** Once opened keep in refrigerator and use within 5 days.	Best before	Supermarket	Not tested	Not tested
FI_092 Dairy chocolate milk substitute	Soy drink with chocolate	SOYA base (Water, Hulled SOYA beans (7%)), Sugar, Fat-reduced cocoa (1.5%), Calcium (Calcium carbonate), Stabilisers (Carrageenan, Guar gum, Xanthan gum), Acidity regulator (Potassium phosphates), Flavouring, Sea salt, Natural flavouring, Vitamins (B2, D2)	**Before:** Can be stored at room temperature before opening. **After:** Store in the refrigerator (+1-7°C). Once opened, it will keep for 5 days in the refrigerator.	Best before	Supermarket	Not tested	Not tested
DE_060 Dairy milk substitute	Drink made from fermented oats with calcium, ultra-high temperature	Water, fermented oats (9 %), sunflower oil, pea protein, calcium carbonate, acidity regulators: potassium phosphate, sodium carbonates; salt, stabiliser: gellan; natural flavourings	**Before:** Ambient **After**: can be kept in the refrigerator for 4 days	Best Before Date	Supermarket	7.5	0.974

4.6 Prevalence of Spore Formers

4.6.1 Prevalence of Presumptive *Bacillus cereus* or *Bacillus* spp.

Eleven project partners analysed 1,113 RTE plant-based dairy, meat and fish substitute samples for presumptive *B. cereus* (n=6 countries) or *Bacillus* spp. (n=5 countries) depending on the testing capacity of the laboratory. Of the 1,113 samples tested, 1,096 (98.5 %) were designated in accordance with Table 8 as satisfactory. This was because either *B. cereus*/*Bacillus* spp. was enumerated at levels <1,000 cfu/g or it was not enumerated above the limit of enumeration for the laboratory analysis method performed (Table 16).

For a small number of samples *B. cereus*/*Bacillus* spp. was enumerated at counts >100 cfu/g but the levels were below 1,000 cfu/g and not considered to pose a risk to public health. According to the Guidance Note 3 (Food Safety Authority of Ireland, 2020), *B. cereus* causes illness by producing toxins in food or the small intestine.

B. cereus causes two types of foodborne disease:

1. Emetic (vomiting) food poisoning – following consumption of food containing pre-formed toxin. The toxin may be formed once *B. cereus* numbers in the food exceed 100,000 cfu/g
2. Diarrhoeal food poisoning – following consumption of food containing high numbers of *B. cereus* cells (usually >1,000,000 cfu/g), which then go on to produce diarrhoeal toxin in the small intestine, or sometimes in the food.

For n=14/1,113 samples (1.3 %) the levels of *B. cereus* or *Bacillus* spp. were enumerated at between 1,000 and 100,000 cfu/g and thus were designated as borderline in accordance with the microbiological guideline criterion for *B. cereus* in Guidance Note 3 (Food Safety Authority of Ireland, 2020). Three samples (0.3 %) were found to have levels in excess of 100,000 cfu/g and were designated as unsatisfactory. However, for the countries that tested the samples for *Bacillus* spp., it should be noted that FSAI Guidance Note 3 (2020) only has a guideline microbiological criterion limit for *B. cereus* and not any other *Bacillus* spp. Thus, the guideline microbiological criterion limit for *B. cereus* in RTE food was used to assess the compliance of all test results.

Borderline levels were found in plant-based substitutes for meat (n=7), cheese (n=4), fish (n=2) made from various plant-based ingredients, three of which had tofu as the main plant-based ingredient, and in dairy cream made from coconut (n=1). Unsatisfactory levels of

>100,000 cfu/g were found in meat substitutes both made from tofu (n=2) and a cheese substitute (n=1) made from cashews (Table 16 and Table 17).

Table 16 Designation of presumptive *B. cereus*[20]/*Bacillus* spp.[21] test results in RTE plant-based dairy and meat substitutes across 11 countries at or near the end of the foods labelled shelf-life[22]

Country	RTE plant-based substitute	Total tested	*Bacillus cereus* <1,000 cfu/g	*Bacillus cereus* 1,000 - ≤100,000 cfu/g	*Bacillus cereus* >100,000 cfu/g
			Satisfactory	Borderline	Unsatisfactory
Austria (*B. cereus*)	Dairy milk	33	33	0	0
	Dairy cream	0	0	0	0
	Cheese	30	30	0	0
	Meat	27	27	0	0
	Fish	4	4	0	0
Belgium (*B. cereus*)	Dairy milk	30	30	0	0
	Dairy cream	0	0	0	0
	Cheese	35	35	0	0
	Meat	27	27	0	0
	Fish	8	8	0	0
Croatia (*B. cereus*)	Dairy milk	44	44	0	0
	Dairy cream	9	9	0	0
	Cheese	15	15	0	0
	Meat	33	32	0	1
	Fish	0	0	0	0
Cyprus (*B. cereus*)	Dairy milk	33	33	0	0
	Dairy cream	3	2	1	0
	Cheese	39	39	0	0

[20] Using the reference method ISO 7932:2004/Amd 1:2020, the test result is reported as 'presumptive *B. cereus*'. The term 'presumptive' is used to acknowledge the fact that it is difficult to distinguish *B. cereus* from other closely related but less commonly encountered *Bacillus* spp., such as *B. anthracis, B. thuringiensis, B. weilhenstephanensis* and *B. mycoides.* Occasionally, *Bacillus* spp. other than *B. cereus* can be pathogenic if toxin producing.

[21] Note FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *B. cereus* in RTE food. It does not have a guideline microbiological criterion limit for any other *Bacillus* spp. Thus, the guideline microbiological criterion limit for *B. cereus* in RTE food was used to assess the compliance of all test results.

[22] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Country	RTE plant-based substitute	Total tested	*Bacillus cereus* <1,000 cfu/g	*Bacillus cereus* 1,000 - ≤100,000 cfu/g	*Bacillus cereus* >100,000 cfu/g
			Satisfactory	Borderline	Unsatisfactory
	Meat	33	33	0	0
	Fish	2	2	0	0
Denmark (*B. cereus*)	Dairy milk	14	14	0	0
	Dairy cream	0	0	0	0
	Cheese	29	29	0	0
	Meat[23]	51	51	0	0
	Fish	6	6	0	0
Estonia (*Bacillus* spp.)	Dairy milk	31	31	0	0
	Dairy cream	0	0	0	0
	Cheese	33	33	0	0
	Meat	35	32	3	0
	Fish	1	1	0	0
Finland (*Bacillus* spp.)	Dairy milk	24	24	0	0
	Dairy cream	2	2	0	0
	Cheese	28	27	1	0
	Meat	44	41	3	0
	Fish	4	4	0	0
France (*B. cereus*)	Dairy milk	17	17	0	0
	Dairy cream	0	0	0	0
	Cheese	36	35	1	0
	Meat	32	31	1	0
	Fish	14	13	1	0
Germany (*B. cereus*)	Dairy milk	23	23	0	0
	Dairy cream	7	7	0	0
	Cheese	33	31	2	0
	Meat	30	30	0	0
	Fish	11	11	0	0
Spain (*Bacillus* spp.)	Dairy milk	35	35	0	0
	Dairy cream	6	6	0	0
	Cheese	25	24	0	1

[23] One sample (plant-based meat) failed to be analysed due to laboratory error.

Country	RTE plant-based substitute	Total tested	*Bacillus cereus* <1,000 cfu/g	*Bacillus cereus* 1,000 - ≤100,000 cfu/g	*Bacillus cereus* >100,000 cfu/g
			Satisfactory	Borderline	Unsatisfactory
	Meat	35	35	0	0
	Fish	2	2	0	0
Sweden[24] (*Bacillus* spp.)	Dairy milk	50	50	0	0
	Dairy cream	0	0	0	0
	Cheese	13	13	0	0
	Meat	31	30	0	1
	Fish	6	5	1	0
			***Bacillus cereus* <1,000 cfu/g**	***Bacillus cereus* 1,000 - ≤100,000 cfu/g**	***Bacillus cereus* >100,000 cfu/g**
		Total tested	**Satisfactory**	**Borderline**	**Unsatisfactory**
Grand total		**1,113**	**1,096**	**14**	**3**

4.6.2 Information on *B. cereus*/*Bacillus* spp. results by country

Austria:

Presumptive *B. cereus* was not detected in any of the samples (<100 cfu/g; n= 94).

Belgium:

In 1 out of 100 samples of plant-based dairy and meat/fish alternatives analysed in this survey in Belgium, presumptive *B. cereus* was detected at the detection limit of the ISO standard enumeration method (1 colony counted on the Mannitol egg Yolk Polymyxin agar (MYP-agar) being confirmed as (presumptive) *B. cereus*) referring to an estimated number of 10 CFU/g in this one sample. This sample (BE_035) was a plant-based fermented alternative to mozzarella based on an almond/cashew preparation with vegetable fibres. The finding of presumptive *B. cereus* at this low level (10 CFU/g) is still a satisfactory result.

Croatia:

[24] *Bacillus* spp. test results were defined on species level (*B. cereus* s.l. or *B. cereus/thuringiensis*) using MALDI-ToF. Dominating colonies were typed as *Peribacillus simplex* and *B. subtilis/pumilis.* It should be noted that of the dominating colonies species typed using MALDI-ToF, *B. cereus* was not identified.

All samples were analysed for *Bacillus* spp. in g using HRN EN ISO 7932:2005 method for *B. cereus* (g). *B. cereus* was enumerated in two samples (HR_068; HR_094) at levels of 350,000 and 20 cfu/g, respectively. Both samples were produced by different manufacturers. The sample with the highest level that was designated as unsatisfactory was a "Tofu grill steak" made of soy, water, shoyu. It was a chilled product labelled with a use by date, and it had a pH of 5.75 and a_w of 0.9832 (Table 17).

The second sample with 20 cfu/g was designated as satisfactory as it had a level <1,000 cfu/g. It was a cheese substitute described as a "Cream Like Mascarpone" with coconut oil as the main plant-based ingredient. It was a chilled product purchased at a supermarket that was labelled with a best before date. The pH and a_w values measured in it were 7.1 and 0.9947, respectively.

Cyprus:

Analyses were performed with the reference method ISO 7932:2004 for presumptive *B. cereus* (accredited). There were no unsatisfactory samples (>100,000 cfu/g). There was one borderline positive sample (1,000 cfu/g; CY_016) that was a dairy cream substitute manufactured containing both coconut cream and desiccated/shredded coconut (Table 17).

There were n=5 positive samples with levels of below <100 cfu/g and thus were designated as satisfactory. *B. cereus* were enumerated at >10 and <100 cfu/g in a locally (Cyprus) produced tofu with herbs (basil and garlic and others, CY_099), which was purchased online. β-glucuronidase positive *E. coli* were also enumerated at 340 cfu/g in this sample. Since three more tofu products samples (CY_098 traditional tofu, CY_100 chilli and tomato, and CY_101 fermented tofu) of the same producer were purchased and tested at the same time with negative results, it was hypothesised that the contamination source was possibly the herbs.

B. cereus was enumerated at 82 cfu/g in CY_014, a creamed coconut sample from Sri Lanka, which was purchased online. *B. cereus* was enumerated at 63 cfu/g in CY_105, a tofu steak sample from Germany. *B. cereus* was present at levels of <40 cfu/g in CY_053, a vegan cheese (Potato, Pea, Coconut, Shea) produced in Cyprus and CY_058, a tofu burger produced in Germany (same producer as CY_105 above). All other 108 samples were negative (<10 cfu/g or <100 cfu/g, depending on the levels of background microbial flora).

Denmark:

Presence of presumptive *B. cereus* was enumerated according to NMKL 67 (2010, version 6). All 100 samples had <100 cfu/g *B. cereus. B. cereus* was verified by plating on *B. cereus* agar and used to calculate the total *B. cereus* cfu/g in the food sample based on initial counting on blood agar plates. One sample (plant-based meat) failed to be analysed due to laboratory error.

Estonia:

Bacillus spp. was not detected in any of the milk substitute samples. Within the cheese substitute category, *Bacillus* spp. was detected at low levels. In four products, counts were below 40 cfu/g, while in an additional four products, enumeration results ranged from 70 to 160 cfu/g. All remaining cheese alternative samples yielded results below the detection limit.

Compared with dairy alternatives, the meat substitute category showed higher levels of contamination. *Bacillus* spp. was detected in 13 out of 35 meat substitute products (37%). Of these, five products contained <100 cfu/g, five products ranged between 100 and 1,000 cfu/g, two products exceeded 1,000 cfu/g, and one product exceeded 10,000 cfu/g.

One vegan snack sausage (EE_039) purchased online showed *Bacillus* spp. counts of 1,300 cfu/g and was designated as borderline. It additionally exhibited low-level presence of *Clostridium* spp. (<40 cfu/g). The product (EE_039) had a pH of 5.56 and a_w of 0.86. According to the product's labelling, it was intended for storage at ambient (room) temperature, and it was labelled with a best before date. A second chilled vegan snack sausage (EE_021) with a use by date and purchased at a supermarket had borderline levels of 4,600 cfu/g, with a pH of 5.66 and a_w of 0.9. One sample of chilled vegan pâté with three chilli flavour and labelled with a use by date (EE_062) was found to have borderline levels of *Bacillus* spp. (11,000 cfu/g). It had >2 plant-based ingredients and was purchased at a supermarket. It had a pH of 5.92 and a_w of 0.98.

Finland:

Bacillus spp. were isolated from food samples using an in-house method. Samples were cultured on blood agar and identified using MALDI-TOF. Species accepted by the List of Prokaryotic Names with Standing in Nomenclature (LPSN) as belonging to the genus *Bacillus* were included in the determination of *Bacillus* spp. *Bacillus* spp. was detected in 26/102 (25%) product samples. Positive samples were detected from 2 dairy milk, 10 cheese and 14 meat substitutes. Enumeration results were mainly low, as only four samples (4%) were found to have enumeration result for *Bacillus* spp. above 1,000 cfu/g. In Finland, there is no recommendation for the levels of *Bacillus* spp. in plant-based products, only for *B. cereus* group. The recommended limits for *B. cereus* group enumeration result in Finland are lower than those presented in this report: borderline 100 cfu/g, and unsatisfactory 1,000 cfu/g (Finnish Food and Drink Industry, 2022). In eight samples (8%), the enumeration result of *Bacillus* spp. was above 100 cfu/g. However, these counts also include other *Bacillus* species in addition to possible *B. cereus* group. In one cheese substitute sample (FI_102) which had >15,000 cfu/g of *Bacillus* spp., *C. perfringens* was present as well at <40 cfu/g (Table 17).

France:

Presumptive *B. cereus* (according to ISO 7932:2004) was found in seven samples. Three of them were contaminated by concentrations higher than 1,000 cfu/g. Among these contaminated samples, five belong to the “cheese substitute”, one to the “meat substitute” and one to the “fish substitute” category. The main plant-based ingredient listed in these seven samples was different (Table 17). The highest concentration of *B. cereus* was found at 15,000 CFU/g in a cheese substitute (alternative to Camembert cheese) with pH=6.95 and a_w=0.96. France used its own national guideline to assess the test results of *B. cereus* (Ministrère De L’Agriculture, De L’Agro-Alimentaire et De la Souverainetè Alimentaire, 2023).

Germany:

B. cereus was detected in 37 of 104 tested products (36 %). Within the different food categories, the prevalence was 0 % for plant-based milk substitutes, 14 % for plant-based cream substitutes, 45 % for plant-based cheese substitutes, 50 % for plant-based meat substitutes and 55 % for plant-based fish substitutes (Figure 5). The limit of detection was 1 cfu/ml for plant-based milk substitute and 10 cfu/g for the other food categories.

Only two samples showed colony counts above 1,000 cfu/g (Table 17). Interestingly, the *B. cereus* isolate from one of these samples (sample DE_024) carried the *ces* gene cluster, which is responsible for the production of the emetic toxin cereulide. Overall, 39 isolates were gathered from the 37 positive samples (two samples contained two different isolates). All of the isolates carried the enterotoxin genes *nheA/B/C*, partially combined with *hblC/D/A* or *cytK-2*. Only one isolate possessed the *ces* gene cluster and none the *cytK-1* gene. Moreover, none of the isolates was able to form parasporal crystals, a characteristic attributed to the species *B. thuringiensis*.

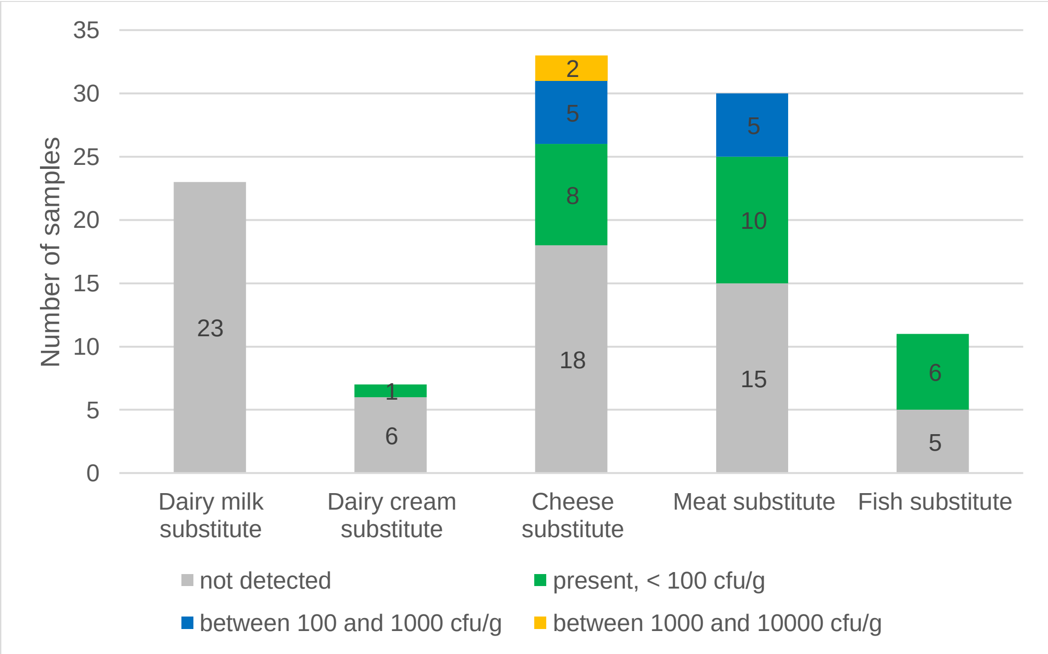


Figure 5 ***B. cereus*** **(*s.l.*) results for different food categories tested in Germany at or near the end of the foods labelled shelf-life[25]**

Spain:

Bacillus spp. was enumerated in all samples according to ISO 7932:2004/Amd 1:2020. Twelve out of 103 samples (11.6%) exceeded the enumeration limits (10 cfu/mL for liquids; 100 cfu/g for solids). Figure 6 shows *Bacillus* spp. levels (log cfu/g or mL) in these samples. According to Guidance Note 3 (Food Safety Authority of Ireland, 2020), eleven samples were classified as satisfactory (<1,000 cfu/g), while one was unsatisfactory (>100,000 cfu/g). The unsatisfactory sample was a Camembert-style cheese substitute (ES_051) with 540,000 cfu/g (Table 17). The same sample had 38,000 cfu/g *E. coli* enumerated in it. A foul odour was identified from the sample when the package was opened for testing. There was no *L. monocytogenes, Listeria* spp. or *Salmonella* spp. found in the sample.

[25] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

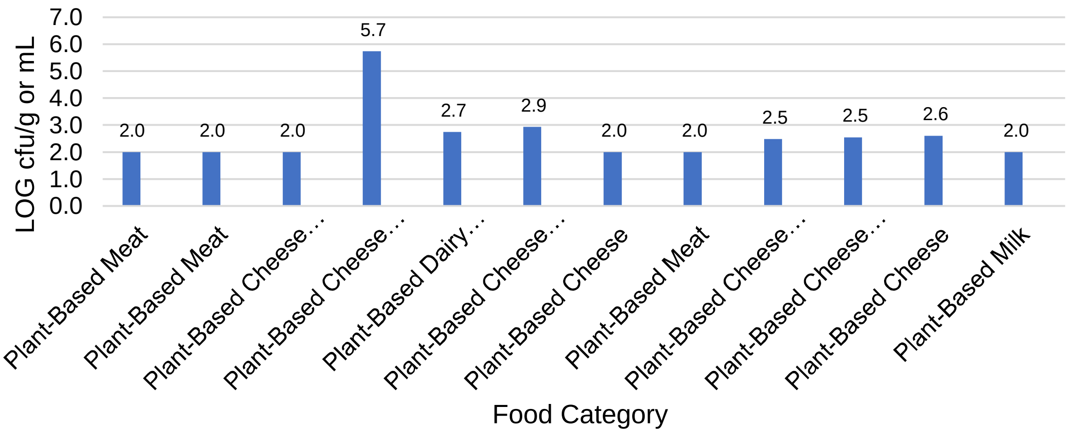


Figure 6 Levels of *Bacillus* spp. (log cfu/g or mL) in twelve plant-based substitutes tested in Spain at or near the end of the foods labelled shelf-life[26]

Half of the *Bacillus*-positive samples (n=6) were fermented products containing cultures, representing 35% of the fermented plant-based substitutes analysed (n=17). Additionally, seven samples were labelled as organic foods. Finally, the following main plant-based ingredients were identified in the positive samples: cashew (n=6), soya (n=2), >2 plant-based ingredients (n=2), coconut (n=1) and oat (n=1).

Sweden:

B. cereus/thuringiensis were found at 100 cfu/g in one product, a sliced deli meat substitute based on rapeseed oil and soya (pH 6.7 and a_w 0.95). In other samples in which growth was noticed, species identification using MALDI-ToF (Matrix-assisted laser desorption ionization–time of flight) was performed showing growth of *Penibacillus simplex* (5.3 $\log_{10}$ cfu/g) in a marinated tofu (pH 6.2 and a_w 0.95) and 3.1 $\log_{10}$ cfu/g *B. subtilis/pumilis* per gram in a vegan sauce Skagen (fish substitute) made from rapeseed oil and pea protein (pH 4.8 and a_w 0.95). Although these test results were designated as unsatisfactory and borderline respectively, it should be noted that of the dominating colonies species typed using MALDI-ToF, *B. cereus* was not identified. Other non-specific colonies that were detected on the agar plates from some samples were strains of *Lactobacillus sakei*, *Weissella Hellenica* and *Enterococcus casseliflavus* (data not shown).

[26] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Table 17 Details of RTE plant-based dairy and meat substitutes in which *B. cereus/Bacillus* spp. was enumerated at >1,000 cfu/g at or near the end of the foods labelled shelf-life[27]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[28]	pH	a_w
HR_068 Meat substitute	Tofu grill steak	Soy, Water, Shoyu	**Before:** Store between +2 and +7°C. **After**: Use within 5 days..	Use By Date	Health food shop	*B. cereus:* 350,000 cfu/g	Unsatisfactory	5.75	0.9832
CY_016 Dairy cream substitute	Santen Creamed Coconut	100% coconut (Sri Lanka)	Store at room temperature. Refrigerate (+4°C) after opening in an air tight container.	Best Before Date	Online Shop	*B. cereus:* 1,000 cfu/g	Borderline	3.78	Not tested
EE_021 Meat substitute	Piri-piri vegan snack sausage	soy protein 34%, rapeseed oil, drinking water, dried onion, coconut oil, table salt, starch, spices, vegetable fat (shea), gluten-free wheat fiber, spice extracts, aromas, smoke aroma, sugar, dried	**Before:** +2+25 C **After**: consume within 2 days	Use By Date	Supermarket	*Bacillus* spp.: 4,600 cfu/g	Borderline	5.66	0.9

[27] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

[28] FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *B. cereus* in RTE food. It does not have a guideline microbiological criterion limit for any other *Bacillus* spp. Thus, the guideline microbiological criterion limit for *B. cereus* in RTE food was used to assess the compliance of all test results.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[28]	pH	a_w
		beetroot 1.2%, antioxidant: sodium -ascorbate, water, vitamin B12, edible coating: gelling agent (alginate, cellulose), stabilizer: calcium chloride							
EE_039 Meat substitute[29]	Vegan snack sausage	Sunflower oil, soya flour 24%, water, tomato flakes 12%, carrots, egg white 2.4%, herbs 1.6%, table salt, tomato powder, starch, spices, dextrose, sodium acetate: ascorbic acid; thickener; sodium alginate; calcium chloride; guar gum;	**Before:** room temp **After**: room temp	Best Before Date	Online shop	*Bacillus* spp.: 1,300 cfu/g	Borderline	5.56	0.86
EE_062 Meat substitute	Vegan pâté with three chilli flavour	Water, carrot, pea protein, onion, garlic, coconut oil, chili mix (chipotle, jalapeno, cayenne pepper) 5,5%, nutritional yeast, salt, black pepper	**Before:** +2+6 C **After**: consume within 5 days	Use By Date	Supermarket	*Bacillus* spp.: 11,000 cfu/g	Borderline	5.92	0.98
FI_025 Meat substitute	Oat-seed balls	Whole grain oat flakes and fiber (26%, flakes from Finland and fiber from EU), water, seeds (sunflower seeds, flax seeds, pumpkin seeds [16%, EU and non-EU]), roasted onion, tomato paste (9%), crushed chili (habanero chili [4%], vinegar, salt), hardened rapeseed oil, malted whole grain rye flakes and flour, wheat flour, syrup, iodized salt, psyllium. May contain soy	Store below +6°C. MAP	Use By Date	Retail shop	*Bacillus* spp.: 2,000 cfu/g	Borderline	Not tested	Not tested
FI_061 Meat substitute	Spiced chicken-style tofu	Tofu (water, SOY beans (38%), rennet (calcium sulphate)), rehydrated SOY and WHEAT protein, vegetable oil [(sunflower,rapeseed),in varying proportions], 5.8% tomato, 5% paprika,	Store 7°C. Once opened, consume within two days.	Best Before Date	Retail shop	*Bacillus* spp.: >15,000 cfu/g	Borderline	Not tested	Not tested

[29] <40 cfu/g *Clostridium* spp. was enumerated in the same sample.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[28]	pH	a_w
		water, SOY protein, thickener (methylcellulose), WHEAT flour, potato starch, herbs and spices, natural vinegar, natural flavouring, sea salt, vitamins (B2, B12), mineral (iron)							
FI_073 Meat substitute	Plant-based balls	SOY PROTEIN (50%), SOY PROTEIN (47%), water, water-soaked WHEAT PROTEIN (14%), vegetable oils (sunflower, rapeseed in varying proportions), corn starch, stabilizers (methylcellulose, carrageenan, guar gum), salt, onion powder, protein hydrolysate (WHEAT), malt extract (BARLEY), yeast extract, beetroot powder, garlic powder, spices, flavors.	Store frozen -18°C or colder. Use immediately after opening, do not refreeze.	Best Before Date	Retail shop	*Bacillus* spp.: 1,000 cfu/g	Borderline	Not tested	Not tested
FI_102 Cheese substitute[30]	Nut and seed-based parmesan alternative	WALNUTS, sunflower seeds, nutritional yeast flakes, BRAZIL NUTS 5%, sea salt.	Store at room temperature	Best Before Date	Retail shop	*Bacillus* spp.: > 15,000 cfu/g	Borderline	Not tested	Not tested
FR079 Meat substitute	Plant-based sausage, fuet style	Pea and broad bean protein, vegan black pudding flavouring, vegetable fibre, modified starch, spices, sunflower oil	Store at the fridge. Use within 3 days after opening.	Best before date	Official Vegan Shop	*B. cereus:* 1,700 cfu/g	Borderline	4.94	0.9335
FR098 Fish substitute	Pizza topping - tofu with tomatoes and seaweed	Tofu, tomato purée, sunflower oil, yeast flakes, sea salt, spices, onions, seaweed	Store at the fridge. Consume quickly after opening	Best before date	Official Vegan Shop	*B. cereus:* 1,100 cfu/g	Borderline	5.32	0.9596

[30] <40 cfu/g *C. perfringens* and >15,000 cfu/g *Bacillus* spp. was enumerated in the same sample.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[28]	pH	a_w
FR043 Cheese substitute	Plant-based alternative to Camembert cheese	Water, cashews	Store at the fridge. Use-by date.	Use-by date	Official Vegan Shop	*B. cereus:* 15,000 cfu/g	Borderline	6.95	0.9506
DE-003 cheese substitute	Vegan spread based on coconut oil and almond protein with cucumber and garlic	water, cucumber (17 %), fully hydrogenated coconut oil (7 %), almond protein (2 %), table salt, modified starch, garlic (1 %), starch, sugar, maltodextrin, citrus fibre, spirit vinegar, dill, spice, stabiliser: guar gum; acidity regulator: sodium carbonate; preservative: potassium sorbate; acid culture	Storage at +2 °C to +8 °C	Best Before Date: 17.07.2025	Super-market	*B. cereus:* 2,100 cfu/g	Borderline	4.3	0.961
DE-024 cheese substitute	Fermented organic tofu preparation with vegetable fat and herbs, rich in omega-3	tofu 68 % (water, soya beans from Italy, lactic acid bacteria), vegetable oils 29 % (coconut oil, cold-pressed linseed oil), herbs & garlic 1.5 %, rock salt	Storage at +2 °C to +8 °C	Best Before Date: 11.07.2025	Super-market	*B. cereus:* 3,000 cfu/g	Borderline	4.4	0.952
ES_051 Cheese substitute[31]	“Camembert cheese” substitute	Cashews (76%), water, salt, vegan cultures.	Keep refrigerated between 2ºC and 4ºC. (No distinction between \`Before´ and \`After´)	Best Before Date	Health food shop	*Bacillus* spp.: 540,000 cfu/g	Unsatisfactory	6.65	0.975
SE_013	Vegan sauce Skagen	Rapeseed oil, pea protein, water, red onion, seaweed extract, lemon juice, dill, cornstarch, salt	Keep refrigerated, use within 2	Best Before Date	Supermarket	*Bacillus* spp.: 1,260 cfu/g[33]	Borderline	4.81	0.95

[31] 3.8 x10^4 cfu/g *E. coli* was found in the same sample which exhibited a foul odour when the package opened for testing.
[33] *B. subtilis/pumilis* was identified using MALDI-TOF. Of the dominating colonies species typed using MALDI-ToF, *B. cereus* was not identified.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[28]	pH	a_w
Fish substitute[32]			days of opening						
SE_020 Meat substitute	Marinated ecological tofu soya and ginger	Soybeans, water, soy sauce, ginger, spices, salt	Keep refrigerated, use within 3 days of opening	Best Before Date	Supermarket	*Bacillus* spp.: 200,000 cfu/g[34]	Unsatisfactory	6.17	0.95

[32] 10 cfu/g *Clostridium sporogenes* enumerated in the same sample.
[34] *Penibacillus simplex* was identified using MALDI-TOF. Of the dominating colonies species typed using MALDI-ToF, *B. cereus* was not identified.

4.6.3 Prevalence of *Clostridium perfringens/Clostridium* spp.

Eight project partners tested the RTE plant-based dairy, meat and fish substitute samples for either *C. perfringens* (n=3) or *Clostridium* spp. (n=5). In total, 810 samples were tested (Table 18). Of these, 712 samples (87.8%) were designated as satisfactory based on the guideline microbiological criterion of <10 cfu/g set for *C. perfringens* in the Food Safety Authority of Ireland Guidance Note 3 *Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market* (Food Safety Authority of Ireland, 2020). There were 99 samples (12.2%) designated as borderline in accordance with the guideline microbiological criterion of between 10 and 10,000 cfu/g set for *C. perfringens* in Guidance Note 3 (Food Safety Authority of Ireland, 2020). None of the samples tested were designated as unsatisfactory (>10,000 cfu/g). It was noted that in Austria, national guidelines (Kommunikationsplattform Verbraucher:innengesundheit, 2025) designate samples with <1,000 cfu/g *C. perfringens* as satisfactory.

According to the risk management actions recommended in Guidance Note 3 (Food Safety Authority of Ireland, 2020), a test result for a batch of food that is designated as borderline is not considered unsafe under Article 14, Regulation 178/2002, as amended. The food business operator should investigate the cause of the elevated levels and take measures as part of their Hazard Analysis and Critical Control Point (HACCP)-based procedures and Good Hygiene Practices (GHP) under its food safety management system to prevent counts in this batch of food (or subsequent batches) reaching unsatisfactory levels.

The physico-chemical characteristics of the RTE plant-based cheese, meat and fish substitutes that have borderline levels of *C. perfringens* or *Clostridium* spp. was measured for some of the samples (Table 19). For the samples tested, the average pH was 5.19 ± 0.74 and the average a_w was 0.92 ± 0.07. According to the Food Safety Authority of Ireland Guidance Note 18 (2022), *C. perfringens* can grow at a minimum pH of 5.5 (optimum pH 7.2) and minimum a_w of 0.93.

Table 18 Results of enumeration method for *Clostridium perfringens/Clostridium* spp.[35] in RTE plant-based dairy and meat substitutes tested in 6 countries at or near the end of the foods labelled shelf-life[36]

Country	RTE plant-based substitute	Total tested	*Clostridium perfringens* <10 cfu/g	*Clostridium perfringens* 10 - ≤10,000 cfu/g	*Clostridium perfringens* >10,000 cfu/g
			Satisfactory	Borderline	Unsatisfactory
Austria[37] **(*C. perfringens*)**	Dairy milk	33	3	30	0
	Dairy cream	0	0	0	0
	Cheese	30	0	30	0
	Meat	27	0	27	0
	Fish	4	0	4	0
Croatia (*Clostridium* spp.)	Dairy milk	44	44	0	0
	Dairy cream	9	9	0	0
	Cheese	15	15	0	0
	Meat	33	33	0	0
	Fish	0	0	0	0
Cyprus (*Clostridium* spp.)	Dairy milk	33	33	0	0
	Dairy cream	3	3	0	0
	Cheese	39	38	1	0
	Meat	33	33	0	0
	Fish	2	2	0	0
Denmark (*C. perfringens*)	Dairy milk	14	14	0	0
	Dairy cream	0	0	0	0
	Cheese	29	29	0	0

[35] Note FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *C. perfringens* in RTE food. It does not have a guideline microbiological criterion limit for any other *Clostridium* spp. Thus, the guideline microbiological criterion limit for *C. perfringens* in RTE food was used to assess the compliance of all test results.

[36] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

[37] All but five results <100 cfu/g (<1,000 cfu/g considered satisfactory in Austria).

Country	RTE plant-based substitute	Total tested	*Clostridium perfringens* <10 cfu/g	*Clostridium perfringens* 10 - ≤10,000 cfu/g	*Clostridium perfringens* >10,000 cfu/g
			Satisfactory	**Borderline**	**Unsatisfactory**
	Meat	52	51	1	0
	Fish	6	6	0	0
Estonia (*Clostridium* spp.)	Dairy milk	31	31	0	0
	Dairy cream	0	0	0	0
	Cheese	33	32	1	0
	Meat	35	32	3	0
	Fish	1	1	0	0
Finland (*Clostridium* spp.)	Dairy milk	23[38]	23	0	0
	Dairy cream	2	2	0	0
	Cheese	27[11]	26	1	0
	Meat	44	44	0	0
	Fish	4	4	0	0
Germany (*C. perfringens*)	Dairy milk	23	23	0	0
	Dairy cream	7	7	0	0
	Cheese	33	33	0	0
	Meat	30	30	0	0
	Fish	11	11	0	0
Sweden (*Clostridium* spp.)	Dairy milk	50	50	0	0
	Dairy cream	0	0	0	0
	Cheese	13	13	0	0
	Meat	31	31	0	0
	Fish	6	5	1	0
			***Clostridium perfringens* <10 cfu/g**	***Clostridium perfringens* 10 - ≤10,000 cfu/g**	***Clostridium perfringens* >10,000 cfu/g**
		Total tested	**Satisfactory**	**Borderline**	**Unsatisfactory**
Grand total		**810**	**711**	**99**	**0**

[38] Two samples (plant-based milk and cheese) were not analysed for *Clostridium* spp.

4.6.4 Information on *C. perfringens/Clostridium* spp. results by country

Austria:

C. perfringens was enumerated at levels <100 cfu/g in 87 samples, two samples were enumerated at <10 cfu/g, and five samples had counts of <1,000 cfu/g. The samples were obtained through official market control and levels <1,000 cfu/g are considered satisfactory in Austria (Kommunikationsplattform Verbraucher:innengesundheit, 2025). The five samples with <1,000 cfu/g are given in detail in

Table 19.

Croatia:

All samples were analysed for *Clostridium spp.* in g using HRN EN ISO 15213-1:2023 method for Sulphite-reducing Clostridia. *Clostridium* spp. was not detected in 25 g in any sample.

Cyprus:

Analyses were performed with the standard method ISO 15213-1:2023 for the enumeration of sulphite-reducing *Clostridium* spp. (not accredited). There were no unsatisfactory samples (>10,000 cfu/g). There was only one borderline positive sample (180 cfu/g), CY_092, a locally produced (Cyprus) blue cheese alternative based on coconut oil. A total of 12 products from this manufacturer were tested, with only one sample yielding a positive result. The colonies isolated from this sample were also tested for confirmation for *C. perfringens* (according to ISO 15213-2:2023), with negative results. All other 109 samples were negative (<10 cfu/g or <100 cfu/g, depending on the levels of background microbial flora).

Denmark:

Enumeration for *C. perfringens* were conducted using NMKL 95 method (2009, Version 5). In short Tryptose sulphite cycloserine agar is used for anaerobic enumeration of black colonies after incubation at 37 °C in 24 h. Black colonies after then further verified based on haemolysis, motility, and lactose fermentation. One sample had one colony (i.e.10 cfu/g) and the remaining had <10 cfu/g *C. perfringens*.

Estonia:

In the majority of samples, *Clostridium* spp. were below the detection limit. However, four products showed low-level presence of *Clostridium* spp., with counts below 40 cfu/g.

Finland:

Clostridium spp. was detected in 1/100 (1%) sample (cheese substitute), but the enumeration result was low at <40 cfu/g. The positive sample was identified as *C. perfringens.* In the same positive sample (FI_102), *Bacillus* spp. was also present at levels exceeding 15,000 cfu/g. It was a nut and seed-based parmesan alternative (Table 17 and Table 19).

Germany:

C. perfringens was not detected in any of the samples (n=104).

Sweden:

The only positive finding of *Clostridium* spp. (10 cfu/g) was in a fish substitute sauce called Skagen. The bacteria were identified as belonging to *C. sporogenes*. *B. subtilis/pumilis* was enumerated in the same sample at levels of 1,260 cfu/g (3.1 $\log_{10}$ cfu/g).

Table 19 Details of RTE plant-based dairy and meat substitutes in which *C. perfringens* or *Clostridium* spp. was enumerated at >10 cfu/g at or near the end of the foods labelled shelf-life[39]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[40]	pH	a_w
AT_009 Meat substitute	Vegetarian cold cuts based on milk and plant protein	Water, plant-based oils and fats (sunflower, canola, coconut fat), 5% milk protein, 3% pea protein, egg protein powder, salt, spices, spice extracts, carrageenan, guar gum, sugar, carotenes, sodium acetate, sodium lactate, monosodium glutamate, ascorbic acid	**Before:** +2°C - +6°C **After**: +2°C - +6°C, consume within 2 days	Best Before Date	Super mark et	*C. perfringens:* <1000 cfu/g	Borderline	6.2	Not tested
AT_016 Meat substitute	Vegan cold cuts based on pea protein, type Toast block	Water, starch, 5.4 % pea protein, canola oil, salt, flavour, radish concentrate, psyllium husks, spice extracts, xanthan gum, konjac, carrageenan, potassium chloride, ascorbic acid, sugar, smoke flavouring, potassium sorbate	**Before:** +2°C - +6°C **After**: +2°C - +6°C, consume as soon as possible	Best before date	Super mark et	*C. perfringens*: <1000 cfu/g	Borderline	5.44	Not tested

[39] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

[40] Note FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *C. perfringens* in RTE food. It does not have a guideline microbiological criterion limit for any other *Clostridium* spp. Thus, the guideline microbiological criterion limit for *C. perfringens* in RTE food was used to assess the compliance of all test results.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[40]	pH	a_w
AT_025 Cheese substitute	Food preparation based on canola oil, ripened with mould	Water, 18% canola oil, potato starch, salt, sugar, potato protein, carrageenan, carotenes, mould	**Before:** +2°C - +8°C **After**: +2°C - +8°C	Best before date	Supermarket	*C. perfringens*: <1000 cfu/g	Borderline	4.00	Not tested
AT_044 Meat substitute	Vegan cold cuts based on pea protein with gherkins, with sugar and sweetener, type 'Extrawurst mit Gurkerl'	Water, starch, 9% gherkins (gherkins, sugar, vinegar, saccharin, flavour), canola oil, 3.6 % pea protein, salt, flavour, spices, radish concentrate, psyllium husks, xanthan gum, konjac, carrageenan, potassium chloride, ascorbic acid, smoke flavouring, potassium sorbate	**Before:** +2°C - +6°C **After**: +2°C - +6°C, consume as soon as possible	Best before date	Supermarket	*C. perfringens*: <1000 cfu/g	Borderline	5.15	Not tested
AT_056 Cheese substitute	Vegan cheese alternative	Water, coconut oil, modified starch, carrot concentrate, smoke flavouring, flavouring, salt	**Before:** +2°C - +8°C **After**: +2°C - +8°C, consume as soon as possible	Best before date	Health food shop	*C. perfringens*: <1000 cfu/g	Borderline	4.73	Not tested
CY_092 Cheese substitute	Blue (Plant based product)	Coconut oil (24%) modified starch, starch, Tapioca Maltodextrin, Olive extract, water, Lactic acid (non-dairy), spirulina flakes, Vitamin B12, Vegan Flavourings	Store chilled at 2-8°C.	Best Before Date	Health Food Shop	*Clostridium* spp.: 180 cfu/g	Borderline	3.95	Not tested
EE_016 Meat substitute	Vegan smoked sausage	Water 45%, wheat protein (36%), plant oil (coconut) 11%, modified starch, soya protein,	0…+6 C; Product can be freezed	Best Before Date	Supermarket	*Clostridium* spp.:	Borderline	5.74	0.97

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[40]	pH	a_w
		dextrose, spices, aroma and flavouring agents, salt, iron oxide				microorganisms are present but less than 40 cfu/g			
EE_025 Meat substitute	Original taste snack sausage	Soy protein 34%, rapeseed oil, water, dried onion, coconut fat, starch, salt, spices, beet powder, iron, B12	+2..+25 C	Use By Date	Supermarket	*Clostridium* spp.: microorganisms are present but less than 40 cfu/g	Borderline	5.65	0.83
EE_039 Meat substitute[41]	Vegan snack sausage	Sunflower oil, soya flour 24%, water, tomato flakes 12%, carrots, egg white 2.4%, herbs 1.6%, table salt, tomato powder, starch, spices, dextrose, sodium acetate: ascorbic acid; thickener; sodium alginate; calcium chloride; guar gum;	room temp	Best Before Date	Online shop	*Clostridium* spp.: microorganisms are present but less than 40 cfu/g	Borderline	5.56	0.86
EE_099 Cheese substitute	Vegan mozzarella	soy drink (water, soybeans), almond puree, coconut oil, tapioca starch, salt, cultures, stabiliser: locust bean gum, xanthan gum.	+3 +8 C	Best Before Date	Supermarket	*Clostridium* spp.: microorganisms are present but less than 40 cfu/g	Borderline	5.9	0.99
FI_102 Cheese substitute[42]	Nut and seed-based parmesan alternative	walnuts, sunflower seeds, nutritional yeast flakes,brazil nuts 5%, sea salt.	Store at room temperature	Best Before Date	Retail shop	*C. perfringens.:* <40 cfu/g	Borderline	Not tested	Not tested

[41] *Bacillus* spp. was enumerated in the same sample at 1300 cfu/g.
[42] <40 cfu/g *C. perfringens* and >15,000 cfu/g *Bacillus* spp. was enumerated in the same sample.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	Enumeration test result	Designation[40]	pH	a_w
SE_013 Fish substitute[43]	Vegan sauce Skagen	Rapeseed oil, pea protein, water, red onion, seaweed extract, lemon juice, dill, cornstarch, salt	Keep refrigerated, use within 2 days of opening	Best Before Date	Supermarket	*Clostridium* spp.: 10 cfu/g	Borderline	4.81	0.95

[43] *C. sporogenes* was identified by MALDI-ToF; *B. subtilis/pumilis* was enumerated in the same sample at levels of 1,260 cfu/g.

4.7 Prevalence of Hygiene Indicators

4.7.1 Prevalence of *Listeria* spp. (non-*monocytogenes*)

L. monocytogenes is a species (spp.) of *Listeria* that is pathogenic (i.e. can cause foodborne disease), and for that reason it is the main focus of the legal requirements in Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005). However, there are other non-pathogenic spp. of *Listeria* that have been isolated from a variety of habitats, including soil, vegetation, silage, sewage, water, and faeces of healthy animals and humans. While other *Listeria* spp. (non-*monocytogenes*)[44] are not considered pathogenic, they serve as valuable indicators of environmental conditions that may support the potential presence of *L. monocytogenes*, as all *Listeria* spp. share the same ecological niche (Spanu and Jordan, 2020; Tompkin, 2002).

Listeria spp. are environmental contaminants capable of persisting in food processing environments including food processing equipment if effective cleaning and sanitation measures are not in place. The detection or enumeration of *Listeria* spp. in RTE food can indicate inadequate heat-treatment or post-processing contamination from the production environment. An unsatisfactory result for a hygiene indicator test does not mean that the batch of food is considered unsafe under Article 14, Regulation 178/2002, as amended (European Commission, 2002). However, unsatisfactory results represent unsatisfactory levels of microbial contamination and indicate that pathogens may be present. The food business operator should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GHP under its food safety management system to in order to ensure levels in subsequent batches of food are satisfactory. For borderline results, the action carried out should be proportional to the levels detected. Suggested risk management actions are provided in Guidance Note 3 (Food Safety Authority of Ireland, 2020).

Regulation 2073/2005, as amended, differentiates between RTE food that can support (food category 1.2) and that cannot support (food category 1.3) the growth of *L. monocytogenes* (European Commission, 2005). Different criterion limits for *L. monocytogenes* apply depending on which category the food belongs to and whether the food business operator has scientific studies

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[44] Use of this term refers to other *Listeria* spp. that are considered non-pathogenic (e.g. *L. innocua, L. ivanovii, L. seeligeri, L. welshimeri,* and *L. grayi*).

sufficient to show how *L. monocytogenes* will grow in their product during its shelf-life. In keeping with the legislation, two different guideline limits are set for *Listeria* spp. in the Food Safety Authority of Ireland's Guidance Note 3 (2020) depending on whether the RTE food can or cannot support the growth of *Listeria* spp.

RTE food that can support the growth of *Listeria* spp. are assessed using a more sensitive method called the detection test (which gives a detected or not detected result) compared to the enumeration test (which gives a count of colony forming units per gram or millilitre (cfu/g or ml) result). The detection test includes an enrichment step which allows injured and stressed *Listeria* spp. to recover thus making their detection more likely. For RTE food that cannot support growth, the enumeration test is used, with an upper guideline limit of 100 cfu/g set for *Listeria* spp. throughout the shelf-life of the food product. The ability of the samples to support the growth of *Listeria* spp. (including *L. monocytogenes*) was not evaluated at the time of sampling for the products tested in this survey. Consequently, all samples were subjected to both detection and enumeration testing for informational purposes. Based on the applied criterion limits for result interpretation, some samples were classified as unsatisfactory when the test results indicated detection in 25 g, while others appeared borderline when evaluated using the enumerated range of 10–100 cfu/g *Listeria* spp., depending on the test method used.

Out of 1,533 samples tested for *Listeria* spp. using both the detection and enumeration method, n=15 (0.98 %) had *Listeria* spp. detected in 25 g and were designated as unsatisfactory (Table 20). For the test results of the enumeration method, n=10 (0.65 %) samples were found to have levels between 10 and 100 cfu/g and were designated as borderline, while n=1 sample (0.07 %) had >100 cfu/g and was designated as unsatisfactory in accordance with the microbiological guideline enumeration limits for *Listeria* spp. in FSAI Guidance Note 3 (Table 20).

In Finland, *L. innocua* was detected at <10 cfu/g in one pea-based chilled meat substitute (FI_039) from a product that was seasoned with tomato and herbs (Table 21). The sample was purchased in a supermarket and was labelled with a use by date. The sample was not tested for pH or a_w. No other pathogen or hygiene indicator was detected or enumerated in the same sample.

In Germany, *Listeria* spp. was detected in 3 of 104 tested products (3 %), in one sample by the detection and enumeration method, and in 2 samples only by one of the two methods (Table 21). Interestingly, one sample was tested positive for *Listeria* spp. only by enumeration method, but not by detection method (sample DE_071). In all cases, the species *L. innocua* was isolated.

Within the different food categories, the prevalence was 0 % for plant-based dairy milk and dairy cream substitutes, 6 % (n=2/33) for plant-based cheese substitute, 3 % (n=1/30) for plant-based meat substitute and 0 % for plant-based fish substitute. One sample (DE_018) had *Listeria* spp. detected at an enumerated level of 3,100 cfu/g and was designated as unsatisfactory. It was a chilled cheese substitute described as a Camembert alternative with fermented cauliflower, based on vegetable oil and starch. It was labelled with a best before date and purchased from an online retailer. It had a measured pH and a_w of 5.6 and 0.958, respectively and thus, it would have been able to support the growth of *Listeria* spp. (including *L. monocytogenes*).

No other pathogen or hygiene indicator was detected or enumerated in the same sample (DE_018). However, a different chilled meat substitute sample with seitan as the main plant-based ingredient (DE_71) which had *Listeria* spp. not detected in 25 g but had *L. innocua* enumerated in it at <40 cfu/g, also had 970 cfu/g *B. cereus* enumerated in the same sample (Table 21). It was purchased in a supermarket, labelled with a best before date and had a pH of 6.0 and a_w of 0.959.

In Ireland, *Listeria* spp. was detected in 5 of 107 samples (4.7 %) at <10 cfu/g. Two of the samples were cheese substitutes, while three were in the meat substitute category (Table 21). Four of the five products which were positive for *Listeria* spp., were purchased from supermarkets and one was purchased from a health food shop. Two were serotyped as *L. ivanovii* (1 meat substitute and 1 cheese substitute sample), 1 as *L. innocua* (meat substitute sample), 1 as *L. grayi* (cheese substitute sample) and 1 with both *L. grayi* and *L. innocua* isolated (meat substitute sample). As shown in Table 21, all meat substitutes and one cheese substitute had physico-chemical characteristics that would have supported growth of *Listeria* spp. (including *L. monocytogenes*), while the other cheese substitute had a pH of 4.2 meaning it could be categorised as not supporting the growth of *L. monocytogenes* in accordance with Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005), Annex I, Chapter 1. Food Safety Criteria, footnote 8.

In the Netherlands, *Listeria* spp. was detected in n=3/63 (4.8 %) meat substitute samples at <10 cfu/g (Table 20). The main ingredient in two samples was tempeh, while the third sample was also soya based (Table 21). All were purchased as chilled products in a supermarket and were labelled with a best before date. All had a pH >5 which would have supported the growth of *Listeria* spp. (including *L. monocytogenes*). The a_w was not tested for these samples.

In Slovakia, *Listeria* spp. was detected in n=1/100 samples. A low level (10 cfu/g) of *Listeria* spp. was enumerated in the same sample (SK_055). Six other samples did not have

Listeria spp. detected in 25 g but low levels of the hygiene indicator were enumerated in them, and they were designated as borderline. Of these samples, n=6/37 were meat substitutes and n=1/37 as a cheese substitute (Table 20). Six of the samples gave results 10 cfu/g *Listeria* spp. and 1 sample (SK_057) which was described as a Hungarian sausage smoked and spicy gave result 20 cfu/g which had a pH of 4.39 and a_w 0.98 (Table 21). Thus, it could be categorised as not supporting the growth of *L. monocytogenes* in accordance with food category 1.3 of Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005).

In Spain, *Listeria* spp. was detected in n=3/103 samples (2.9 %), comprising two meat substitutes (ES_007, ES_018) and one dairy cream substitute (ES_052) (Table 20). Additionally, in one of the positive meat substitutes described as Organic "Red" vegan slices (ES_018) *Listeria* spp. was enumerated at 20 cfu/g. Another different meat substitute (ES_017; Altervizo slices, chorizo style) contained *Listeria* spp. at 10 cfu/g, although it was not detected by the detection method (Table 21). Two of the positive meat substitutes with pH values of 5.32 and 6.08, in combination with a_w values of 0.98 and 0.975, respectively would be able to support the growth of *Listeria* spp. (including *L. monocytogenes*). The remaining positive meat substitute and cheese substitute had lower pH and a_w values of 4.77 and 0.944, and 3.92 and 0.991, respectively, and would not support the growth of *Listeria* spp. (including *L. monocytogenes*), in accordance with footnote 8 of the Food Safety Criteria of Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005). With regards to the type of establishment, one sample was collected from a supermarket, while the other three were obtained from different health food shops. Furthermore, two of the positive samples — the dairy cream substitute (ES_052) and one meat substitute (ES_018) — were organic. The main ingredient registered for all positive samples was ">2 main plant-based ingredients."

Table 20 Prevalence of *Listeria* spp. and *E. coli* in RTE plant-based dairy, meat and fish substitutes at or near the end of the foods labelled shelf-life[45]

Country	RTE plant-based substitute	Total tested	*Listeria* spp. Detected in 25 g	*Listeria* spp. 10 - ≤100 cfu/g	*Listeria* spp. >100 cfu/g	*E. coli* >20 - ≤100 cfu/g	*E. coli* >100 cfu/g
			Unsatisfactory	**Borderline**	**Unsatisfactory**	**Borderline**	**Unsatisfactory**
Austria	Dairy milk	33	0	0	0	0	0
	Dairy cream	0	0	0	0	0	0
	Cheese	30	0	0	0	0	0
	Meat	27	0	0	0	0	0
	Fish	4	0	0	0	0	0
Belgium	Dairy milk	30	0	0	0	Not tested	Not tested
	Dairy cream	0	0	0	0	Not tested	Not tested
	Cheese	35	0	0	0	Not tested	Not tested
	Meat	27	0	0	0	Not tested	Not tested
	Fish	8	0	0	0	Not tested	Not tested
Croatia	Dairy milk	44	0	0	0	0	0
	Dairy cream	9	0	0	0	0	0

[45] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Country	RTE plant-based substitute	Total tested	*Listeria* spp. Detected in 25 g	*Listeria* spp. 10 - ≤100 cfu/g	*Listeria* spp. >100 cfu/g	*E. coli* >20 - ≤100 cfu/g	*E. coli* >100 cfu/g
			Unsatisfactory	Borderline	Unsatisfactory	Borderline	Unsatisfactory
	Cheese	15	0	0	0	0	0
	Meat	33	0	0	0	0	2
	Fish	0	0	0	0	0	0
Cyprus	Dairy milk	33	0	0	0	0	0
	Dairy cream	3	0	0	0	0	0
	Cheese	39	0	0	0	0	0
	Meat	33	0	0	0	0	1
	Fish	2	0	0	0	0	0
Denmark	Dairy milk	14	0	0	0	Not tested	Not tested
	Dairy cream	0	0	0	0	Not tested	Not tested
	Cheese	29	0	0	0	Not tested	Not tested
	Meat	52	0	0	0	Not tested	Not tested
	Fish	6	0	0	0	Not tested	Not tested
Estonia	Dairy milk	31	0	0	0	0	0
	Dairy cream	0	0	0	0	0	0
	Cheese	33	0	0	0	0	0
	Meat	35	0	0	0	0	0
	Fish	1	0	0	0	0	0
Finland	Dairy milk	24	0	0	0	0	0
	Dairy cream	2	0	0	0	0	0
	Cheese	28	0	0	0	0	0
	Meat	44	1	0	0	0	0
	Fish	4	0	0	0	0	0
France	Dairy milk	17	0	0	0	Not tested	Not tested

Country	RTE plant-based substitute	Total tested	*Listeria* spp. Detected in 25 g	*Listeria* spp. 10 - ≤100 cfu/g	*Listeria* spp. >100 cfu/g	*E. coli* >20 - ≤100 cfu/g	*E. coli* >100 cfu/g
			Unsatisfactory	Borderline	Unsatisfactory	Borderline	Unsatisfactory
	Dairy cream	0	0	0	0	Not tested	Not tested
	Cheese	36	0	0	0	Not tested	Not tested
	Meat	32	0	0	0	Not tested	Not tested
	Fish	14	0	0	0	Not tested	Not tested
Germany	Dairy milk	23	0	0	0	0	0
	Dairy cream	7	0	0	0	0	0
	Cheese	33	2	0	1	0	2
	Meat	30	0	1	0	0	0
	Fish	11	0	0	0	0	0
Ireland	Dairy milk	34	0	0	0	0	0
	Dairy cream	1	0	0	0	0	0
	Cheese	35	2	0	0	0	0
	Meat	36	3	0	0	0	0
	Fish	1	0	0	0	0	0
Latvia	Dairy milk	33	0	0	0	0	0
	Dairy cream	9	0	0	0	0	0
	Cheese	29	0	0	0	0	0
	Meat	27	0	0	0	0	0
	Fish	4	0	0	0	0	0
Netherlands	Dairy milk	15	0	0	0	0	0
	Dairy cream	6	0	0	0	0	0
	Cheese	26	0	0	0	0	0
	Meat	63	3	0	0	0	0
	Fish	0	0	0	0	0	0

Country	RTE plant-based substitute	Total tested	*Listeria* spp. Detected in 25 g	*Listeria* spp. 10 - ≤100 cfu/g	*Listeria* spp. >100 cfu/g	*E. coli* >20 - ≤100 cfu/g	*E. coli* >100 cfu/g
			Unsatisfactory	Borderline	Unsatisfactory	Borderline	Unsatisfactory
Slovakia	Dairy milk	25	0	0	0	0	0
	Dairy cream	0	0	0	0	0	0
	Cheese	37	0	1	0	0	0
	Meat	37	1	6	0	0	0
	Fish	1	0	0	0	0	0
Spain	Dairy milk	35	0	0	0	0	0
	Dairy cream	6	1	0	0	0	0
	Cheese	25	0	0	0	0	1
	Meat	35	2	2	0	0	0
	Fish	2	0	0	0	0	0
Sweden	Dairy milk	50	0	0	0	0	0
	Dairy cream	0	0	0	0	0	0
	Cheese	13	0	0	0	0	0
	Meat	31	0	0	0	0	0
	Fish	6	0	0	0	0	0
Grand total samples analysed		**1,533**	***Listeria* spp. Detected in 25 g Unsatisfactory**	***Listeria* spp. 10 - ≤100 cfu/g Borderline**	***Listeria* spp. >100 cfu/g Unsatisfactory**	***E. coli* 20 - ≤100 cfu/g Borderline**	***E. coli* >100 cfu/g Unsatisfactory**
Total number tested for *Listeria* spp.		**1,533**	**15**	**10**	**1**		
Total number tested for *E. coli*		**1,229**[46]				**0**	**6**

[46] Two samples were not analysed for *E. coli* in Finland and The Netherlands, respectively (n=4).

Table 21 Details of RTE plant-based dairy and meat substitutes in which *Listeria* spp. (non-*monocytogenes*) was detected and/or enumerated at or near the end of the foods labelled shelf-life[47]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*Listeria* spp. detection method	*Listeria* spp. enumeration method	pH	a_w
FI_039 Meat substitute	Pea-based cold cut tomatoes and herbs	Water, pea protein, canola oil, coconut fat, thickeners (carrageenan, konjac gum), iodized salt, yeast extract, spices (e.g. tomato, oregano, garlic, basil) and spice extracts, dextrose, flavorings, preservative (lactic acid), colorant (iron oxide).	Store at +2-+6°C.	Use by date	Supermarket	Detected in 25 g (*L. innocua*)	<10 cfu/g	Not tested	Not tested
DE_018 Cheese substitute	Camembert alternative with fermented cauliflower, based on vegetable oil and starch	water, cauliflower (25 %), palm oil (24 %), starch (12 %), sunflower protein, carrots, salt, thickening agents: xanthan gum, guar gum; vegan fermentation and noble mould cultures	Keep refrigerated at max. +7 °C. Once opened, consume as soon as possible.	Best Before Date	Online retailer	Detected in 25 g (*L. innocua*)	3,100 cfu/g	5.6	0.958
DE_027 Cheese substitute	Mild, naturally matured, grated alternative to cheese based on coconut oil	water, coconut oil, potato starch, lupin flour, rock salt, turmeric extract, ripening cultures	Unopened at max. +7 °C best before date	Best Before Date	Retail shop	Detected in 25 g (*L. innocua*)	< 10 cfu/g	4.2	0.944

[47] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*Listeria* spp. detection method	*Listeria* spp. enumeration method	pH	a_w
	and potato starch								
DE_71 Meat substitute[48]	Vegan organic smoked cold cuts	85 % seitan (water, wheat protein), high-oleic sunflower oil, rock salt, yeast extract, onions, thickener (locust bean gum and guar gum), spices, beechwood smoke	At +7 °C best before date: Once opened, keep the pack covered in the refrigerator.	Best Before Date	Retail shop	Not detected in 25 g (*L. innocua*)	< 40 cfu/g	6.0	0.959
IE_045 Meat substitute	Hoisin Tofu	Soybeans, Soy sauce, Sunflower oil	**Before:** Keep refrigerated. **After:** Once opened, consume within 24 hours.	Use by	Retail	Detected in 25 g (*L. ivanovii*)	<100 CFU/g	5.8	0.97
IE_060 Cheese substitute	Organic Smoked Paprika & Onion Cream Cheese Alternative from Fresh Cultured Cashew	Cashews cream (Water, cashew nuts, sea salt), smoked paprika and cultures	**Before:** Refrigerate below 5°C. **After:** Best eaten within 7 days.	Use by	Retail	Detected in 25 g (*L. ivanovii*)	<100 CFU/g	4.2	1
IE_068 Meat substitute	Pulled Seitan Teriyaki	Seitan (66%), Soy sauce	**Before:** **After:** Refrigerate after opening and use within 2 days.	Best before	Retail	Detected in 25 g (*L. grayi/ innocua*)	<100 CFU/g	5.7	0.96
IE_074 Meat substitute	Yakko Tofu (Japanese	Water, soybeans	**Before:** Store cool: 2-7°C. **After:**	Use by	Health Food Shop	Detected in 25 g (*L. innocua*)	<100 CFU/g	5.3	1

[48] *B. cereus* (970 cfu/g) was enumerated in the same sample

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*Listeria* spp. detection method	*Listeria* spp. enumeration method	pH	a_w
	Speciality Soft Tofu)								
IE_095 Cheese substitute	Artisan Smokey Chilli Cheeze	Activated cashew nuts, Coconut oil, Miso (Soy)	**Before:** Keep refrigerated below 5°C. **After:** Once opened, use within 5 days.	Use by	Retail	Detected in 25 g (*L. grayi*)	<100 CFU/g	4.5	0.97
NL_035_2025 42469 Meat substitute	Tempeh (block)	No photo of sample. Typical ingredients: soybean, water, rice flour, starter culture.	**Before:** Store chilled. **After**: No photo.	Best Before Date	Super-market	Detected in 25 g	< 10 cfu/g	7.03	Not tested
NL_036_2025 42867 Meat substitute	Marina-ted tempeh cubes	58% soybean, sunflower oil, water, molasses, soy sauce (water, soybeans, salt, vinegar), lemon juice, garlic, white vinegar, maltodextrin, modified starch (corn), starter culture, salt, natural aroma, rice flour, spices, preservative (potassium sorbate)	**Before:** Store chilled. **After**: Store chilled, con-sume within 3 days.	Best Before Date	Super-market	Detected in 25 g	< 10 cfu/g	5.13	Not tested
NL_043_2025 45393 Meat substitute	Plant based chicken pieces	Water, vegetable protein 18.7% (soy 16%, wheat 2.7%), vegetable oils (sunflower, rapeseed), (chicken) egg white, wheat starch, spices (onion powder, garlic powder, white pepper, ginger), vinegar, natural aroma, preservative (E261), acid (citric acid), salt, mineral (iron), vitamin B12	**Before:** Store chilled. **After**: Store chilled, max. 3 days.	Best Before Date	Super-market	Detected in 25 g	< 10 cfu/g	5.52	Not tested
SK_059_7584 Meat substitute	Sausage exclusive spicy	Soya, chilli, rice flour, pea protein	Store at +2 - +6°C	Use by date	supermarket	Not detected	10 cfu/g	5.72	0.94
SK_055_7580 Meat substitute	Klobana smoked spicy	Chickpeas, brown rice, gluten free oat flour	Store at +0 - +8°C	Use by date	supermarket	Detected in 25g	10 cfu/g	4.37	0.98
SK_030_7554 Meat substitute	Vegan product with oyster mushroom	Oyster mushroom, gluten, wheat protein	Store at +0 - +8°C	Use by date	supermarket	Not detected	10cfu/g	5.98	0.96

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*Listeria* spp. detection method	*Listeria* spp. enumeration method	pH	a_w
SK_036_7560 Meat substitute	Tofu spread with roast pork flavour	Soya beans, roasted onion	Store at +2 - +8°C	Use by date	supermarket	Not detected	10 cfu/g	5.53	0.97
SK_044_7569 Cheese substitute	Tofu for Greek style	Tofu, soya beans, brain	Store at +2 - +8°C	Use by date	supermarket	Not detected	10 cfu/g	5.47	0.98
SK_056_7581 Meat substitute	Hungary sausage smoked	Wheat, soya, barley, paprika	Store at +0 - +8°C	Use by date	supermarket	Not detected	10 cfu/g	4.40	0.98
SK_057_7582 Meat substitute	Hungary sausage smoked spicy	Wheat, soya, spicy paprika	Store at +0 - +8°C	Use by date	supermarket	Not detected	20 cfu/g	4,39	0.98
ES_007 Meat substitute	Vegetal slices with vegetables	Egg white (53%), vegetables (red bell pepper, carrot) (13%), olives (7%), water, sunflower oil, corn starch, stabilizers (guar gum, xanthan gum), flavourings, salt, fruit and vegetable concentrate, buffered vinegar powder. Lactose content: < 0.01%	**Before:** Keep refrigerated between 0-5ºC. **After**: Once opened, use within 24 hours.	Best Before Date	Supermarket	Detected in 25 g	<10 cfu/g	5.32	0.980
ES_017 Meat substitute	Altervizo slices (chorizo style)	Water, refined sunflower oil, soy protein (13%), stabilizers (methylcellulose, processed Eucheuma seaweed, gluconic delta-lactone), smoked paprika (4.2%), garlic, corn starch, tapioca starch, flavourings, smoke flavouring, vegetable fiber, salt, pea protein, tomato powder, gelling agent (agar-agar), potassium salt, acidity regulator (citric acid), preservative (potassium sorbate), colours (paprika extract), antioxidant (tocopherol-rich extract), iron (ferrous sulphate), vitamin B12. It may content traces of egg.	**Before:** Keep between 0ºC and 5ºC. **After**: Once opened keep refrigerated and use within 2 days.	Best Before Date	Health food shop	Not detected in 25 g	20 cfu/g	4.77	0.944
ES_018 Meat substitute	"Red" vegan slices. Organic	Water, canola oil, stabilizers (carrageenan, locust bean gum, xanthan gum), pea protein (3.5%), spices (1.1%), maltodextrin, vinegar, sea salt, natural vinegar flavouring, natural herbs and spices flavouring, natural vegetable extract flavouring, beet concentrate, tomato concentrate, acidity regulator	**Before:** Keep between 0ºC and 7ºC. **After:** Once opened, keep refrigerated and	Use By Date	Health food shop	Detected in 25 g	10 cfu/g	6.08	0.975

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*Listeria* spp. detection method	*Listeria* spp. enumeration method	pH	a_w
		(calcium citrate). It may contain gluten, celery, mustard and nuts.	use within 2 days.						
ES_052 Dairy cream substitute	Fraïs with chives. Organic fermented creamy plant-based spread made with almonds, cashews, and chives.	Water, coconut fat, almonds (6.1%), cashews (3.9%), tapioca starch, yeast, chives (1%), salt, thickener (carrageenan), garlic paste (garlic, salt), firming agent (calcium chloride), cultures.	**Before:** Keep between 4ºC and 8ºC. **After:** Once opened use within 5 days.	Best Before Date	Health food shop	Detected in 25 g	<10 cfu/g	3.92	0.991

4.7.2 Prevalence of *E. coli*

The *E. coli* test is used to assess the hygiene status of a food product and can indicate that faecal pathogens could be present. *E. coli* are killed by adequate heat processing and should not be present in heat processed food. *E. coli* are also removed from the food processing environment by effective cleaning and sanitisation. *E. coli* counts of <20 cfu/g food are considered satisfactory. Unsatisfactory results, although not inherently a hazard, can indicate:

- Faecal contamination of raw materials
- Inadequate heat processing
- Cross-contamination
- Inadequate cleaning and sanitisation
- Poor temperature and time control
- Increased likelihood of the presence of pathogens.

An unsatisfactory result for a hygiene indicator test does not mean that the batch of food is considered unsafe under Article 14, Regulation 178/2002 (European Commission, 2002). However, unsatisfactory results represent unsatisfactory levels of microbial contamination and indicate that pathogens may be present. The food business operator should investigate the cause of the elevated levels and take measures as part of their HACCP-based procedures and GHP to in order to ensure levels in subsequent batches of food are satisfactory. For borderline results, the action carried out should be proportional to the levels detected. Recommended risk management actions for elevated level of *E. coli* are provided in Guidance Note 3 (Food Safety Authority of Ireland, 2020).

Twelve project partners assessed the RTE plant-based samples of dairy, meat and fish substitutes for the presence of the hygiene indicator *E. coli.* Of 1,229 samples tested, n=6 (0.49 %) enumerated *E. coli* at levels in excess of 100 cfu/g (Table 20). These samples were designated as unsatisfactory in accordance with the microbiological guideline limits set for *E. coli* in the Food Safety Authority of Ireland's Guidance Note 3 (2020). No samples were found to have borderline levels of *E. coli* between 20 and 100 cfu/g.

In Croatia, *E. coli* was found in n=2/33 (6.1 %) chilled meat substitutes, both of which had 180 cfu/g and were made from Tofu by the same manufacturer (Table 22). They were both purchased in a health food shop and were labelled with a use by date. The pH and a_w values were 5.07 and 0.9887, and 5.66 and 0.9968, for each sample respectively.

In Cyprus, *E. coli* was found at 340 cfu/g in n=1/33 (3 %) chilled meat substitute (CY_099) made from tofu with herbs (basil and garlic and others), which had a best before date and was purchased online (Table 22). It had a pH of 7.21, a_w was not tested. Presumptive *B.cereus* were also enumerated in this sample at between 10 and 100 cfu/g (result calculated according to ISO 7218:2004). Three more tofu products samples (CY_098 traditional tofu, CY_100 chilli and tomato, and CY_101 fermented tofu) manufactured by the same producer were tested at the same time with negative results. It was hypothesised that the added herbs were the most likely contamination source for this particular product.

In Germany, *E. coli* was enumerated in n= 2/104 (1.9%) samples, with counts of 720 and 910 cfu/g, respectively (Table 22). Both samples were fermented soft cheese alternatives with cashew as the main ingredient. Both were chilled products purchased online and labelled with best before dates. They had a combined pH and a_w of 5.6 and 0.97, and 6.9 and 0.94, respectively.

In Spain, *E. coli* was enumerated in one sample (ES_051) at a notably high level of 38,000 cfu/g and was designated as unsatisfactory (Table 22). The product was described as a camembert cheese substitute made from cashews (76%) and contained vegan cultures. It was a chilled product, purchased in a health food shop and labelled with a best before date. It had a pH of 6.65 and a_w of 0.975. It was noted that the product exhibited a foul odour when the package was opened for testing. The same sample (ES_051) had 540,000 cfu/g *Bacillus* spp. enumerated in it (Table 17). There was no *L. monocytogenes, Listeria* spp. or *Salmonella* spp. found in the sample.

Table 22 Details of RTE plant-based dairy and meat substitutes in which *E. coli* was enumerated at >20 cfu/g at or near the end of the foods labelled shelf-life[49]

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*E. coli* test result	Designation[50]	pH	a_w
HR_59 Meat substitute	Smoked tofu	Soy Milk (98%), Nigan, Garlic, Salt	**Before:** Refrigerate between 4 and 8 Celsius **After**: Use within 3 days	Use by Date	Health food shop	180 cfu/g	Unsatisfactory	5.07	0.9887
HR_60 Meat substitute	Fresh tofu	Soy Milk (99 %), Nigan	**Before:** Refrigerate between 4 and 8 Celsius **After**: Keep in cold water and use within 2 days	Use by Date	Health food shop	180 cfu/g	Unsatisfactory	5.66	0.9968
CY_099 Meat substitute	Tofu Basil n' Garlic	soya, spinach, basil, parsley, garlic	Store for 3 months after production, chilled at 0-4°C. After opening use within 4 days.	Best Before Date	Online	340 cfu/g	Unsatisfactory	7.21	Not tested
DE_19 Cheese substitute	Fermented vegan camembert alternative	water, cashew nuts (45 %), green pepper (5.0 %), salt, macadamia nuts, vegan fermentation and mould cultures	Keep refrigerated at max +7°C	Best Before Date	Online	720 cfu/g	Unsatisfactory	5.6	0.97

[49] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.
[50] Test result designation basis: FSAI Guidance Note 3 Guidelines for the Interpretation of Results of Microbiological Testing of RTE Foods Placed on the Market

Sample ID and sample type	Sample description	Ingredients	Storage conditions before and after opening	Best Before or Use By Date	Retail stage	*E. coli* test result	Designation[50]	pH	a_w
DE_20 Cheese substitute	Fermented vegan blue cheese alternative	cashew nuts (44 %), water, coconut milk, salt, starter cultures, edible mould cultures	Keep refrigerated at max +7°C	Best Before Date	Online	910 cfu/g	Unsatisfactory	6.9	0.94
ES_051 Cheese substitute	“Camembert cheese” substitute	Cashews (76%), water, salt, vegan cultures. May contain traces of other tree nuts.	Keep refrigerated between 2ºC and 4ºC. (No distinction between `Before´ and `After´)	Best Before Date	Health food shop	38,000 cfu/g	Unsatisfactory	6.65	0.975

4.8 Summary of positive test results

Table 23 presents a summary of the key findings from the present survey on the microbiological quality of dairy, meat and fish substitutes at or near the end of the foods labelled shelf-life[51]. Across 1,533 total samples, *L. monocytogenes* was detected in only 1 sample (from a chilled oat-based dairy milk substitute). *Salmonella* spp. was not detected in any of the samples tested (n=1332). Five PCR positive but culture negative results for STEC were identified, however no viable organisms were cultured confirmed and the samples were not designated as unsatisfactory.

Apart from the single detection of *L. monocytogenes* at <1 cfu/g in a chilled oat-based drink, dairy milk and cream substitutes had the best microbiological quality. Cheese and meat substitutes seemed to have the most quality issues, which may indicate hygiene and processing issues at manufacturing level, and/or poor maintenance of the cold chain. Borderline (n=14) and unsatisfactory (n=3) levels of *B. cereus* or *Bacillus* spp. (depending on the test the laboratory carried out) were found mainly in meat (7 borderline, 2 unsatisfactory), cheese (4 borderline, 1 unsatisfactory). Five of the *Bacillus*-positive samples that had levels >1,000 cfu/g were made from tofu as the main plant-based ingredient (3 borderline, 2 unsatisfactory). This may suggest cross-contamination from the raw materials, and/or temperature abuse or handling issues in the manufacturing of these products. Borderline counts of *C. perfringens* or *Clostridium* spp. (depending on the test the laboratory carried out) were found in cheese substitutes (33 borderline), meat substitutes (31 borderline) and fish substitutes (5 borderline). No unsatisfactory results of >10,000 cfu/g were found.

Listeria spp. were detected in dairy cream (n=1), cheese (n=4) and meat (n=10) substitutes. Borderline levels of *Listeria* spp. were enumerated in cheese (n=1) and meat (n=9) substitutes. One camembert cheese substitute made from fermented cauliflower (DE_018) had a high unsatisfactory count of 3,100 cfu/g *Listeria* spp. (Table 21). There was a low occurrence overall of *E. coli*, however unsatisfactory results (>100 cfu/g) were found in a small number of cheese (n=3) and meat (n=3) substitutes. High levels of *E. coli* and *B. cereus* were enumerated in the same sample of camembert cheese substitute made from cashews (ES_051) (Tables 17 and 22).

[51] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Table 23 Summary of results for all microbiological test parameters conducted in RTE plant-based dairy, meat and fish substitutes at or near the end of the foods labelled shelf-life[52]

RTE plant-based substitute	*L. monocytogenes*			*Salmonella* spp.		STEC		*B. cereus / Bacillus* spp.[53]			*Clostridium perfringens / Clostridium* spp.[54,55]			*Listeria* spp.				*E. coli*		
	Total tested	Unsatisfactory (detected in 25 g)	Unsatisfactory (>100 cfu/g)	Total tested	Unsatisfactory (detected in 25 g)	Total tested	Unsatisfactory (detected in 25 g)	Total tested	Borderline (1,000 - ≤100,000 cfu/g)	Unsatisfactory (>100,000 cfu/g)	Total tested	Borderline (1000 - ≤10000 cfu/g)	Unsatisfactory (>10000 cfu/g)	Total tested	Unsatisfactory (Detected in 25 g)	Borderline (10 - ≤100 cfu/g)	Unsatisfactory (>100 cfu/g)	Total tested	Borderline (20 - ≤100 cfu/g)	Unsatisfactory (>100 cfu/g)
Dairy milk	**441**	1	0	**377**	0	217	0	**334**	0	0	251	30	0	**441**	0	0	0	378	0	0
Dairy cream	**43**	0	0	**43**	0	28	0	**27**	1	0	21	0	0	**43**	1	0	0	43	0	0
Cheese	**443**	0	0	**401**	0	237	0	**316**	4	1	219	33	0	**443**	4	1	1	342	0	3
Meat	**542**	0	0	**459**	0	324	0	**378**	7	2	285	31	0	**542**	10	9	0	430	0	3
Fish	**64**	0	0	**52**	0	25	0	**58**	2	0	34	5	0	**64**	0	0	0	36	0	0
Grand total	**1533**	**1**	**0**	**1332**	**0**	**831**	**0**	**1113**	**14**	**3**	**810**	**99**	**0**	**1533**	**15**	**10**	**1**	**1229**	**0**	**6**

[52] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

[53] FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *B. cereus* in RTE food. It does not have a guideline microbiological criterion limit for any other *Bacillus* spp. Thus, the guideline microbiological criterion limit for *B. cereus* in RTE food was used to assess the compliance of all test results. Note that for one of the borderline fish substitute samples tested by Sweden, *B. subtilis/pumilis* was identified using MALDI-TOF. *Penibacillus simplex* was identified by MALDI-ToF in a second Swedish sample which was one of the meat substitutes designated as unsatisfactory. For both of these samples it should be noted that of the dominating colonies species typed using MALDI-ToF, *B. cereus* was not identified.

[54] Note FSAI Guidance Note 3 (2020) only has a published guideline microbiological criterion limit for *C. perfringens* in RTE food. It does not have a guideline microbiological criterion limit for any other *Clostridium* spp. Thus, the guideline microbiological criterion limit for *C. perfringens* in RTE food was used to assess the compliance of all test results. *C. sporogenes* (10 cfu/g) was identified by MALDI-ToF in 1 fish substitute sample (Sweden). *B. subtilis/pumilis* was enumerated in the same sample at levels of 1,260 cfu/g.

[55] Different national microbiological guideline criterion limits are used to assess *C. perfringens* test results in Austria, where <1,000 cfu/g is designated as satisfactory for RTE samples placed on the market (Kommunikationsplattform Verbraucher:innengesundheit, 2025).

5 Discussion

The primary objective of this collaborative European retail prevalence survey funded by the EFSA TMA initiative was to identify potential emerging food safety risks associated with RTE plant-based dairy (milk, cream and cheese), meat and fish substitutes. The findings of the present survey contribute towards the publication of evidence-based food safety guidance tailored to food businesses manufacturing these types of RTE plant-based products. It also underpins risk communication advice and recommendations to consumers to help minimise the public health risk of foodborne illness resulting from their consumption.

The overall findings of the survey provide microbiological prevalence data that can help EU risk managers in food safety authorities across the EU decide whether current measures for comparable animal-derived products should be adapted for RTE plant-based substitutes. Since RTE plant-based products often use manufacturing methods that differ from the traditional time-temperature controls applied to the animal-derived foods they replace, they may retain microbiological hazards not eliminated during production, making it essential to understand emerging risks to ensure their safety and protect public health.

The literature review carried out as part of this survey found that knowledge gaps exist in relation to the public health risk associated with these types of products and the competence of the food business operators that produce them. Foodborne pathogens *L. monocytogenes*, *B. cereus* and *S. aureus* were occasionally identified in prevalence surveys assessing the microbiological quality of RTE and non-RTE plant-based dairy and meat substitutes (Table 2 and Table 6). *L. monocytogenes* was detected at levels that were below the limit of enumeration in tofu, meat substitutes, and vegan deli slices, while borderline levels of *B. cereus* (1,000 to <100,000 cfu/g) were found in a small number of cheese substitutes. Both *B. cereus* and *S. aureus* were detected in meat substitutes at levels of 2 and 2.7 $\log_{10}$ cfu/g respectively, in a Swiss retail study of 100 RTE and non-RTE samples (Barmettler *et al.,* 2025). Elevated levels of other non-pathogenic *Listeria* spp., *E. coli* and Enterobacteriaceae tested as hygiene indicators were found in meat and cheese substitutes (Barmettler *et al.,* 2025; Van Paepeghem *et al.,* 2024; Willis *et al.,* 2023; Canadian Food Inspection Agency, 2023). The literature search also identified previous outbreaks of foodborne illness linked to RTE plant-based dairy substitutes contaminated with pathogens (Table 1). This was most likely due to poor control of the relevant food safety hazards in the implicated food business operation(s) under GHP and HACCP-based procedures.

During the collaborative survey conducted across 15 Member States, it was observed that while some participating countries have established national guidelines including recommended microbiological criteria for monitoring the contamination of RTE products on the market, this is not uniformly the case across all Member States. The development and implementation of harmonised microbiological criteria for evaluating the microbiological quality of RTE plant-based dairy, meat, and fish substitutes would be valuable in promoting consistency in official assessments of the microbiological compliance of these products.

All of the products sampled were tested at or near the end of the foods labelled shelf-life[56] for *L. monocytogenes* and *Listeria* spp. (n=1,533), with the physico-chemical characteristics of pH (n=1,421) and a_w (n=1,113) assessed for a large majority of samples. Varying numbers of samples were also tested for *Salmonella* spp. (n=1,332), STEC (831), *B. cereus* or *Bacillus* spp. (n=1,113), *Clostridium* spp. or *C. perfringens* (n=810) and *E. coli* (n=1,229) depending on the capacity of the laboratory that analysed the samples. The survey assessed pH and a_w values in the samples to determine whether they could support growth of foodborne pathogens should the products become contaminated at manufacturing level. Most had pH > 4.4 or a_w > 0.92 or did not have a combined pH and a_w of ≤ 5.0 and ≤ 0.94, respectively, placing them under Food Category 1.2 unless justified otherwise under Footnote 8 of Commission Regulation (EC) No 2073/2005 (European Commission, 2005).

The microbiological quality of the products tested in the present survey was generally good (Table 23). Across 1,533 total samples, *L. monocytogenes* was detected in only 1 sample (from a chilled oat-based dairy milk substitute) at <1 cfu/g. While it is reasonably foreseeable that a consumer could store the unopened product for longer than the labelled shelf-life duration as it was marked with a best before date, it is important to note that the analysis began one day before the end of the best before date that was labelled on the product, and no measurable growth was present. This absence of measurable growth can support the interpretation that the product does not enable the growth of *L. monocytogenes*.

[56] All chilled or ambient RTE plant-based milk, cream, cheese, meat, and fish substitutes with a use-by or best before date that expired during the survey period (1st March–30th November 2025) were tested at the end of their labelled shelf-life. Ambient RTE plant-based milks with best-before dates that extended beyond 30th November 2025 were tested at the end of the survey period as close to the expiry date as possible.

Salmonella spp. was not detected in any of the samples tested (n=1,332). Five PCR-positive but culture-negative results for STEC were identified from n=831 samples, however no viable organisms were cultured confirmed and the samples were not designated as unsatisfactory.

Fourteen of 1,113 samples (1.3 %) enumerated for either *B. cereus* or *Bacillus* spp. at between 1,000 and 100,000 cfu/g and thus were designated as borderline. Three samples (0.3 %) had levels in excess of 100,000 cfu/g and were designated as unsatisfactory. However, it is important to note that for two of the test results designated as unsatisfactory and borderline in the Swedish dataset, *B. cereus* was not identified in the dominating colonies species typed using MALDI-ToF. *Penibacillus simplex* was identified by MALDI-ToF (5.3 $\log_{10}$ cfu/g) in a marinated tofu (pH 6.2 and a_w 0.95) and 3.1 $\log_{10}$ cfu/g *B. subtilis/pumilis* per gram in a vegan sauce Skagen (fish substitute) made from rapeseed oil and pea protein (pH 4.8 and a_w 0.95). The *Bacillus*-positive test results were mainly found in plant-based substitutes for cheese, meat and fish, with one sample of dairy cream substitute having a level that was designated as borderline (Table 16 and 17).

Of 810 samples tested for *C. perfringens* (n=3 countries) or *Clostridium* spp. (n=5 countries), n=99 (12.2%) were found to have borderline levels in cheese, meat and fish substitutes. No unsatisfactory results of >10,000 cfu/g were found. The presence of *Listeria* spp. (non-*monocytogenes*) assessed in the samples collected as a hygiene indicator in the present survey. While *Listeria* spp. (non-*monocytogenes*) are not considered pathogenic, they serve as valuable indicators of environmental conditions that may support the potential presence of *L. monocytogenes*, as all *Listeria* spp. share the same ecological niche (Spanu and Jordan, 2020; Tompkin, 2002). *Listeria* spp. was detected in n=15 (0.98%) of samples. They were identified in a dairy cream (n=1), cheese (n=4) and meat (n=10) substitutes. There were also low levels of *Listeria* spp. enumerated in cheese (n=1) and meat (n=9) substitutes.

One cheese substitute had an unsatisfactory count of 3,100 cfu/g *Listeria* spp. This product was a camembert alternative with fermented cauliflower, based on vegetable oil and starch purchased from an online retailer and labelled with a best before date (DE_018) (Table 21). While the presence of *Listeria* spp. may not constitute a regulatory non-compliance, it indicates contamination potential and highlights the importance of robust hygiene controls and environmental monitoring, particularly for these RTE plant-based products (Food Safety Authority of Ireland, 2026). This is particularly relevant given that the pH and a_w of these products would in general not prevent microbial growth, given that 68.7% (n=976/1,421) of samples had a pH ≥5.1 and 92.9 % (n=1,034/1,113) of samples had an a_w ≥0.94.

There was very low occurrence overall of the hygiene indicator *E. coli.* Of 1,229 samples tested, n=6 (0.49 %) enumerated *E. coli* at levels in excess of 100 cfu/g and were designated as unsatisfactory (Table 20). No samples were found to have borderline levels of *E. coli* between 20 and 100 cfu/g. Unsatisfactory results (>100 cfu/g) were found in cheese substitutes (n=3) and meat substitutes (n=3). Of note, *E. coli* was enumerated in one sample (ES_051) at a high level of 38,000 cfu/g described as a camembert cheese substitute made from cashews (76%) and contained vegan cultures (Table 22). The same sample (ES_051) had 540,000 cfu/g *Bacillus* spp. enumerated in it (Table 17). There was no *L. monocytogenes, Listeria* spp. or *Salmonella* spp. found in the sample.

While the general microbiological quality of the samples tested in the present survey was good, some issues were noted. Based on the findings of the present microbiological prevalence survey, continued official control surveillance of RTE plant-based cheese and meat substitutes are recommended. This survey found microbiological contamination mainly in plant-based products containing tofu, pea protein and cashews as a primary ingredient. Smaller numbers of positive samples were identified which contained other ingredients such as other nuts, oat, coconut, seitan and tempeh, as well as in those with blended formulations. Periodic targeted monitoring for pathogens and hygiene indicators in these products would contribute towards the evidence base for identifying emerging risks. It would also support the implementation of appropriate preventive controls at manufacturing level that are tailored to controlling microbiological hazards associated with plant-based products of this nature.

The overall results for the present survey highlight that the presence of foodborne pathogens in plant-based RTE dairy, meat and fish substitutes while not common, can occur sporadically. Appropriate control of the possible risks should be taken into account under the food business operator’s GHP and HACCP-based procedures under its food safety management system. If food business operators do not manage these hazards appropriately through the PRP’s of good supplier control of raw materials, adequate hygiene programmes, staff training, and proper maintenance and temperature control of the cold chain (European Commission, 2022), there is a risk of any contamination with pathogens growing to levels which may cause serious foodborne illness, similar to what would be expected for animal-derived RTE dairy, meat and fish products.

Some potential contamination issues were identified in relation the presence of non-pathogenic *Listeria* spp. which acts as a hygiene indicator for the pathogen *L. monocytogenes. L. monocytogenes* was detected at <1 cfu/g in one sample of a chilled oat-based dairy substitute in

Finland which was designated as unsatisfactory. In 2024, a significant listeriosis outbreak of 20 cases (15 hospitalisations and three fatalities) was associated with chilled almond, oat, and coconut plant-based milk products (Public Health Agency of Canada, 2024). Additionally, an oat-based milk substitute in Finland and Estonia was the source of a *B. cereus* outbreak in 2022 (Ruokavirasto – Finnish Food Authority, 2022).

While no viable STEC isolates were culture confirmed, there was 5 presumptive PCR positives of STEC in three dairy milk substitutes (2 oat-based, 1 soya-based), and two meat substitutes (2 pea-based). Certain animal-derived versions of these products, such as raw milk, raw milk cheese, and fermented meats, have been associated with cases of foodborne illness. However, to date, there have been no reported incidents of foodborne illness linked to RTE plant-based dairy or meat substitutes. It is increasingly understood that certain plant-based ingredients such as grains may be exposed to STEC contamination via contaminated water, natural fertilisers or wild animal faeces. Since 2009, STEC foodborne outbreaks have been reported in the USA, Canada and France due to the contamination of flour products and dough (BfR (German Federal Institute for Risk Assessment), 2024).

Many plant-based products, especially those derived from cereals and legumes, can be vulnerable to contamination with spore-forming organisms. While *C. perfringens/Clostridium* spp. were not detected at levels of concern, higher numbers of *B. cereus/Bacillus* spp. were enumerated in certain products. This may suggest cross-contamination from the raw materials, and/or temperature abuse or handling issues in some products. While toxin production was not assessed in the present survey, the high level of *B. cereus* detected in some samples may indicate insufficient process control or post-processing contamination and could represent a potential risk if such products are mishandled or stored improperly. The German partners in the present survey did report that the sample they identified with the highest colony count of *B. cereus (*DE-024, a tofu-based cheese substitute) contained an isolate harbouring the *ces* gene cluster, which is responsible for the production of the emetic toxin cereulide.

In Livsmedelsverket (2025), the conclusions were that surveys have shown a low pathogen occurrence in RTE products but with a relatively high proportion of spores. Further that *C. botulinum* spores have been detected in plant-based sausages (Pernu *et al.,* 2020). However, predictive modelling indicated that product properties, such as low pH and/or a_w, did not support growth (and hence toxin production) of *C. botulinum* at refrigerated temperatures (8 °C) (Livsmedelsverket 2025).

It was noted in the present survey that the cheese and meat/fish alternatives are mainly refrigerated RTE foods and have a shelf-life allocation ranging from one to several weeks up to several months.

They were labelled on several occasions with a best before date, allowing consumers to decide whether to consume the product after that date based on their assessment of its sensory qualities, such as visual appearance, odour, and taste. Seven of the products with borderline or unsatisfactory test results were purchased from online shops where the risk of the cold chain not being maintained properly during distribution to the final consumer is reasonably foreseeable. This could enable temperature conditions that might increase the risk of pathogens growing in the product should it be contaminated.

Furthermore, the present survey cannot make a comment on to what extent food business operators allocate shelf-life based on sufficient knowledge of (inter-batch) product characteristics or by using predictive modelling or challenge tests to assess the growth (inhibition) of pathogens such as *L. monocytogenes or B. cereus.* Further research is needed to identify points of attention and CCPs in the production processes of RTE plant-based dairy (milk, cream and cheese), meat and fish substitutes. Such studies would inform the further development of tailored guidelines for GHP, time/temperature parameters, and product formulation recommendations to ensure microbiological safety during processing and preservation. Additionally, fit-for-purpose predictive models and challenge testing should support shelf-life determination, providing food business operators with science-based guidance for shelf-life allocation.

Finally, consumers often perceive plant-based foods as safer than their animal-based counterparts. Continued vigilance by regulators and food business operators is warranted to maintain food safety standards and to address isolated findings that could pose a potential public health concern if left unmanaged. It is recommended that food safety authorities, in collaboration with relevant stakeholders, should review and update risk communication for consumers related to storage and use of RTE plant-based products. RTE plant-based chilled products sampled in the present survey were often labelled with best before dates rather than use by dates. Under food safety legislation, food business operators must take consumer behaviour into account by assessing the reasonably foreseeable conditions of distribution, storage and use, and the normal consumption of a food product when determining in a food is unsafe (European Commission, 2002; 2005). Temperature abuse and long shelf-life durations of product labelled with a best before date, which some consumers may choose to eat long after the labelled date has passed, may facilitate the growth of foodborne pathogens to levels that are considered unsafe. It is vital that clear risk communication information is provided to consumers regarding the importance of respecting the labelled storage conditions on products and to ensure proper handling of plant-based RTE products - particularly after opening, when refrigerated storage time is limited - to help ensure food safety all the way to the plate.

6 Recommendations

Based on the findings of this survey, the following recommendations are proposed –

For Food Business Operators:

1. Food business operators should review and strengthen food safety management systems, with emphasis on post-heat-treatment handling and equipment hygiene programmes. Environmental monitoring programs may be beneficial in identifying potential sources of contamination, particularly for *L. monocytogenes* (Food Safety Authority of Ireland, 2026; Joint FAO/WHO Food Standards Programme CODEX Committee on Food Hygiene, 2025).
2. The time-temperature combinations used for heat inactivation to 'inactivate' spores of *B. cereus* should be validated to prevent substantial outgrowth (and emetic toxin formation) of *B. cereus* during fermentation/soaking (e.g. of nuts/fermented cheese alternatives) (Ellouze *et al.,* 2021). Effective thermal processing strategies for plant-based dairy alternatives, are essential for ensuring safety and stability of this growing and sustainable food category (Karamcheti *et al.,* 2025).
3. Food businesses should account for how consumers actually store and use products, recognising that temperature abuse and extended consumption beyond the best before date can allow harmful microbes to grow if they are present. Clear, prominent risk communication on storage, handling, and limited refrigerated shelf-life after opening is recommended on the label of the products to ensure food safety. This would help reduce the risk of foodborne pathogens growing to levels that could cause illness in consumers.

For Regulators:

1. Continued official control surveillance of plant-based RTE products placed on the market is recommended. In particular, the present survey identified microbiological contamination issues in RTE plant-based products containing tofu (12 products), pea protein (8 products) and cashews (7 products) as a primary ingredient. Microbiological issues were also observed in a smaller number of products primarily made from other nuts (3 products), oat (4 products), coconut (4 products), seitan (2 products) and tempeh (2 products). The remaining products with positive test results were blended formulations that contained more than two plant-based ingredients in varying proportions. Periodic targeted official control monitoring of pathogens and hygiene indicator organisms in these products may help identify emerging risks and support preventive control measures.

2. While national guidelines with recommended microbiological criteria to monitor microbiological contamination of RTE products placed on the market are available in some EU Member States that participated in the present survey, this is not the case for others. Harmonised microbiological criteria to assess the microbiological quality of RTE plant-based dairy, meat and fish substitutes would be welcomed to ensure consistency when assessing the compliance of these products under official controls.
3. It is recommended to conduct a risk profiling of these plant-based alternatives and their main raw materials based on the present (and other) survey results. This will enable an understanding of the most critical raw material-product type–pathogen combinations for prioritisation in annual monitoring programs by food safety authorities or food industry self-monitoring systems.
4. It is recommended to critically review the shelf-life validation of RTE plant-based dairy, meat and fish substitutes. It was noted in the present survey that the cheese and meat/fish substitutes sampled were mainly refrigerated RTE foods with a shelf-life duration ranging from one to several weeks, up to several months. The products were labelled on several occasions with a best before date. To ensure the food safety of these products, it is critical that the food business operator uses appropriate studies such as physico-chemical characterisation of the products when establishing and validating shelf-life based on reasonably foreseeable conditions of distribution, storage, and use as per Annex II of Commission Regulation (EC) No 2073/2005, as amended (European Commission, 2005). For example, determination of shelf-life should be based on a comprehensive understanding of inter-batch product characteristics, application of predictive modelling and/or challenge testing to evaluate the growth or inhibition of pathogens such as *L. monocytogenes* or *B. cereus*.

For Consumers:

1. Risk communication advice to consumers should recommend that they always follow storage instructions on the RTE plant-based dairy, meat and fish substitutes carefully. In particular, it is important to maintain the refrigeration conditions for products labelled with dates, and to observe good hygiene handling practices after opening. Although the products tested were generally of good microbiological quality, proper storage and timely consumption remain important to minimise the risk of foodborne illness.

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