

Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH). Part 3 (Fresh-whole FVH process water management plan)

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

Abstract

Water used in post-harvest handling and processing operations is an important risk factor for microbiological cross-contamination of fruits, vegetables and herbs (FVH). Industrial data indicated that the fresh-whole FVH sector is characterised by very variable operational cycle duration (between 8 and 900 h), large product volumes (e.g. more than 6000 tonnes) and process water at 2.8–25.0°C. Intervention strategies were based on water disinfection treatments, mostly using chlorine-based disinfectants. Water replenishment was not observed within studied industries. The industrial data, which included 29 scenarios were used to develop a guidance for a water management plan (WMP) for the fresh-whole FVH sector. A WMP aims to maintain the fit-for-purpose microbiological quality of the process water and consists of (a) identification of microbial hazards and hazardous events linked to process water; (b) establishment of the relationship between microbiological and physico-chemical parameters; (c) description of preventive measures; (d) description of intervention measures, including their validation, operational monitoring and verification; and (e) record keeping and trend analysis. A predictive model was used to simulate water management outcomes, highlighting the need for water disinfection treatments to maintain the microbiological quality of the process water and the added value of water replenishment. Relying solely on water replenishment (at realistic feasible rates) does not avoid microbial accumulation in the water. Operational monitoring of the physico-chemical parameters ensures that the disinfection systems are operating effectively. Verification includes microbiological analysis of the process water linked to the operational monitoring outcomes of physico-chemical parameters. Food business operators (FBOs) should set up and validate a tailored WMP to identify physico-chemical parameters, as well as microbial indicators and their threshold levels, as performance standards for maintaining the fit-for-purpose microbiological quality of the process water during post-harvest handling and processing operations.

KEYWORDS

fit-for-purpose water, industry, operational monitoring, validation, verification, wash water, washing, water disinfection, water quality, water safety

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Biological Hazards (BIOHAZ) to provide a scientific opinion on the microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (ffFVH) to provide guidance on the use of water in the production of fffVH and to describe the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water. In particular, the Panel was asked: (1) to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of fffVH and the routes and rates of contamination of the water and the fffVH; (2) to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water, used for post-harvest handling and processing operations of fffVH, taking into account their impact on the physiological state of the microbiological hazards present in the water; and (3) to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH.

The mandate includes five outputs (scientific opinions). The already published Part 1 opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire replies. The Part 2 Opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in fffVH. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively. This Part 3 Opinion is specific to the fresh-whole FVH sector with an emphasis on data generated from the EFSA outsourced activities described in Gil et al. (2025). These data were analysed to understand the industrial practices followed by the industrial collaborators included in this tender, and the relevant outputs were used to address the TORs.

First, the SO describes the post-harvest handling and processing operations using water and highlights the main characteristics of the sector. Based on the data obtained from the food business operators (FBOs) (EFSA outsourced activities – Gil et al., 2025), the main characteristics of the sector were identified. Results showed that common post-harvest handling operations include hydrocooling, dumping, pre-sorting and washing. The water temperature was usually not controlled and often above 10°C, reaching as high as 25°C, to avoid chill injuries for certain climacteric fruits, such as mangoes and avocados. The water used during these operations is sourced from municipal, well and surface waters, with limited instances of recycled water. The operational cycle of some scenarios lasted about 4–5 h, while in other scenarios, operational cycles lasted more than 1 month. The amount of product processed during the operation cycles varied between hundreds and thousands of tonnes (e.g. 6000 tonnes). The most common water volumes of the water tanks varied between 5000 L and 10,000 L, but water volumes above 10,000 L were also observed. The product-to-water contact time during operations varied from a few seconds (9–10 s) to several minutes (> 10 min), but in most of the cases was below 5 min.

Second, this SO addresses the main components of a water management plan (WMP) aiming to maintain a fit-for-purpose microbiological quality of process water. The WMP covers (a) the identification of microbial hazards and hazardous events linked to process water; (b) the establishment of relationships between microbial and physico-chemical parameters of the process water; (c) a description of preventive measures; (d) a description of intervention measures, including their validation, operational monitoring and verification; and (e) record keeping and trend analysis. There are common aspects applicable to the three FVH sectors included in the specific scientific opinions Parts 3, 4 and 5.

Across the three sectors, certain microorganisms – such as *Listeria monocytogenes*, *Salmonella* spp. and pathogenic *Escherichia coli* – are consistently identified as the most important microbiological hazards, particularly in cases where water is repeatedly reused or insufficiently disinfected. Common hazardous events for all the sectors include (i) an incomplete removal of contaminated water and/or inadequate cleaning and disinfection between operations and (ii) using the same water to wash large volumes of product during an operational cycle without a well-managed intervention strategy.

In order to analyse the microbiological data of process water obtained from industrial scenarios (EFSA outsourced activities – Gil et al., 2025), three layers of analyses were performed: (i) graphical representation (box-plot) of the levels of potential microbial indicators for process water samples in which pathogens were either detected or not detected, allowing calculation of the percentage of observations exceeding different thresholds of the potential microbial indicators; (ii) calculation of the odds ratio (OR) of detecting pathogens in relation to different thresholds of potential microbial indicators, aggregating data for sampling visit; and (iii) multivariable logistic mixed-effect modelling using the entire data set to assess the effect of microbial indicator levels on pathogen detection, accounting for the hierarchical structure of the data set. Statistically significant ORs were found in all the sectors, suggesting potential useful threshold levels for some microbial indicators. However, this logistic model revealed that pathogen detection in process water samples is influenced by multiple factors – such as the specific FVH product, type of operation and operational conditions – introducing a substantial random effect tied to each specific scenario for pathogen detection. The complexity of the data set (EFSA outsourced activities – Gil et al., 2025), which was not initially generated to establish associations between microbial indicators and pathogens, further complicated the identification of microbial indicators and setting of thresholds that could reliably predict pathogen detection in process water. Therefore, the suitability of any microbial indicator for verification purposes within a WMP should be validated under the specific operational conditions of each FBO.

Preventive measures aim to minimise the microbiological contamination in process water during post-harvest handling and processing operations. These measures are primarily based on Good Hygiene Practices (GHPs) and Good Manufacturing Practices (GMPs), which help maintain water quality throughout handling and processing. The key preventive measures include (a) infrastructure and fit-for-purpose buildings and equipment, (b) cleaning and disinfection of equipment and the

environment, (c) technical maintenance and calibration, (d) water and air quality control, (e) personnel management and (f) working methodology. These preventive measures will help to establish a basic level of water quality control before further interventions are implemented.

A primary intervention measure commonly used was the application of chemical disinfectants. Chlorine-based treatments, peroxyacetic acid (PAA) and hydrogen peroxide (H_2O_2) were commonly applied by the industry. An effective disinfectant application to the water requires real-time monitoring of different parameters such as residual disinfectant levels, pH and other physico-chemical parameters. No water replenishment practices were observed in any industrial scenario across the sectors; instead, smaller volume refilling strategies were used to sustain constant water levels. In the fresh-whole sector, intervention strategies were based on water disinfection treatments using chlorine-based disinfectants, followed by PAA and hydrogen peroxide.

In order to assist FBOs in implementing optimal intervention strategies and understanding the impact of these on process water quality, simulations of the effect of hypothetical scenarios representative of each sector (EFSA outsourced activities – Gil et al., 2025) were carried out through a mathematical model developed in the Part 2 Opinion (EFSA BIOHAZ Panel, 2025) and made available as a user-friendly tool (<https://r4eu.efsa.europa.eu/app/WaterManage4You>). Model simulations indicated that effective water management using chlorine-based disinfectants requires continuous operational monitoring and adjustment of physico-chemical parameters, such as the disinfectant residual levels and pH, to maintain process water microbiological quality within acceptable ranges. Conversely, the simulations showed that using water replenishment (at realistic feasible rates) alone as an intervention strategy was insufficient to maintain fit-for-purpose microbiological quality. A combination of water disinfection and replenishment provided a more effective water management strategy.

Each FBO should conduct a validation study to assess the efficacy of intervention measures, which will also support the selection of physico-chemical parameters (e.g. residual disinfectant, pH) as well as of the specific microbial indicators and corresponding thresholds (performance standards) to be used in the operational monitoring and verification procedures, respectively. This study should account for the fit-for-purpose water concept, tailored to the specific handling and processing operations, variability in operating conditions and the intended use of FVH, among other factors.

Specifically for the fresh-whole FVH sector, the most relevant microorganisms identified as hazards in process water included *L. monocytogenes* and norovirus. *L. monocytogenes* was frequently detected in scenarios where large product volumes were processed over extended operational cycles. *Salmonella* spp. was confirmed by PCR in one sample of water used for dumping apples. However, enteric pathogenic *E. coli* (STEC or the O157:H7 serotype) was not detected in any sample. In addition to the common hazardous events listed above, the use of contaminated water sources and the uncontrolled water temperature have been identified as additional hazardous events.

The potential relationship between the detection of enteric pathogens and levels of microbial indicators was evaluated. When a threshold of 100 CFU/100 mL of *E. coli* was considered, the OR for *L. monocytogenes* indicated that the odds of detecting the pathogen in the process water with levels of *E. coli* above this threshold is 11.6-fold (95% CI: 1.9–70.6, $p=0.008$) the odds when the indicator keeps below the threshold. These findings suggest a possible association between high levels of *E. coli* and the detection of *L. monocytogenes*. However, the above-mentioned logistic regression model showed that the detection of *L. monocytogenes* in process water is an event influenced by multiple factors/variables (e.g. a specific combination of FVH product, type of operation, operational conditions), which leads to an important random effect mainly linked to the scenario. Therefore, the suitability of any potential microbial indicator for verification purposes within the WMP should be validated under the specific operational conditions of each FBO.

It is recommended that relevant stakeholders use the developed mathematical model for their FVH sector to understand the impact of certain parameters and intervention measures on the process water quality, using specific data generated in their industrial settings. Evaluation of potential chemical hazards associated with the use of water disinfectants was outside the remit of this opinion. However, these need to be assessed in a WMP and linked to the fit-for-purpose microbiological quality of the post-harvest process water to be used.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

There has been an increase in the number of reported outbreaks, cases, hospitalisations and deaths associated with food of non-animal origin (FoNAO) in the EU from 2008 to 2011 (EFSA BIOHAZ Panel, 2013). A tendency has been observed for the outbreaks associated with FoNAO to involve more cases but be less severe than those associated with food of animal origin (Da Silva Felício et al., 2015). Reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) show an increasing trend in the implication of foodstuffs of FoNAO on the total burden of foodborne outbreaks in Europe (Machado-Moreira et al., 2019). Moreover, frozen vegetables and fruit have also been associated with major outbreaks (Murray et al., 2017; Soon et al., 2020). There has been an increase in the number of reported outbreaks associated with fresh produce in Europe and North America in recent years (Aiyedun et al., 2021), as well as in the number of fresh and frozen berry-linked viral outbreaks globally (Bozkurt et al., 2021).

Potential sources of contamination of FoNAO attributed to primary production and processing operations have been reviewed by EFSA for various commodities, including fresh and frozen fruit and vegetables (EFSA BIOHAZ Panel, 2011, 2013, 2014a, 2014b, 2014c, 2014d, 2014e, 2020). Water use during harvesting and processing has been identified as an important risk factor for contamination of fruits, vegetables and herbs (FVH). Special attention has been given to microbiological hazards associated with the use of contaminated water during harvest, post-harvest handling and processing, with a special emphasis on cross-contamination during the washing of fresh and frozen fruits, vegetables and herbs (fffVH) (EFSA BIOHAZ Panel, 2014a). The process water used after blanching vegetables in the deep-freezing industry is also important (EFSA BIOHAZ Panel, 2020). The microbiological quality of the water that comes into contact with fffVH is an important consideration and should be controlled by an operational prerequisite program (oPRP) activity to avoid cross-contamination (EFSA BIOHAZ Panel, 2020; FAO/WHO, 2019).

Large volumes of water are used during harvest and post-harvest handling and processing operations (e.g. washing, rinsing, the use of a flume, chilling, cooling, and for general cleaning, sanitation and disinfection purposes), as well as during fresh-cut/freeze value-added operations, distribution and end-user handling of fffVH. Therefore, most post-harvest processors favour using the same water during many hours of processing operations for sustainability reasons (i.e. to save water and energy) and because, in some regions, access to potable water is limited or very expensive. According to current practices, potable water is used to fill the equipment and tanks during the first hour in the morning, and the water is not replaced for several hours or even several days in some cases, during which large volumes of fffVH may be processed. Hence, organic matter, microorganisms, including pathogens, and chemical residues can accumulate in the water, thus causing cross-contamination between batches, which is a major concern (FAO/WHO, 2019). The quality of water used in post-harvest handling practices and during processing operations of fffVH should be monitored and controlled to avoid an accumulation of microbiological hazards.

Most current recommendations specify that post-harvest water that comes in contact with fffVH and that is not usually subjected to an upstream microbiological inactivation or reduction treatment should be of potable quality during all post-harvest handling operations (FAO/WHO, 2019).

According to Council Directive 98/83/EC, 'water intended for human consumption'¹ shall mean, among others, 'all water used in any food-production undertaking for the manufacture, processing, preservation or marketing of products or substances intended for human consumption unless the national competent authorities (CAs) are satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form'.

Annex II– Chapter VII of Regulation (EC) No 852/2004 on the hygiene of foodstuffs² states that recycled water used in processing or as an ingredient is not to present a risk of contamination. It is to be of the same standard as potable water unless the CA is satisfied that the quality of the water cannot affect the wholesomeness of the foodstuff in its finished form.

Additionally, paragraph 7.3.4.3.c in the EU Commission Notice (2017/C 163/01)³ on guidance documents addressing microbiological risks in fresh fruits and vegetables (fFVs) at primary production through good hygiene indicates that, for primary production and associated operations at the place of such production (harvest and post-harvest), the washing water used should be at least of clean water quality for the initial washing stages. Water used for final rinses has to be of potable quality if the fFVs are often consumed as ready-to-eat (e.g. tomatoes, apples, pears, young carrots, spring onions).

According to paragraph 7.3.4.3.f in the EU Commission Notice (2017/C 163/01) as well as in relevant research papers (FAO/WHO, 2019; Gombas et al., 2017), if water is contaminated during washing and then used to process large quantities of fffVH, it can be a vehicle for cross-contamination.

In order to avoid cross-contamination of the product due to the use of contaminated water, water disinfection treatments are needed to eliminate or reduce, to an acceptable level, microorganisms of public health concern, but these

¹Council Directive 98/83/EEC of 3 November 1998 on the quality of water intended for human consumption (OJ L 330, 5.12.1998, p. 32–54) has been replaced by Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) (OJ L 435, 23.12.2020, p. 1–62).

²Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–23.

³European Commission, 2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

treatments should not adversely affect the quality and safety of the produce. Therefore, regardless of the wash method used, growers and processors should follow good practices that ensure and maintain appropriate water quality.

National rules within Member States exist and may create trade barriers since some prohibit using water disinfection treatments in the process water, while such practice is common in others. These risk management decisions are often based on different considerations about the reduced risk associated with microbiological contamination versus the potential added chemical risk associated with their use.

Moreover, concerns may arise regarding the maintenance of the microbiological quality of process water as well as the application of water disinfection treatments by the food business operators (FBOp). The proper operation of water disinfection treatment (e.g. application rate, in-use concentration and residual concentration on fffVH), as well as monitoring the efficacy, has to be conducted properly and safely. As established by FAO/WHO (2019), water quality must be maintained throughout the processing operation, and special attention must be paid to common wash and flume systems and reused water.

Water quality and use in post-harvest handling and processing operations are an increasing concern at the global level, mostly because there is an expected reduction in the availability of water of drinking quality due to climate change (CXC 53–2003).⁴ During the 43rd session of the Codex Alimentarius Commission on the Joint FAO/WHO Food Standards Programme in Autumn 2020, the future development of guidelines for the safe use and reuse of water in food production was approved. These guidelines will contain a specific Annex on the use and reuse of water in fresh produce production.

Terms of reference

The BIOHAZ Panel is asked to issue a scientific opinion on microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables and herbs (fffVH) to provide guidance on the use of water in the production of fffVH, the establishment of microbiological requirements for water quality and the available prevention and control measures that can be implemented to maintain the appropriate microbiological quality of the water.

More specifically, EFSA is requested to address the following terms of reference (TORs):

TOR 1 aims to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of fffVH and the routes and rates of contamination of the water and the fffVH.

TOR 1.1: Which are the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for fffVH?

TOR 1.2: What are the routes of water contamination and the rates of contamination (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) in the water used in different post-harvest handling and processing operations for fffVH?

TOR 1.3: Which are the contamination rates (increase in microbiological and pathogen load over time) for the most relevant microbiological hazards (identified in TOR 1.1.) between different fffVH batches during different post-harvest handling and processing operations using the same water?

TOR 2 aims to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment rates, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH, taking into account their impact on the physiological state of the microbiological hazards present in the water.

ToR 2.1: Which good hygiene practices are recommended to ensure appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH?

TOR 2.2: Which are the most efficacious water disinfection treatments (dose and mode of application) to maintain the appropriate microbiological quality requirements of water used during different post-harvest handling and processing operations of fffVH?

TOR 2.3: What is the impact of different water disinfection treatments on the induction of the viable but non-culturable (VBNC) state or injury state in bacteria in water used for different post-harvest handling and processing operations of fffVH?

TOR 2.4: Which are the relevant parameters to establish efficacious water replenishment rates needed to maintain the appropriate microbiological quality requirements of water used for different post-harvest handling and processing operations of fffVH?

TOR 3 aims to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH.

TOR 3.1: Which relevant parameters can be used to validate and/or verify the appropriate microbiological quality requirements of the water intended to be used for different post-harvest handling and processing operations of fffVH?

TOR 3.2: Which relevant parameters can be used to monitor the appropriate microbiological quality requirements of water that is being used during different post-harvest handling and processing operations for fffVH?

⁴CAC (Codex Alimentarius Commission), 2003. Code of Hygienic Practice for Fresh Fruits and Vegetables CXC 53–2003, p. 1–39.

1.2 | Additional information

The Mandate on Microbiological Hazards in Water Use during Postharvest Operations of Fresh and Frozen Fruits, Vegetables and Herbs (ffFVH) is a self-task mandate from the BIOHAZ Panel, including multiple outputs. It integrates a work package that consists of outsourced activities, including tasks such as literature reviews, experimental data collection in industrial settings and modelling, as detailed in the external scientific report (Gil et al., 2025).

The mandate includes five outputs (scientific opinions), as illustrated in Figure 1. The already published Part 1 opinion (EFSA BIOHAZ Panel, 2023) contains the literature review and analysis of the outbreak data and stakeholder questionnaire. The Part 2 Opinion contains a summary of the development of a dynamic mass balance model for processing operations using water in ffFVH. Parts 3, 4 and 5 Opinions focus specifically on the fresh-whole, fresh-cut and frozen FVH sectors, respectively. The same approach and structure are used for each sector-specific opinion (Parts 3–5), aiming to produce concise opinions offering sector-specific guidance. This is achieved by extracting information from the experimental data generated through EFSA's outsourced activities coupled with modelling based on these outcomes. A user-friendly tool has been also developed to allow FBOs to analyse their data and use predictive mathematical modelling to understand the impact of their intervention measures on microbial indicator levels (<https://r4eu.efsa.europa.eu/app/WaterManage4You>).

This opinion only covers some of the sub TORS from TOR1 (i.e. TOR 1.1), mostly because industrial data were unavailable to provide further knowledge apart from what has been already included in the Part 1 Opinion. However all the sub TORS from TOR 2 and TOR 3 were addressed. Throughout the text, all the opinions from this mandate will be referred to as “Part 1 opinion”, “Part 2 opinion”, etc.

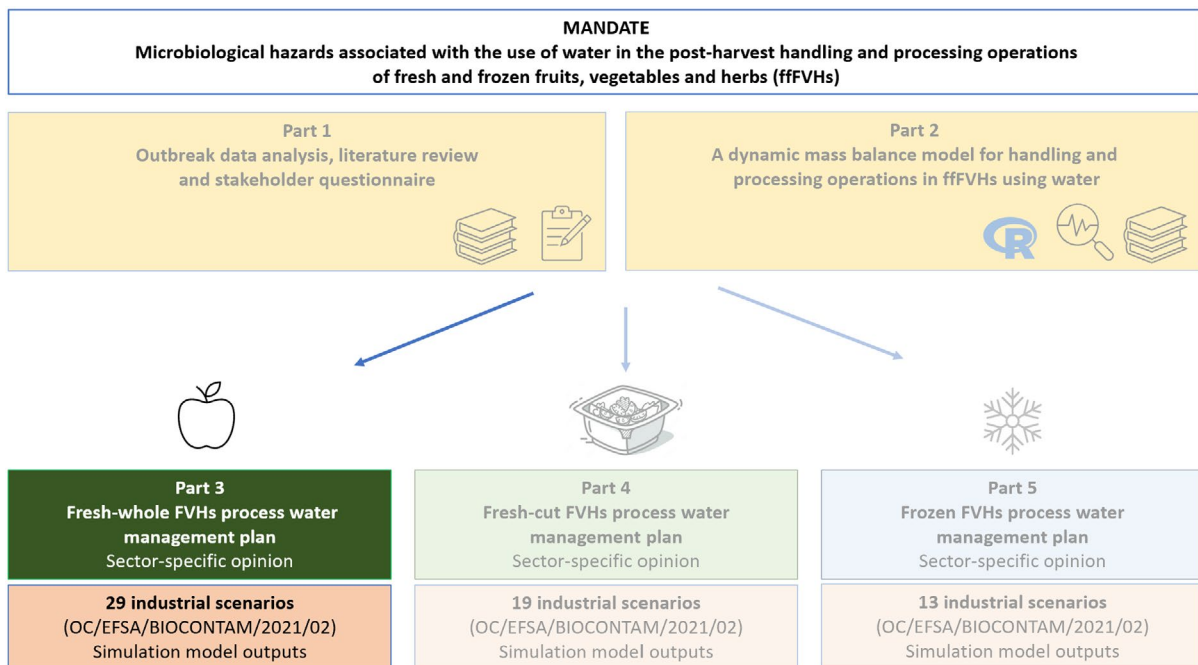


FIGURE 1 Outputs from the mandate on ‘Microbiological hazards associated with the use of water in the post-harvest handling and processing operations of fresh and frozen fruits, vegetables, and herbs (ffFVH)’ (including EFSA outsourced activities – Gil et al., 2025).

1.3 | Interpretation of the Terms of Reference

This scientific opinion for the fresh-whole FVH sector focuses on the evaluation of microbiological hazards that might be present in the process water used in different handling and processing operations. Chemical hazards are out of the remit of this Scientific Opinion. The aim is to identify the main microbiological hazards and understand the routes of contamination of the process water used in different operations of the fresh-whole FVH together with the strategies that can be applied by the FBOs to keep the microbiological quality of the process water according to its use, i.e. fit-for-purpose quality.⁵ The transfer of microorganisms from the process water to the product as well as from product to product (TOR 1.3) is out of the remit of this opinion.

The fresh-whole FVH sector-specific guidance focuses on all the TORs previously described in the Part 1 EFSA scientific opinion (EFSA BIOHAZ Panel, 2023), with an emphasis on evidence generated from the EFSA outsourced activities

⁵Fit-for-purpose water (in this opinion used as a synonym of the concept ‘process water’): encompassing potable water, clean water, recirculated water or recycled water that could be fit for different post-harvest handling and processing operations provided they do not compromise the safety of the final product for the consumer. While water quality will be different in each context, it can be fit to use for certain purposes. Deciding whether water is fit-for-purpose, assessment of the source water, potential hazards linked to this water source, treatment options and their efficacy, multiple barrier processes and the end use of the food product (e.g. if eaten raw) must be considered (FAO and WHO, 2019).

described in Gil et al. (2025). This data set was analysed to understand the industrial practices followed by the industrial collaborators included in this tender, and the relevant outputs were used to address the TORs.

Based on these findings, it is important to clarify the following terms when referring to the process water management practices:

1) **Refilling:** Adding a small amount of water (e.g. potable water, process water) during the operations to maintain a constant volume in the water tank/equipment/process lines. In this case, the water added during the refilling replaces the water lost from the water tank by the movement of the product or due to spilling during the process. This volume is not enough to dilute the organic matter and microorganisms accumulated in the process water due to the introduction of FVH. Therefore, it is not considered as a water management intervention strategy. Based on the data obtained from the industrial settings sampled in the context of EFSA's outsourced activities (Gil et al., 2025), the volumes added during refilling are usually small (< 100 L/h in a 2500 L tank). This agrees with available information (Barrera et al., 2012; Gil et al., 2016). Moreover, the volume of water added is not usually monitored by the FBOp.

2) **Replenishment (or Refreshment):** In the Part 1 Opinion of this mandate (EFSA BIOHAZ Panel, 2023), water replenishment was defined as the 'practice of replacing used water with fresh water during the cleaning and rinsing of fresh fruits and vegetables'. Water replenishment was considered as a potential intervention strategy to dilute the organic matter and the microorganisms accumulated in the water tank. To achieve a diluting effect of organic matter and microorganisms in the process water due to the water replenishment strategy, the volume of water added to the water tank should be considerably high (e.g. $\geq 50\%$ of the total volume, 1000 L/h in a 2500 L water tank) (Allende et al., 2016; Gil et al., 2016). Based on the information retrieved from the industrial settings sampled in the context of EFSA's outsourced activities (Gil et al., 2025), water replenishment is currently not performed by the Fresh whole FVH sector. Only the water refilling is done as described above.

3) **Complete removal:** In the fresh-whole FVH sector, after a certain time, the water tank/equipment/processing lines are (almost) fully emptied and the tank is filled again with new (fresh) water. This means that almost all the water (e.g. 90% or more water from a tank) present in the water tank is removed and replaced by new fresh water (e.g. municipal tap water, reconditioned water).

Data from the industrial settings sampled in the context of EFSA's outsourced activities ($n=61$, considering the 3 sectors for fresh-whole, fresh-cut and frozen FVH) were obtained from two distinct sampling visits (Gil et al., 2025). In each sampling visit, six sampling time points were selected within one operational cycle, and duplicate samples were collected. Exceptionally, in this sector, for six scenarios, only one sampling visit could take place during the operational cycle.

The working group defined the 'operational cycle' as the period between (almost completely) filling and emptying the water tank used for the handling and/or processing operation. Sampling time points were distributed from the start of the operation (process start), generally it coincided with the filling of the water tank which is the start of the operational cycle, but it was not always possible. In some cases, the operation may begin sometime after the tank is filled with water, and emptying may occur after the completion of the sampling and handling activities. It should be noted that if the filling and emptying times of the water tank were unknown or not applicable, the operational cycle was considered to be the time between the start and end of the handling process.

In the fresh-whole FVH sector, the duration of the operational cycle varied significantly. In some cases, it reached over 800 or even 900 h, while in others, it was much shorter, around 8–10 h. For shorter cycles, samples were taken at regular intervals, such as every 2 h for an 8-h operational cycle (i.e. 0, 2, 4, 5, 6 and 8 h). For longer cycles, sampling time points were adjusted accordingly to ensure that data was collected more or less evenly distributed throughout the entire process. For example, in one scenario with an 122-h operational cycle, samples were taken at 1, 7, 25, 48, 55 and 75 h. Overall, the sampling time points were strategically distributed to capture changes in water quality throughout a significant portion of the operational cycle, ensuring a comprehensive assessment.

2 | DATA AND METHODOLOGIES

2.1 | Data

2.1.1 | Literature review

The information retrieved from the literature searches for the Part 1 scientific opinion was used in this sector-specific opinion. Details of the methodology followed for the literature search can be found in (EFSA BIOHAZ Panel, 2023).

2.1.2 | Data collection

The external scientific report describing EFSA's outsourced activities contains data representative of the fresh-whole FVH industry settings. It is relevant to address some of the specific assessment questions in this mandate. The main objective of this tender was to gain insights into the characteristics of the process water and practices followed by the industry to maintain water quality used during the post-harvest handling and processing operations for fffvvh. The case-studies (scenarios)

selected by the tenderer included three types of fFVH: (i) fresh-whole FVH, (ii) fresh-cut FVH and (iii) frozen FVH. Data include the characterisation of the water used in different post-harvest handling and processing operations of fFVH with the aim of evaluating the microbiological and physico-chemical quality of the process water in industry settings. Several physico-chemical parameters were included in the assessment (water temperature, pH, oxidation–reduction potential (ORP), electrical conductivity (EC), residual concentration of disinfectant, total chlorine, total dissolved solids (TDS), turbidity, total soluble solids (TSS), chemical oxygen demand (COD), unfiltered and filtered UV-absorbance, redox potential). The assessment of the microbiological quality of the process water included enumeration of total bacterial count (TBC), total coliforms (TC), *E. coli*, *Listeria* spp., moulds and yeasts, F-specific phages, total phages and human gut-associated DNA bacteriophage named crAssphage. However, bacteriophages were only determined in selected samples. Detection of foodborne pathogens was also performed, including *Salmonella* spp., *L. monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC) (including O157:H7), norovirus (GI and GII) and *Cryptosporidium* spp. Levels of VBNC cells were determined in selected samples, including TBC, TC, *E. coli* and *Listeria* spp., as well as the capsid integrity of norovirus. Spores of *C. perfringens* have also been analysed in specific samples. In this scientific opinion, data obtained for the fresh-whole FVH sector were considered. Not all the data generated in EFSA's outsourced activities have been used in the assessment of this scientific opinion. The most relevant data were selected based on the objective of the analyses. The results of all the analyses performed during the two sampling visits to each FBOp are included in Annex A, which contains all the data collected.

2.2 | Methodologies

Data collected for fresh-whole FVH from EFSA's outsourced activities were used to answer the different TORs. Data were compiled in an Excel file, including information related to the characterisation of the post-harvest handling and/or processing operations (e.g. volume of product, volume of water, characteristics of the process water, type of intervention) and the results regarding the physico-chemical parameters and microbiological analysis obtained in different sampling points and visits to the industries covering different scenarios.

2.2.1 | Data analysis

Data regarding the outputs of the handling and processing operations generated by EFSA's outsourced activities (Gil et al., 2025), which included 29 industrial scenarios of the fresh-whole FVH sector, were analysed in three steps.

First, results of the levels of potential microbial indicators (CFU/100 mL) corresponding to each individual sample were \log_{10} transformed and plotted in figures to facilitate the exploratory analysis.

In addition, the counts of potential microbial indicators (TBC, TC, *E. coli* and *Listeria* spp.) for samples in which pathogens were detected and not detected were graphically represented through box-and-whisker plot using R (version 4.3.2) and R Studio (version 2023.2.3.561). Graphical representation included the median, interquartile range (IQR as boxes) and min-max range (as whisker). Potential outlier dots included values out of 1.5 times the IQR below Q1 and above Q3. Results of potential microbial indicators below their respective LOD were set as 0 \log_{10} CFU/100 mL by setting these results as 0.01 CFU/mL and then transformed into 1 CFU/100 mL. Thresholds (in \log_{10} scale) for microbial indicators were defined and used to compute the percentage of observations with microbial indicator levels exceeding these thresholds, which could be considered as potential process water performance standards for verification purposes. This was done using all the results of analysed process water samples for the fresh-whole FVH sector.

TABLE 1 Relevant thresholds selected for the potential microbial indicator groups TBC, TC, *E. coli* and *Listeria* spp.

| Indicator | Levels of microbial indicator |
|----------------------|--|
| TBC | 4, 5 and 6 \log_{10} CFU/100 mL (10^4 , 10^5 , 10^6 CFU/100 mL) |
| TC | 2, 3 and 4 \log_{10} CFU/100 mL (10^2 , 10^3 , 10^4 CFU/100 mL) |
| <i>E. coli</i> | 0, 1 and 2 \log_{10} CFU/100 mL (1, 10 and 100 CFU/100 mL) |
| <i>Listeria</i> spp. | 0, 1 and 2 \log_{10} CFU/100 mL (1, 10 and 100 CFU/100 mL) |

Secondly, the relationship between the occurrence of potential microbial indicators above the thresholds indicated in Table 1 and the detection of pathogens (as an outcome) was explored by aggregating observations within each sampling visit. In the aggregated data, the pathogen was considered detected if it was identified at least once in any of the sampling time points and replicated samples within each visit. The odds ratio⁶ (OR) of detecting the pathogen depending on the threshold (Th) of the microbial indicator (MI) was calculated as:

$$OR = \frac{\text{Odds MI} \geq Th}{\text{Odds MI} < Th} = \frac{a/b}{c/d}$$

⁶Odds measure how likely the detection of the pathogen is, and is calculated when the indicator is below and above the given threshold.

Where:

a is the number of visits in which the pathogen was **detected** in the process water, and the level of the microbial indicator was **equal to or above** the potential threshold, at least in one of the samples of process water analysed within the operational cycle.

b is the number of visits in which the pathogen was **never** detected in the process water and the level of the microbial indicator was **equal to or above** the potential threshold, at least in one of the samples of process water analysed within the operational cycle.

c is the number of visits in which the pathogen was **detected** in the process water, and the level of the microbial indicator was **below** the potential threshold in all the samples of process water analysed within the operational cycle.

d is the number of visits in which the pathogen was never **detected** in the process water, and the level of the microbial indicator was **below** the potential threshold in all the samples of process water analysed within the operational cycle.

The OR confidence interval at 95% and the statistical significance (*p* value) of the *z* statistic were calculated with MedCalc⁷ for the three different thresholds defined above for TBC, TC, *E. coli*, or *Listeria* spp. as potential microbial indicators.

It is worth noting that the OR is equivalent to the exponential of the regression parameter of a univariable logistic model that includes a single microbial indicator group as explanatory variable, dichotomised according to each of the three thresholds as 'below/above' and the probability of detection of pathogens (logit-transformed) as dependent variable. An OR greater than one suggests increased odds of detection of the pathogen when the indicator exceeds the selected threshold.

Thirdly, a multivariable logistic mixed effect model was developed with the entire data set to assess the potential effect of the levels of the potential microbial indicators (in log₁₀ CFU/100 mL) on the detection of the relevant pathogen (*L. monocytogenes*) in process water. Food sectors included in the model for *L. monocytogenes* were only fresh-whole FVH and frozen FVH since no positive results were observed in fresh-cut FVH. The hierarchical structure in the data was accounted for considering nested random factors (i.e. (i) the scenarios, (ii) the sampling visits nested into scenarios and (iii) sampling time points nested into sampling visits and scenarios). To reduce the complexity of the model, the replicates effect was not considered, i.e. the microbial indicator levels of the two sample replicates of each sampling time point were averaged (before log-transformation), and the pathogen detection was determined as positive if detected in at least one of the two replicates. The interactive effects of the microbial indicator levels by food sectors were included as fixed factors. Only a subset of the four microbial indicators was included in each model to avoid multicollinearity of the explanatory variables.

When modelling the detection of *L. monocytogenes*, an additional fixed factor was included to consider whether the detection of the pathogen was confirmed by PCR or carbohydrate fermentation testing. Four different models for *L. monocytogenes* were set and compared for their ability to fit the data. The complete statistical analysis performed, including exploratory analysis and the modelling, is described in Annex B.

In order to identify appropriate physico-chemical parameters, the industrial data were used to detect potential relationships between the physico-chemical and microbiological parameters. A principal component analysis (PCA) was performed among microbiological and physico-chemical characteristics of process water using the JMP Pro 15.0.0 (390308) (SAS Institute Inc., Cary, NC, USA). The analysis aimed to reduce data dimensionality while preserving essential information, enabling, if possible, the identification of underlying patterns and relationships between the variables.

2.2.2 | Identification of intervention measures

The dynamics of the effect of the different intervention measures applied in the different post-harvest handling and/or processing operations were addressed by consulting modelling approaches described in the literature. Despite the available information, none of the existing models could readily simulate the contamination/inactivation dynamics of all possible post-harvest handling and/or processing operations. As such, substantial amendments and customisations in the model structures and assumptions were required to tailor the models to different processes at an industrial scale. The model described in the Part 2 Model opinion, which was developed within EFSA's outsourced activities was fed with data obtained in industrial cases from the fresh-whole FVH sector to simulate different scenarios which mimic situations observed in the sampled industrial settings (Gil et al., 2025). A user-friendly tool allowing the simulation of different scenarios of intervention strategies (e.g. water replenishment and water disinfection treatments) as well as the management of these aiming to avoid cross-contamination of FVH by process water has also been developed (<https://r4eu.efsa.europa.eu/app/WaterManage4You>).

2.3 | Uncertainty analysis

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

⁷MedCalc Software Ltd. Odds ratio calculator. https://www.medcalc.org/calc/odds_ratio.php (Version 22.023; accessed April 7, 2024).

context of the TORs of the mandate, which do not include any assessment request, the uncertainty analysis was restricted to an overview of the uncertainty sources affecting the different TORs/AQs (Table A.1 in Appendix A).

3 | ASSESSMENT (FRESH-WHOLE FVH PROCESS WATER MANAGEMENT PLAN)

3.1 | Post-harvest handling and processing operations using water

3.1.1 | Flow charts of the processing lines and identification of water sources

Data generated by EFSA's outsourced activities include industrial scenarios for apples, avocados, carrots, celery, cherries, fruit mixes, mangoes, nectarines/peaches, pears, peppers and (uncut) vegetable mixes (Gil et al., 2025) (Appendix B). Both ready-to-eat (RTE) foods (e.g. apples, tomatoes, peppers) and non-RTE foods (e.g. uncut vegetable mixes) are included. Based on the European Commission Notice, the minimum water quality requirements established for process water use in post-harvest handling and processing operations are different for RTE and non-RTE foods. One can consult Annex II of the EU Commission Notice (2017/C 163/01)⁸ for details.

Handling and processing operations using water for fresh-whole FVH have been extensively described in the Part 1 opinion. Figure 2 shows a general flow chart for the handling and processing of fresh-whole fruits, vegetables and herbs (FVH), which includes steps where water can be used, like hydrocooling, pre-sorting, dumping, trimming, brushing, pre-washing, washing and rinsing (Appendix B). The EFSA outsourced activities included scenarios using water during product hydrocooling, dumping, pre-sorting, pre-washing and washing. It is relevant to mention that in the case of fresh-whole fruits, the dumping step replaces the pre-washing and washing operations, as in many cases, after dumping, the fresh-whole fruits are subjected directly to (air)drying.

⁸European Commission. (2017). Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

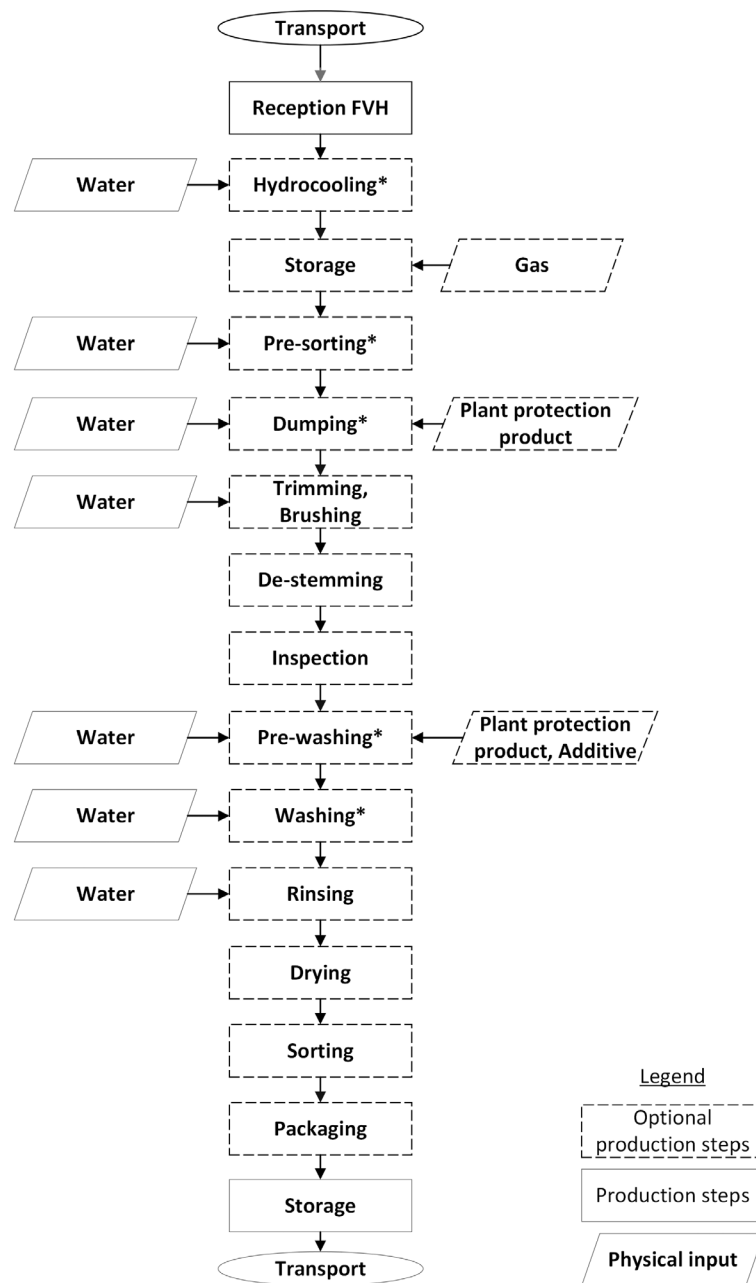


FIGURE 2 A general flow chart for handling and processing fresh-whole fruits, vegetables and herbs (FVH), which includes steps where water can be used. The flow chart can vary between FBOs (e.g. following order of steps, optional steps present or not) and represents a general description of the post-harvest processing and handling operations. *Production steps sampled during EFSA's outsourced activities (Gil et al., 2025).

3.1.2 | Characterisation of the industrial handling and processing operations for the fresh-whole FVH sector

Data generated by EFSA's outsourced activities included a total of 29 industrial scenarios for fresh-whole FVH processing (Gil et al., 2025). The water sources used in this sector included municipal tap water (12 scenarios), well water (9 scenarios) and surface water (7 scenarios) (Appendix B). In some scenarios, it has been declared that surface water is treated before being used in the different operations. Recycled water was only used in one scenario. Among the 29 industrial scenarios evaluated by the tender, 8 scenarios did not apply any intervention strategy aiming to maintain the microbiological quality of the process water (i.e. no water replenishment or water disinfection treatments). The other 21 scenarios made use of water disinfection treatments. The water disinfection treatments include chlorine-derived compounds (sodium and calcium hypochlorite (NaClO₂ and CaClO₂)), peroxyacetic acid (PAA) and hydrogen peroxide (H₂O₂) (Appendix B). The operational cycle varied among the different scenarios. Some operational cycles lasted about 4–5 h, while in other scenarios, operational cycles lasted more than 1 month.

The industrial scenarios included in EFSA's outsourced activities included two sampling visits to the same packing house or processing facility (Gil et al., 2025). In several scenarios, the two visits were performed in a short interval of time, mostly due to the production season of certain commodities. For example, in the scenarios for cherries, avocados and mangoes, production was concentrated to a few weeks of the year or only one sampling visit was performed (e.g. mangoes). Figure 3

shows the accumulated mass of fresh-whole FVs processed during an operational cycle. Differences were observed among scenarios, with a maximum amount of product being processed up to 6000 tonnes in almost 42 days (1000 h). In this sector, fruits are the most predominant commodity with 24 scenarios, followed by the rest of the commodities with 2 scenarios including bulbs and roots, 1 scenario including leafy greens and 2 including fruit or vegetable mix.

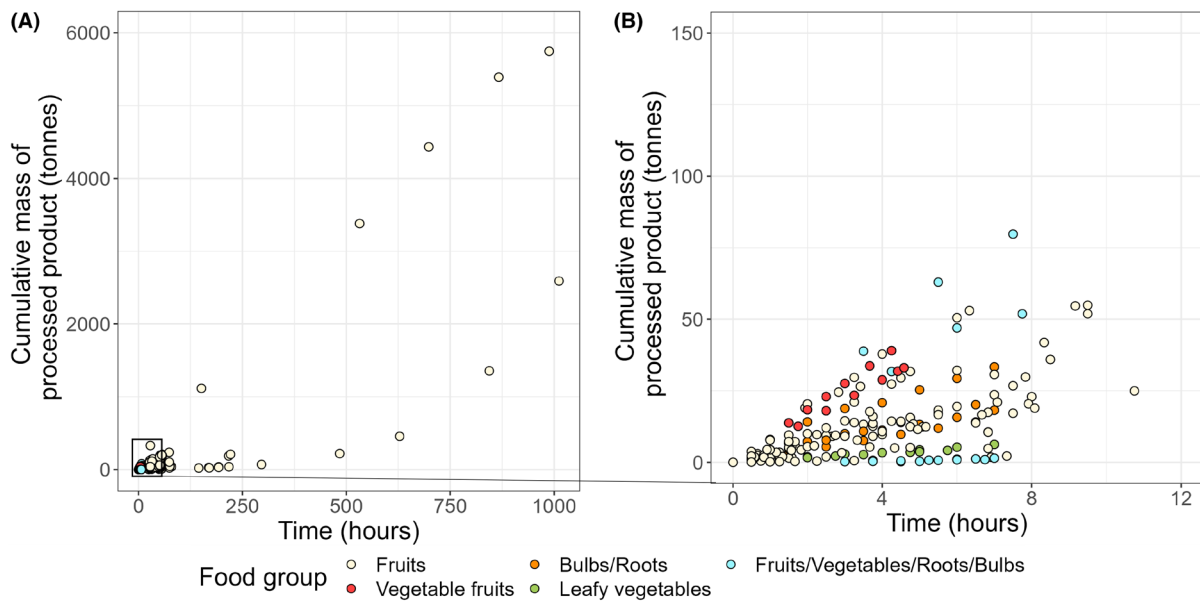


FIGURE 3 Cumulative mass of the product (tonnes) being processed during each sampling time point of the operational cycle (A) and (B) shows a zoomed-in picture of the values observed between 0 and 12 h of production. Source: EFSA outsourced activities (Gil et al., 2025).

Despite the large mass of product being processed (Figure 3), most of the FBOs only reported a minimal partial refilling of the water tank with unknown volumes of water in this sector. Based on the physico-chemical and microbiological characteristics of the process water during the production, it could be concluded that water replenishment (or refreshment) is not applied as an intervention strategy (EFSA outsourced activities – Gil et al., 2025). FBOs only refill the water tank to maintain the volume of water in the tank during the operational cycle.

Figure 4 shows the results of the measured water temperature and COD of process water of fresh-whole FVH along the operational cycle in scenarios with no water treatment and in those using chlorine-based, hydrogen peroxide and PAA disinfectants.

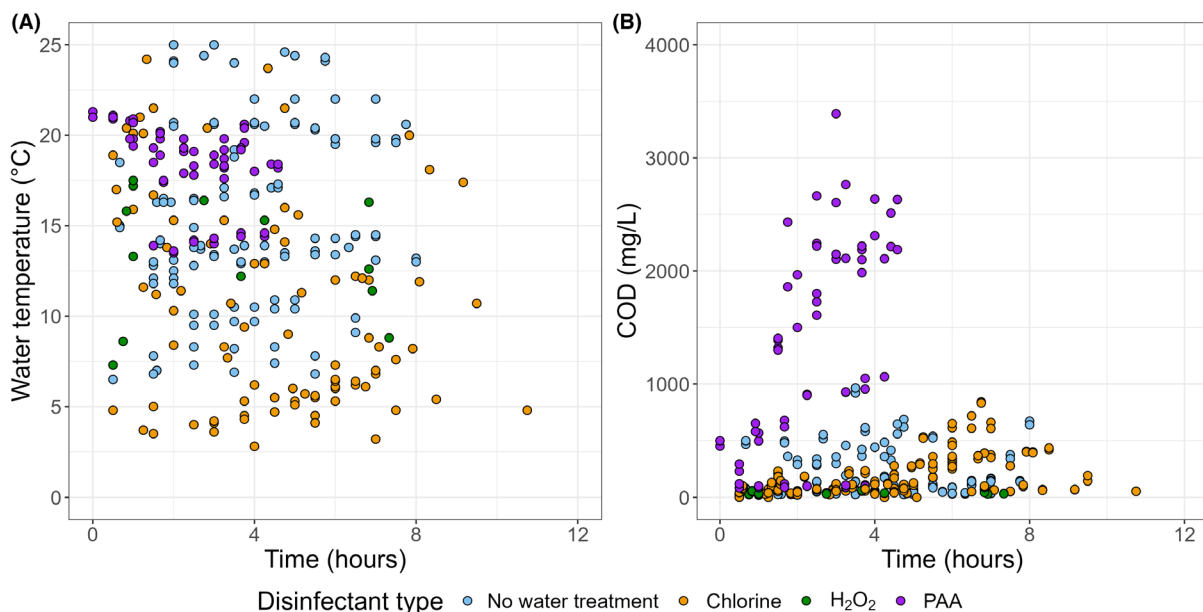


FIGURE 4 Water temperature (°C) (A) and chemical oxygen demand (COD, mg/L) (B) of the process water applied for fresh-whole FVH during each operational cycle. Selection is restricted between 0 and 12 h of production. H₂O₂: hydrogen peroxide; PAA: peroxyacetic acid. Source: EFSA outsourced activities (Gil et al., 2025).

One characteristic of the fresh-whole FVH sector is that, in many cases, the temperature of the process water is not cooled. Most of the recorded temperatures in process water were above 10°C, although some scenarios showed cooled conditions (3–5°C) (Figure 4A). Cooled conditions are recommended to keep the quality of the product as well as to avoid microbial growth (EFSA BIOHAZ Panel, 2023). However, as explained in the Part 1 scientific opinion, climacteric fruits such as mango and avocado do not sustain low temperatures due to chilling injury (e.g. < 10–15°C). So, for these commodities, process water temperature needs to be higher than cooled conditions.

The COD, which illustrates the oxygen consumption resulting from the chemical oxidation of organic matter accumulated in the process water, showed differences among the different scenarios (Figure 4B). In some cases, COD significantly increases during the operational cycle. There are different hypotheses to explain the increase of COD values of process water, including (1) the large mass of product being washed, (2) only using refilling and (3) the type of water disinfection treatment applied. For instance, the use of PAA to maintain the microbiological quality of process water significantly increases the COD of the water (EFSA BIOHAZ Panel, 2023). Adding PAA, an organic acid, to the process water increases the organic load of the process water. Therefore, in most of the cases, those scenarios where PAA is applied showed the highest COD values. Detailed information related to the industrial data can be found in Gil et al. (2025).

The pH of process water was, in most of the cases, higher than 7 (Figure 5). The industrial data include scenarios with and without water disinfectant treatment. The lowest pH values (pH ≤ 5.26) observed belonged to those scenarios using PAA as a water disinfectant (EFSA outsourced activities – Gil et al., 2025). The addition of peroxyacetic acid, an organic acid, considerably reduces the pH of the process water (pH 3.1–5.3). The remaining scenarios show pH values of between 6.5 and 9.0 (with 70% of the scenarios showing pH values above 7.0). Based on the recommendations found in the scientific literature (EFSA BIOHAZ Panel, 2023), when chlorine-derived compounds such as sodium or calcium hypochlorite are applied, the pH of process water should be maintained between 6.0–6.5 to ensure that the disinfectant (free chlorine, FC) is in its active form (hypochlorous acid). However, none of the industrial scenarios using chlorine-based compounds maintained the pH within these values. In those scenarios where water disinfectants were used the residual concentration was variable. When chlorine-based disinfectants were used, FC concentrations varied from 0 to 150 mg/L, while in the case of PAA, residual concentrations varied from 2 to ~600 mg/L. In the case of H₂O₂, residual concentrations changed from 0 to 130 mg/L. Detailed information related to the industrial data can be found in Gil et al. (2025).

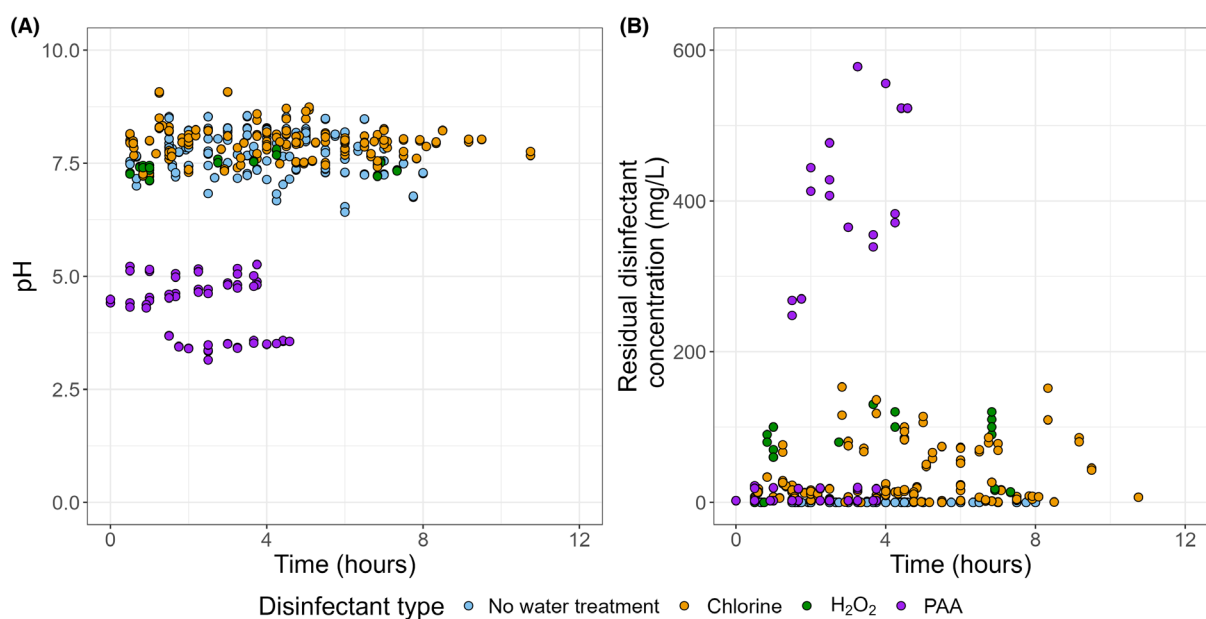


FIGURE 5 pH (A) and residual concentration of disinfectant (mg/L) (B) of the process water applied for fresh-whole FVH during each operational cycle. Selection is restricted between 0 and 12 h of production. H₂O₂: hydrogen peroxide; PAA: peroxyacetic acid. Source: EFSA outsourced activities (Gil et al., 2025).

The volumes of water applied were variable (Figure 6A). Most of the water tanks (13 scenarios) had a water volume between 5000 and 10,000 L. Only 5 scenarios showed water tank volumes higher than 10,000 L. The contact times between water and product were also variable, ranging from only a few seconds (9–10 s) to several minutes (> 10 min). However, in most cases, the contact times were below 5 min (Figure 6B). Detailed information related to the industrial data can be found in Gil et al. (2025).

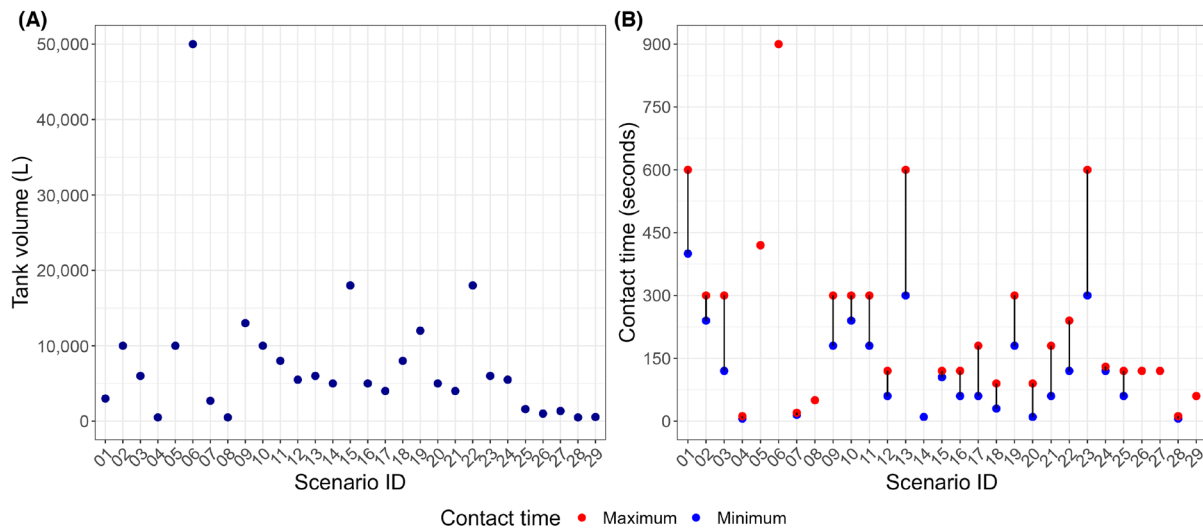


FIGURE 6 Tank volume (L) (A) and contact time (seconds) between product and water (B) in the process water samples from the fresh-whole FVH scenarios ID 1 to ID 29 during each operational cycle. The line segments in graph B show the range of contact times observed for each scenario from the minimum (blue dot) to the maximum (red dot) values. Source: EFSA outsourced activities (Gil et al., 2025).

The microbial accumulation in the process water during processing and handling operations of the fresh-whole FVH sector is represented in Figure 7.

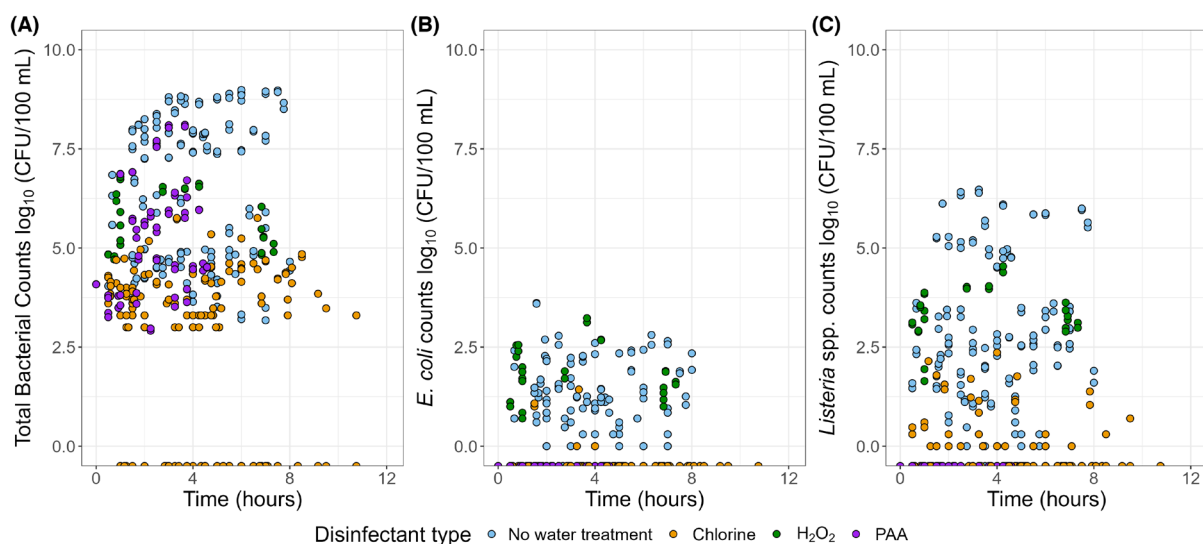


FIGURE 7 Distribution of total bacterial counts (A), *E. coli* counts (B) and *Listeria* spp. counts (C) (in \log_{10} (CFU/100 mL)) in process water treated with chlorine, H_2O_2 , PAA or without water treatment in industrial scenarios during each operational cycle of the fresh-whole FVH. Selection is restricted between 0 and 12 h of production. Bacterial counts for the three microbial groups (A, B and C) below the respective limit of detection (LOD) are represented below the '0' value in the Y-axis to distinguish these results from the real '0' observations (i.e. 1 CFU/100 mL for *E. coli* and *Listeria* spp.). H_2O_2 , hydrogen peroxide; PAA, peroxyacetic acid. Source: EFSA outsourced activities (Gil et al., 2025).

Levels of TBCs in the process water were very variable among the different scenarios (ranging from 3 up to 8–9 \log_{10} CFU/100 mL) (Figure 7A). The highest TBC were observed for those scenarios where no water disinfection treatments were applied (blue). When PAA (violet) or H_2O_2 (green) were used, the TBC in process water were between 5 to 7 \log_{10} CFU/100 mL. Low TBC ($<4 \log_{10}$ CFU/100 mL) were mostly observed in those scenarios where chlorine-based disinfectants were applied (orange) (EFSA outsourced activities – Gil et al., 2025). *E. coli* was mostly detected in those scenarios where no water disinfection treatment or H_2O_2 was applied (Figure 7B). A few samples of process water treated with chlorine showed detectable levels of *E. coli*. A similar trend was observed for *Listeria* spp. when comparing the microbiological quality of process water when different strategies were applied. *Listeria* spp. was mostly detected in those scenarios where no water disinfection or H_2O_2 were applied (Figure 7C). In those cases where chlorine-based disinfectants and PAA were applied, *Listeria* spp. levels were always low and even undetected. Detailed information related to the industrial data can be found in Gil et al. (2025).

The levels of VBNC bacterial cells in process water were determined in 3 scenarios, 1 of them corresponded to no application of a water disinfection treatment (ID 05), while the other 2 were scenarios where when PAA was applied (EFSA outsourced activities – Gil et al., 2025). In all the cases, high levels of TBC were observed, and, significantly, the levels of VBNC

cells were about 1 or 2 log₁₀ higher than that of the culturable, indicating no significant differences among the 3 scenarios with and without disinfection treatments and it has been observed that these treatments allow the induction of VBNC. A similar trend was also observed for TC. Due to the limited amount of data on VBNC bacterial cell levels, the results should be interpreted with caution. Given the data scarcity, it is not possible to draw conclusions comparing scenarios that use disinfectants and those that do not.

Based on the industrial data collected through EFSA's outsourced activities, the most relevant characteristics of the fresh-whole FVH sector are:

- (i) A wide range of water temperatures during operations is reported (2.8–25.0°C), which indicates that process water is not always kept at cold temperatures. This could be due to the fact that for climacteric fruits, such as mango and avocado, a higher water temperature may be needed to avoid chilling injury.
- (ii) During the operational cycle, large amounts of product can be processed. In some dumping operations, more than 6000 tonnes were reported by a FBOp. However, reported volumes of product varied depending on the post-harvest handling and processing operation where water is used, and these are cumulative during the production cycle. Also characteristic of this sector is the non-uniform addition of product, with high peaks of product volume being added at certain time points.
- (iii) Intervention strategies are limited to the use of water disinfection treatments such as chlorine-based disinfectants ($n=15$), PAA ($n=3$) and H₂O₂ ($n=3$). The most common water disinfection treatments reported are chlorine-based compounds such as sodium (7 industrial cases) and calcium hypochlorite (8 industrial cases).
- (iv) Water replenishment (or refreshment) is not applied as an intervention strategy. Only water refilling is used just to maintain the volume of the water tank or process lines.
- (v) A wide variability of water volumes in operation tanks (range: 500–50,000 L) was reported, and various water sources were used, including municipal tap water, surface water, well water and recycled water.
- (vi) A wide variability of product-to-water contact times during operations (range: 6–900 s), indicating that not all contact times are 'short' (e.g. less than 2–3 min).
- (vii) Seasonal work: some commodities are only available for a few weeks during the year, and the production is concentrated during these weeks (e.g. for products like cherries, avocados and mangoes) with long operational cycles (up to 6 weeks).

3.2 | Identification of microbiological hazards and hazardous events linked to process water

In the Part 1 opinion, it was concluded that *L. monocytogenes*, *Salmonella* and STEC were the main pathogens based on an EFSA FoNAO opinion (EFSA, 2013), reported European outbreaks between 2014 and 2020, and a literature review (publications published between 2010 and 15-2-2022) (EFSA BIOHAZ Panel, 2023). The prioritised hazard-product combinations identified are listed in Table 2.

TABLE 2 Prioritised microbiological hazard-product combinations in water used for post-harvest handling and processing operations for fresh-whole fruits, vegetables and herbs.

| General fresh-whole FVH food category | Specific fresh-whole FVH food category | Microbiological hazards |
|---------------------------------------|--|---|
| Leaves | Leafy greens eaten raw as salads | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, <i>Shigella</i> spp.*, norovirus, <i>Yersinia</i> spp., <i>Cryptosporidium</i> spp. |
| Leaves | Fresh herbs | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, <i>Shigella</i> spp.* |
| Fruits | NA | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, |
| Vegetable fruits | Tomatoes, peppers and aubergines | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC |
| Vegetable fruits | Fresh pods, legumes and grain (only those not dried) | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, <i>Shigella</i> spp.* |
| Sprouted seeds | NA | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC |
| Fruit: soft fruits | Berries | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, norovirus, HAV |
| Root and tuberous vegetables | Carrots | <i>L. monocytogenes</i> , <i>Salmonella</i> spp., STEC, <i>Yersinia</i> spp. |

Abbreviations: HAV, hepatitis A virus; NA, not available; STEC, Shiga toxin-producing *Escherichia coli*.

*If imported raw product.

In EFSA's outsourced activities, analysis of *Salmonella* spp., *L. monocytogenes*, STEC (including O157:H7), norovirus and *Cryptosporidium* spp. was performed at 6 sampling time points in 29 different scenarios (Gil et al., 2025). All findings for pathogens are listed in Appendix C. *L. monocytogenes* and norovirus were the biological hazards most commonly detected. *Salmonella* spp. was confirmed by PCR in one sample of water used for dumping apples. *L. monocytogenes* was detected in water used for dumping and pre-washing, more often after larger volumes of fresh-whole FVH had been processed, e.g.

at later sampling time points. When *L. monocytogenes* was detected at the second sampling time point, surface water was the water source. STEC was never detected (Appendix C).

Norovirus and *Cryptosporidium* spp. were only analysed at a single sampling time point (i.e. at the last out of the six defined sampling time points) for each FBOp scenario. Whereas norovirus genomes were detected in several process water samples, *Cryptosporidium* spp. was never detected. The detection method used for norovirus GI after the addition of PMAXx indicates the presence of intact virions but not their infectivity (Appendix C). In one scenario in which no microbial indicator groups were detected, norovirus was detected. It is worth noting that chlorine-based disinfectants act at the genome of naked viruses, and if the DNA is not destroyed in the specific amplicon, it will lead to a false positive result.

Based on the industrial data, including these pathogen findings, and the processing conditions described in Gil et al. (2025), different hazardous events can be expected. The most important identified for the fresh-whole processing industry are:

- a) **Contaminated water source.** In the Part 1 EFSA opinion (EFSA BIOHAZ Panel, 2023), the FVH were suggested as the most common route of contamination of process water for most hazards. Several water samples in the fresh-whole FVH sector (at least 10 records from at least 4 FBOp) showed the presence of *Listeria* spp. and *E. coli* at the beginning of the operational cycle (sampling time point 1). Different sources of water were used in these scenarios: municipal water, well water and surface water. The single *Salmonella* positive finding in the process water of fresh-whole FVH (EFSA outsourced activities – Gil et al., 2025) had surface water as source water. It was detected at the first sampling time point (after 60 min). Surface water was the source when *L. monocytogenes* was detected relatively early in the dumping process, at the second sampling time point (Appendix C). Hence, water as a potential source of contamination cannot be excluded. The presence of *E. coli*, *Listeria* spp. and *Salmonella* already found at the first sampling time point could be attributed to several causes: (1) water was already contaminated before it entered the production (e.g. use of well water/surface water); (2) contamination occurred in the facility due to a lack of a proper cleaning or disinfection and/or technical maintenance of the water storage and distribution systems, and/or (3) water was not fully replenished/completely removed before the operational cycle started again and this remaining water contaminated the new operational cycle.
- b) **Use of the same water to wash large volumes of product during the operational cycle without a well-managed intervention strategy.** In several industrial scenarios, it was observed that the same water was used in a single handling and/or processing operation without the application of a well-managed physical and/or intervention strategy during the whole operational cycle. The use of the same water in contact with large volumes of product for long processing operations without water replenishment will build up contamination in the water and may contribute to batch-to-batch contamination. Such situations may lead to the transfer of pathogens from the process water to the FVH at later stages in the process. As an example, *L. monocytogenes* was detected in almost all the water samples obtained from the pre-washing of fresh-whole avocado during the operational cycle (150 min, > 10,000 kg of fruit processed) (Appendix C). A similar situation was observed in the dumping tank of several FBOp, where the same water is used for an extended time, leading to a high product-to-water ratio enabling cross-contamination as previously described.
- c) **Incomplete removal of the water used in the water tank.** Different combinations were identified based on the industrial data collected through EFSA's outsourced activities. In about 1/3 of the sampling occasions ($n=10$), it was observed that the water of the tank used for the different operations (e.g. dumping, pre-washing, washing) was fully replaced once a day, usually at the end of the working day. This means that the whole production of the day (e.g. 100–5000 tons per day) are all in contact with the same water (e.g. 1000–10,000 L). Another third of the sampling occasions ($n=10$) indicated that the water in the tank is replaced 2–3 times per week, which means that the same water is used for different working days. The water at the beginning of the working day is not fresh and contains high concentrations of organic matter and microorganisms. In the rest of the sampling occasions ($n=7$), the water of the tank was replaced once a week, and a few FBOps only replaced water every 2–3 weeks ($n=2$) or less than once a month ($n=4$).
- d) **Water temperature.** As previously mentioned in section 3.1.3, post-harvest water used in the industry of fresh-whole FVH is kept above 15°C, with some scenarios with temperatures higher than 20°C. For some commodities, e.g. climacteric and/or tropical fruits such as avocado, a higher water temperature is needed to avoid chill injury. However, the data retrieved from EFSA's outsourced activities did not show a relationship between non-cooled water (> 15°C) and microbial accumulation in the process water (Gil et al., 2025).

3.3 | Analysis of microbiological and physico-chemical parameters of process water (data from EFSA's outsourced activities)

The data collected through EFSA's outsourced activities represent a unique and comprehensive database of industrial scenarios, offering valuable insights into the contamination dynamics of process water across various operations in the fresh whole FVH sector (Gil et al., 2025). This database has been thoroughly analysed to explore potential relationships among the different parameters evaluated. The goal is to identify relationships among microbiological parameters as well as microbiological parameters and physico-chemical factors that can be used for the validation, verification and monitoring within the water management system procedures.

3.3.1 | Relationship among microbiological parameters

In order to understand the relationship between the levels of potential microbial indicators and the detection of pathogens in process water of fresh-whole FVH, the microbiological data was analysed in three steps:

3.3.1.1 | Exploratory analysis of the results of individual samples

Bacterial pathogens were detected in 24 out of 624 water samples analysed, belonging to 6 out of 29 scenarios. *L. monocytogenes* was the most frequently detected pathogen (in 22 samples, of which 11 belonging to the scenario ID 26). *Salmonella* spp. was only detected in 1 water sample out of 624. STEC was not detected in any of the water samples from the 29 scenarios. Given these results, data associated with the detection of *L. monocytogenes* were used to assess the suitability of TBC, TC, *E. coli* and *Listeria* spp. as potential microbial indicators of pathogen contamination.

In Figure 8, the counts of TBC, TC, *E. coli* and *Listeria* spp. in \log_{10} CFU/100 mL for individual samples (including and excluding scenario ID 26) are displayed as a combined box plot for samples in which *L. monocytogenes* was detected and not detected (shown in different colours).

A special case was found in scenario ID 26, which corresponds to the pre-washing of avocados, where 12 out of 24 samples were found to be positive for *L. monocytogenes*. However, no detection of *E. coli* or *Listeria* spp. was reported. The high values of COD (up to 2000 mg/L) and high levels of TBC (up to 6 \log_{10} CFU/100 mL) indicate a high accumulation of organic matter and microorganisms.

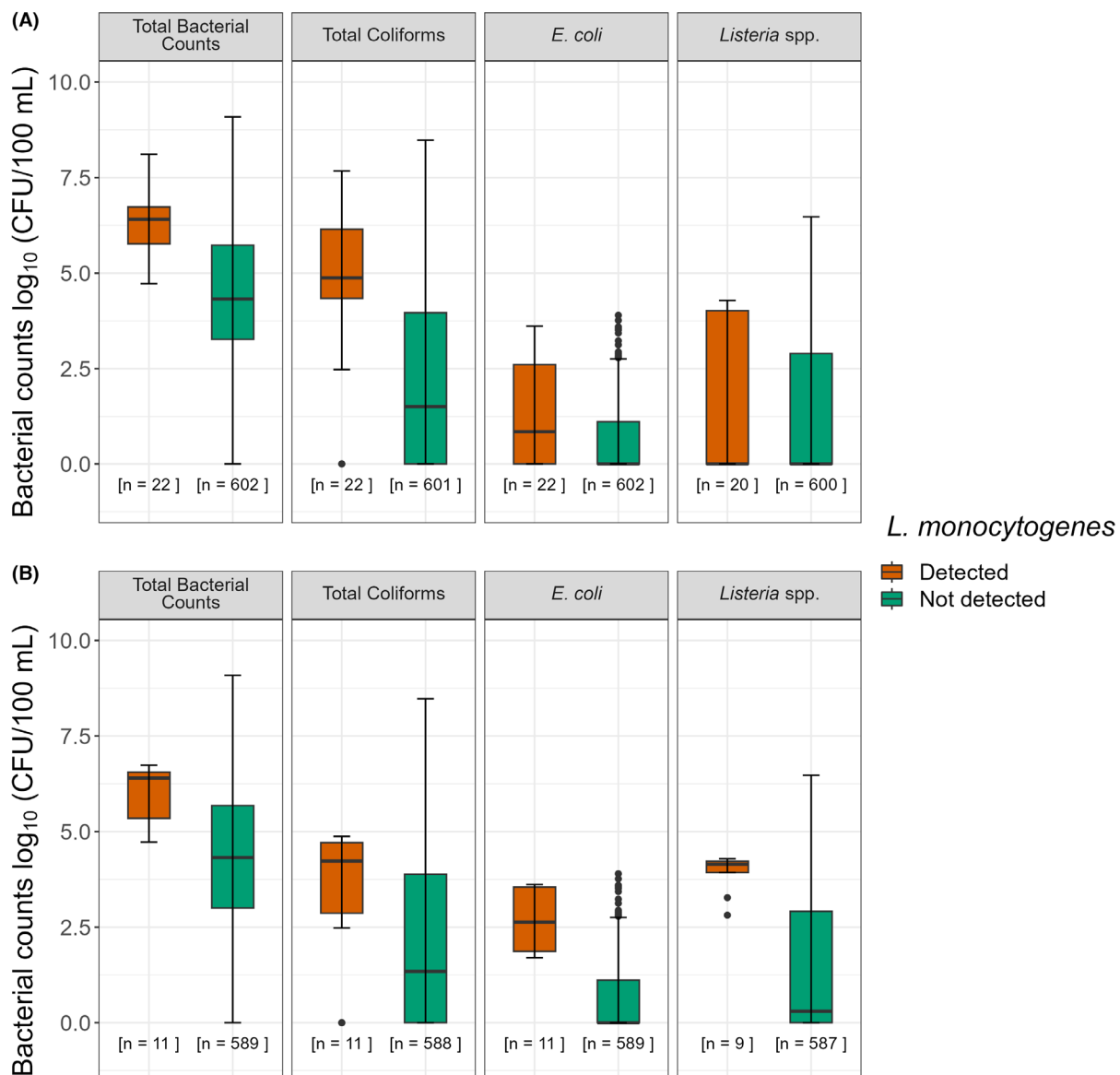


FIGURE 8 Box plot of levels (\log_{10} CFU/100 mL) of total bacterial counts (TBCs), total coliforms (TC), *E. coli* and *Listeria* spp. in individual samples of process water taken from different processing operations of fresh-whole FVH scenarios, including (A) and excluding (B) data from scenario ID 26, in which *L. monocytogenes* was detected (orange) and not detected (green). The numbers in square brackets [n=] represent the number of process water samples in each box plot. The results of the microbial indicators below the respective LOD were set to 0 \log_{10} CFU/100 mL. Source: EFSA outsourced activities (Gil et al., 2025).

The descriptive analysis of the empirical distribution of the data considering each individual sample, without accounting for the association among the sampling observations due to the multilevel hierarchical structure of the data, was performed first. The main results are described below.

The levels of **TBC** were generally higher in samples positive for *L. monocytogenes* compared to samples in which the pathogen was not detected. When *L. monocytogenes* was detected, levels of TBC were above $4.5 \log_{10}$ CFU/100 mL. More specifically:

- TBC were above 10^6 CFU/100 mL in 73% of the samples positive for *L. monocytogenes* and in 21% of the samples negative for *L. monocytogenes*.
- TBC were above 10^5 CFU/100 mL in 93% of the samples positive for *L. monocytogenes* and in 34% of the samples negative for *L. monocytogenes*.
- TBC were above 10^4 CFU/100 mL in 100% of the samples positive for *L. monocytogenes* and in 59% of the samples negative for *L. monocytogenes*.

The levels of **TC** found in samples positive for *L. monocytogenes* were generally higher than in samples negative for the detection of the pathogen. When *L. monocytogenes* was detected, levels of TC were above or equal to $2.5 \log_{10}$ CFU/100 mL (except in two samples, which were < LOD). More specifically:

- TC were above 10^4 CFU/100 mL in 78% of the samples positive for *L. monocytogenes* and in 24% of the samples negative for the pathogen.
- TC were above 10^3 CFU/100 mL in 87% of the samples positive for *L. monocytogenes* and in 40% of the samples negative for the pathogen.
- TC were above 10^2 CFU/100 mL in 94% of the samples positive for *L. monocytogenes* and in 45% of the samples negative for the pathogen.

According to these findings, the distribution of the TBC and TC levels is shifted up (higher values) for positive samples as compared to negative samples for *L. monocytogenes*. The difference in the distribution between positive and negative cases could not be formally tested, considering that the observations were not independent.

For samples positive for *L. monocytogenes*, the levels of ***E. coli*** were generally higher, and, when detected, in most of the cases, the levels were close to or above 100 CFU/100 mL, compared with samples in which *L. monocytogenes* was not detected, which showed levels below 100 CFU/100 mL in most of the cases (Figure 8B). More specifically:

- *E. coli* was above 100 CFU/100 mL in 33% of the samples positive for *L. monocytogenes* and in 11% of the samples negative for the pathogen.
- *E. coli* was above 10 CFU/100 mL in 50% of the samples positive for *L. monocytogenes* and in 28% of the samples negative for the pathogen.
- *E. coli* was above 1 CFU/100 mL in 52% of the samples positive for *L. monocytogenes* and in 36% of the samples negative for the pathogen.

Levels of ***Listeria spp.*** tend to be higher in samples positive for *L. monocytogenes* compared to samples in which the pathogen was not detected (Figure 8B). Similar percentages of positive and negative samples for the pathogen were observed for different thresholds of *Listeria spp.* (1, 10 and 100 CFU/100 mL). These results make it difficult to use *Listeria spp.* as a potential microbial indicator for the detection of *L. monocytogenes*.

3.3.1.2 | Analysis of pathogen detection within the operational cycle (by sampling visit)

The data were also analysed to detect pathogens within each sampling visit (lasting for the duration of the processing operation) of each scenario. Within the total 52 different visits, *L. monocytogenes* was detected in at least one sample of 7 sampling visits, which corresponded to the following scenarios: ID 02, ID 03, ID 12, ID 17, ID 23, ID 26 (two visits positive) (Appendix C). *Salmonella spp.* was found in only 1 sample from scenario ID 13, and enteric pathogenic *E. coli* (STEC or the O157:H7 serotype) was not detected in any sample. Given these results, the data associated with the detection of *L. monocytogenes* was used to assess the suitability of TBC, TC, *E. coli* and *Listeria spp.* counts as potential microbial indicators of pathogen contamination. The low number of positive samples from *Salmonella spp.* did not allow to draw any conclusions. In scenario ID 26, despite *L. monocytogenes* being detected in 3 out of 6 sampling time points of the two sampling visits, no *Listeria spp.* or *E. coli* was enumerated.

The relationship between the occurrence of potential microbial indicator groups above a given threshold and the detection of *L. monocytogenes* within each sampling visit was assessed. For this purpose, the OR,⁹ its 95% CI and the statistical significance were calculated for three different thresholds for TBC, TC, *E. coli* and *Listeria spp.* (Appendix D).

⁹Odds measure how likely the detection of the pathogen, and is calculated when the indicator is below and above the given threshold.

The OR for the detection of *L. monocytogenes* considering TBC thresholds $\leq 10^5$ CFU/100 mL could not be calculated because the odds of detecting the pathogen in the process water with levels of TBC $\leq 10^5$ CFU/100 mL were zero. The odds of detecting *L. monocytogenes* for samples with TBC higher than 10^6 CFU/100 mL was 6.9-fold (95% CI: 1.2–40.3, $p < 0.001$) the odds calculated for samples with TBC below this level.

Using a threshold for TC of 100 CFU/100 mL resulted in an OR of detecting *L. monocytogenes* of 4, although this value was not statistically significant (95% CI: 0.4–36.1, $p = 0.285$). These results hamper the consideration of TC as an appropriate microbial indicator of the microbiological quality of the process water in the fresh-whole FVH sector.

If the threshold of 100 CFU/100 mL of *E. coli* is established, the OR for *L. monocytogenes* indicates that the odds of detecting the pathogen in the process water with levels of *E. coli* above this threshold is 11.6-fold (95% CI: 1.9–70.6, $p = 0.008$) the odds when the indicator keeps below the threshold. Lower *E. coli* thresholds also resulted in higher odds of detecting the pathogen when the thresholds were exceeded, though in this case, the OR was not statistically significant. These findings suggest a possible association between high levels of *E. coli* and the detection of *L. monocytogenes*.

For *Listeria* spp., the odds of detection of *L. monocytogenes* for samples with more than 10 or 100 CFU/100 mL of *Listeria* spp. were about 6.9-fold (95% CI: 1.2–40.3, $p < 0.05$) and 7.2-fold (95% CI: 1.3–39.6, $p < 0.05$), respectively the odds calculated for samples with the *Listeria* spp. level below these thresholds.

3.3.1.3 | Analysis by multivariable logistic mixed effect modelling (hierarchical structure)

A multivariable logistic mixed effect model was developed to explain the potential effect on the detection of the microbial pathogens of the microbial indicator levels considering data from all the FVH sectors and, only for *L. monocytogenes*, also considering the effect of the analytical methods applied to confirm the detection (carbohydrate fermentation testing vs. PCR). The hierarchical structure of the data was accounted for using random effects (more details about the complete model are available in Annex B).

For this sector, after controlling for the effect of the analytical methods and accounting for the hierarchical structure in the data (random effects), none of the potential microbial indicator levels appeared to influence the odds of detecting *L. monocytogenes*. A large sampling uncertainty characterises all the model estimates as reflected by the large standard error and size of the confidence intervals (Annex B, Tables 16 and 18). This does not allow conclusions to be drawn based on the model. Purely qualitative considerations can be made regarding the impact of the analytical method used to confirm presumptive *L. monocytogenes* colonies. The upper bound of the intercept of the model when the method used was PCR is lower than the one for the carbohydrate fermentation-based method. However, this result must also be interpreted with caution, considering the large uncertainties in the estimates and the correlation between the method used and the FVH sectors.

The only conclusion that is consistent among all the models about the relationship between the potential microbial indicators and the detection of pathogens in process water is that random effects (i.e. 'scenario', 'sampling visit' and 'sampling time points') play an important role. The largest effect on the variability of the odds of detecting *L. monocytogenes* was driven by 'sampling time points', probably due to the long operational cycles characterising the two sectors (fresh-whole and frozen FVH) to which the estimates of the random effects explain the variability of *L. monocytogenes* were based. The 'scenario' and 'sampling visit' appeared to have a secondary influence in the explanation of the variability for this pathogen.

It is worth noting that the 'scenario' combines several production features, such as the specific combination of FVH product, type of operation and operational conditions, each including additional specific characteristics. The individual effect of each of these features and characteristics could not be investigated through modelling and remains an open issue.

Therefore, the suitability of these potential microbial indicators for verification purposes within the water management system should be validated under the specific operational conditions of each FBO, as described in Section 3.5.

3.3.2 | Relationship between microbiological and physico-chemical parameters

Despite comprehensive statistical analysis, including techniques such as principal component analysis (PCA), the data set for fresh-whole FVH did not reveal any critical parameters suitable for use in operational monitoring. Statistical data analysis tools, such as PCA and others, rely on variation within a data set to identify meaningful relationships between factors and datasets lacking variation hinder the ability to draw strong conclusions.

The studies performed under real industrial settings conditions (as the ones observed in the scenarios sampled in EFSA's outsourced activities) often suffer from this limitation because the actual practices applied in commercial processing and handling conditions minimise variation in most of the physico-chemical parameters. On several occasions, the microbial loads fall below detectable levels or are randomly distributed.

Despite the lack of statistical association, it is worth mentioning that within the scenarios using chlorine-based disinfection, *E. coli* was counted only in samples with a residual FC concentration below 15 mg/L, irrespective of the water source (municipal tap water, surface water, or well water). *Listeria* spp. was detected in the treated process water at higher residual chlorine concentrations (up to 71.9 mg/L); however, in these samples, the pH of the process water ranged from 7.0 to 8.3, which is above the effective pH range in which the active hypochlorous acid predominates with minimal dissociation. *E. coli* could still be detected in water treated with hydrogen peroxide (with a residual concentration of up to 130 mg/L), regardless of whether the water source is municipal tap water or surface water.

To ensure real-time monitoring of process water quality, identifying critical physico-chemical parameters that correlate with the microbiological quality of the process water is essential. It would allow the online monitoring of physico-chemical parameters as a proxy for microbiological quality.

3.4 | Preventive measures: Good hygiene and good manufacturing practices in water management, distribution and storage systems

All 29 scenarios were systematically screened, and hazardous events were identified (Section 3.2). In this section, these hazardous events are linked to specific preventive measures. In Table 3, specific points of attention for the fresh-whole FVH production are formulated as pre-start measures (i.e. to set up the water management system), restart measures (i.e. to start up again after technical interference or temporary, seasonal work) and routine measures (i.e. in the daily routine operation of an industry) in the frame of specific PRPs. Therefore, not all PRPs are included in Table 3. A full description of generic pre-requisite measures (13 PRPs) can be found in the EC Commission Notice 2022/C 355/01¹⁰ and pre-requisite measures specific to water management, distribution and storage systems in the Part 1 Opinion (EFSA BIOHAZ Panel, 2023).

¹⁰Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.

TABLE 3 Specific points of attention in the preventive measures, as defined in the EC Commission Notice 2022/C 355/01¹¹ and Part 1 Opinion (EFSA BIOHAZ Panel, 2023), related to Good Hygiene and Good Manufacturing Practices in water management, distribution and storage systems for post-harvest water use in handling and/or processing operations of fresh-whole FVH, based on the identified hazardous events (Section 3.2).

| Preventive measure | Specific attention to preventive measures for fresh-whole FVH | | |
|---|---|--|---|
| | Prestart measures | Restart measures | Routine measures |
| PRP 1: Infrastructure and fit-for-purpose building and equipment | <p>Hygienic design and special expertise are needed to set up the infrastructure of the water management system to avoid problems and reduce routine inspection/maintenance.</p> <p>Set up a well-designed and managed water disinfection system as an intervention measure (installation of pumps, dosing systems, sensors, valves, etc.).</p> | Every time the processing line is restarted, the infrastructure and equipment should be revised. | <p>A monthly visual check based on a checklist of infrastructure (hygiene and condition).</p> <p>Monitoring the proper working of the water management system (see Section 3.6).</p> |
| PRP 2: Cleaning and disinfection | The equipment used for the cleaning and disinfection of the water distribution and storage systems needs to be revised before activities are started. | In the case of restart after a prolonged stand-still (as in the case of seasonal activities), cleaning and disinfection need to be conducted to prevent microbial contamination of water and avoid biofilm formation. | <p>Periodically performing cleaning and disinfection procedures, including deep cleaning and disinfection procedures. Activities might include effective soil removal, biofilm prevention and removal, and pathogen inactivation.</p> <p>Dedicated time surely needs to be foreseen when continuous and/or long-time runs occur in the processing of whole FVH.</p> <ul style="list-style-type: none"> – Spot visual checks – Daily visual checks <p>Perform microbiological environmental testing.</p> |
| PRP 4: Technical maintenance and calibration | Proper equipment installation is needed to prevent contamination of fit-for-purpose water with potentially contaminated water (such as between potable water fill lines and dump tank drain lines). | Proper re-installation of equipment is needed (e.g. connecting pipelines) to prevent contamination of fit-for-purpose water with potentially contaminated water (such as between potable water fill lines and dump tank drain lines), surely after a prolonged period of not using the water management system, e.g. due to seasonal work. | <p>Routine inspection and maintenance of equipment are needed to prevent contamination of fit-for-purpose water with potentially contaminated water (such as between potable water fill lines and dump tank drain lines).</p> <p>Monitoring programmes of technical maintenance may consist of (a) inspections of records of the functionality and maintenance status of equipment and (b) inspections of the abstraction area and the treatment, storage and distribution infrastructure without prejudice to monitoring requirements.</p> <p>Monitoring equipment needs to be calibrated, e.g. thermometers, water flow meters and disinfection dosing systems, so that proper use can be guaranteed.</p> |

(Continues)

¹¹Commission notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01). 16.9.2022, p. 1–58.

TABLE 3 (Continued)

| Preventive measure | Specific attention to preventive measures for fresh-whole FVH | | |
|--|--|--|--|
| | Prestart measures | Restart measures | Routine measures |
| Pre-requisite (EC Commission Notice 2022/C 355/01) | | | <p>In case a deviation is found during the routine inspections and/or cleaning activities, a replacement of water distribution systems needs to be foreseen to avoid contamination due to biofilms, e.g. filters, tubing, fittings to connect tubes or nozzles to make small water droplets in hydrocooling installations.</p> <p>In seasonal production peaks, a preventive technical maintenance regime should also be included.</p> |
| PRP 8: Water and air control | <p>Cooling post-harvest water to reduce microbial growth. Remark: in this sector, climacteric commodities are processed for products like mangoes and avocados, so cold temperatures are not appropriate for these commodities; however, temperatures around 10–15°C can be recommended.</p> <p>The frequency of complete removal of water in tanks/ processing lines needs to be established.</p> | <p>Cooling post-harvest water to reduce microbial growth.</p> <p>Every time the processing line is restarted, the water in the processing lines/tanks should be completely removed and new fresh water inserted.</p> | <p>Cooling post-harvest water to reduce microbial growth. The microbiological quality of ice should also be considered to avoid potential contamination (in the case of the use of ice to cool products).</p> <p>Routine inspection of the frequency of complete removal of the water in the processing lines/ tanks is needed to avoid prolonged use of the process water.</p> |
| PRP 9: Personnel (hygiene, health status, training) | <p>Appropriated training for operators and team leaders due to the nature of manual labour (e.g. manual sorting) and create a positive food safety culture and awareness of hygiene and microbiological food safety.</p> | | <p>Retake the training with a certain frequency to create awareness among personnel on microbiological hygiene and the safety of water and produce.</p> |
| PRP 12: Working methodology | <p>Personnel following work descriptions and standard operating procedures (SOP).</p> | <p>Personnel following work descriptions and standard operating procedures (SOP).</p> | <p>Personnel following work descriptions and standard operating procedures (SOP).</p> <p>SOP including logistic management: e.g. optical dirty after optical clean products, or if different product categories are processed: covered (e.g. peas) before above ground (open, e.g. green leaves, tomatoes, peppers) before root vegetables (carrots, potatoes).</p> <p>SOP includes reusing water: use the process water applied for 'cleaner' processing steps as input water for more dirty steps, e.g. last washing/ rinsing water to be reused for first washing of FVH.</p> <p>SOP includes the use of disinfection techniques: clear explanation of the dose, monitoring, etc., and what to do in case the disinfection is not working appropriately (e.g. actions to take to process and product).</p> |

3.5 | Intervention measures: Water disinfection and/or water replenishment

Intervention measures include steps in the post-harvest handling and processing operations aiming to avoid the microbiological contamination of the process water, thus preventing the accumulation of microorganisms and organic matter in the water and the consequent cross-contamination of the processed fresh-whole FVH. In the Part 1 opinion (EFSA BIOHAZ Panel, 2023), two main intervention measures were identified: Water replenishment and water disinfection treatments. However, based on EFSA's outsourced activities, only chemical water disinfection treatments were applied by some FBOs from the fresh-whole FVH sector (Gil et al., 2025) (Table 4). In this case, as presented in Table 5, four Scenario Groups (SGs) were identified, which cover the above-mentioned intervention measures. It is critical to note that the application of an intervention measure needs to be tailored to the product and processing line, and it should be integrated into the water management strategy, which includes validation, operational monitoring and verification to demonstrate its performance. Therefore, the information provided in this section should be considered as illustrative.

TABLE 4 Types of intervention measures considered in this scientific opinion and the number of scenarios found in the fresh-whole FVH industrial data included in EFSA's outsourced activities ($n = 29$ scenarios from one European country) (Source: EFSA outsourced activities (Gil et al., 2025)).

| Intervention measures | Number of scenarios from EFSA's outsourced activities |
|--|---|
| None | 8 |
| Water Disinfection (WD) | 21 |
| Water Replenishment (WR) | 0 |
| Water Disinfection and Water Replenishment (WD + WR) | 0 |

To illustrate the different SG identified within the industrial cases included in the EFSA outsourced activities and also other situations that were not found among the industrial cases, representing adequate water safety management (e.g. SG 2.2 and SG 4) (Table 5), a mathematical model was used to simulate hypothetical scenarios representative of the fresh-whole FVH sector (Section 2.2.2). The mathematical model has been implemented in a public user-friendly tool (<https://r4eu.efsa.europa.eu/app/WaterManage4You>).

To compare the outputs of the model when different intervention measures are applied, all the simulations refer to a common hypothetical fresh-whole product (i.e. peaches), based on scenario ID20, sampling visit 1 (Gil et al., 2025), with an operational cycle of 75.6 h, running under the same operational conditions (e.g. volume of water 5000 L and total amount of product 48,019 kg) as shown in Figures 9 and 10 and Table 5. In this case, the product load fluctuates and irregularly peaks during the operational cycle, which is characteristic of this sector, and challenges the management of the microbiological quality of process water.

Tables E.1 and E.2 in Appendix E summarise all the model parameters and input data used for variables in the model simulations of all the SG in the fresh-whole FVH sector.

TABLE 5 Conditions^a of application of the simulated intervention measures and physico-chemical parameters (FC, pH and temperature) used as input values for model simulations of all the scenario groups representative of the fresh-whole FVH sector.

| Scenario group | Intervention measures conditions | | | Physico-chemical parameters | |
|--------------------|---------------------------------------|--|-------------------------|-----------------------------|-------------------------|
| | Initial chlorine concentration (mg/L) | Mode and dose of disinfectant addition | Water addition (L/min) | pH | T (°C) |
| Scenario Group 1 | 0 | None | Water refilling: 0.9 | Not adjusted: 7.9–8.2 | Not adjusted: 20.4–21.9 |
| Scenario Group 2.1 | 20 | Discrete: 10,000 mg every hour | Water refilling: 0.9 | Not adjusted: 7.9–8.2 | Not adjusted: 20.4–21.9 |
| Scenario Group 2.2 | 20 | Continuous: 550 mg/min | Water refilling: 0.9 | Adjusted: 6 | Adjusted: 5 |
| Scenario Group 3 | 0 | None | Water replenishment: 50 | Not adjusted: 7.9–8.2 | Not adjusted: 20.4–21.9 |
| Scenario Group 4 | 20 | Continuous: 550 mg/min | Water replenishment: 50 | Adjusted: 6 | Adjusted: 5 |

Abbreviations: FC, free chlorine; FVH: fruit, vegetables and herbs.

^aBased on scenario ID 20, sampling visit 1 from EFSA's outsourced activities (Gil et al., 2025), with an operational cycle of 75.6 h, running under the same operational conditions (e.g. volume of water 5000 L and total amount of product 48,019 kg, contact time 1 min).

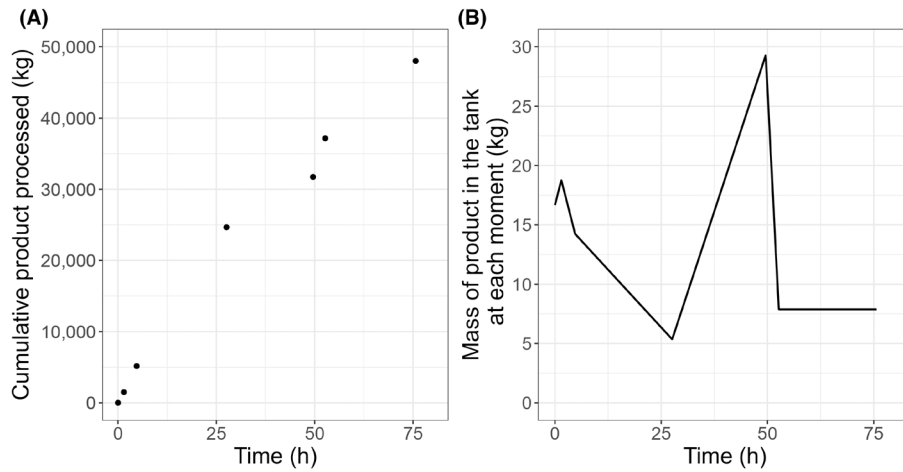


FIGURE 9 Cumulative mass of the product being processed (A) and mass of the product in the tank at each moment (B) during the 75.6 h operational cycle used in the simulations of the scenario groups selected as representative of the fresh-whole FVH sector.

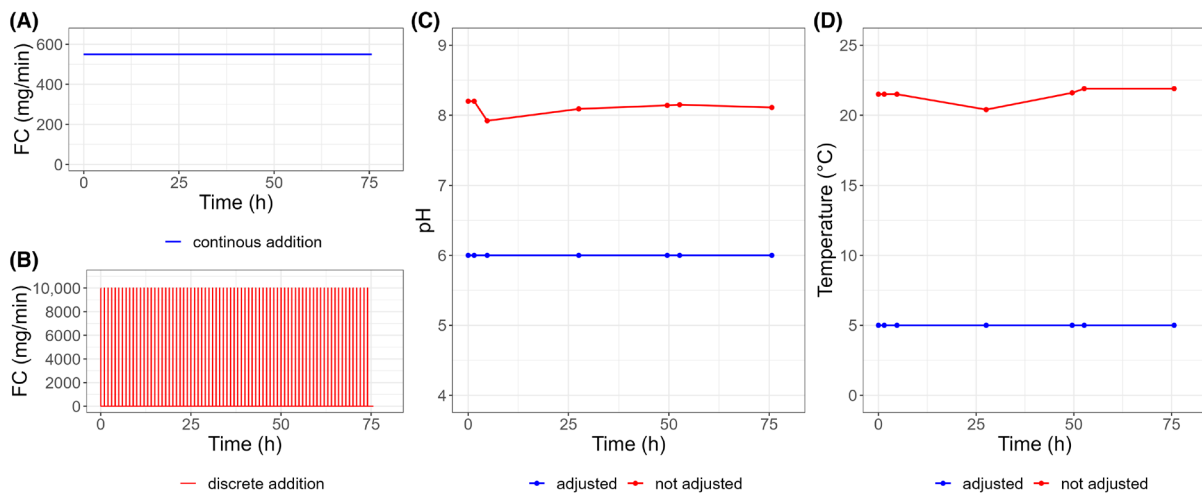


FIGURE 10 Changes in physico-chemical parameters of free chlorine (FC) dosing (A: Continuous addition, B discrete addition), pH (C) and temperature (D) used as model inputs for the simulation of the scenario group of the fresh-whole FVH sector, corresponding to a discrete application of water disinfection treatments and no adjustment of the physico-chemical characteristics of the water (SG 2.1), and the scenario groups associated with a continuous application of water disinfection treatments and adjustment of the physico-chemical characteristics of the water (SG 2.2 and SG 4).

Figure 11 shows the simulations provided by the mathematical model for different parameters, including FC and hypochlorous acid (HOCl) (A), accumulation of COD (B) and levels of total bacterial counts (C) based on the different defined scenario groups (SGs 1, 2.1, 2.2, 3 and 4). Each column of Figure 11 refers to one type of SG.

Scenario Group 1: The simulations included in this group represent a situation commonly observed within the industrial data collected through EFSA's outsourced activities (Gil et al., 2025), where no intervention strategies were applied (i.e. no water disinfection and no water replenishment). In this SG, no FC is detected (Figure 11A), and only water refilling at a constant rate (0.9 L/min) is considered (Table 5). The model simulations clearly show that in the absence of any water disinfection intervention strategy, the COD (Figure 11B) and the microbial load (Figure 11C) of the initially clean water constantly increase throughout the operational cycle, reaching levels up to ca. $7 \log_{10}$ CFU/100 mL and 2500 mg/L, respectively (Figure 11). Under these conditions, the water management system is not able to control the accumulation of bacteria in the process water.

Scenario Group 2 resembles a handling and processing operation with chlorine-based disinfection treatment of process water without water replenishment, but only water refilling at a constant rate (0.9 L/min) (Table 5). Two different sub-scenario groups were addressed:

- **Sub-scenario group 2.1** represents a scenario in which the physico-chemical parameters indicated that the water management system was not adjusted to achieve the fit-for-purpose microbiological quality. Water disinfection treatment was applied without properly adjusting the chlorine dosing regimen or the physico-chemical parameters. In this scenario, the quantity of disinfectant added during the operational cycle was too low and was applied discretely; the pH was not optimal, and the temperature was not adjusted. Under such conditions, the concentration of FC in water, as well as hypochlorous acid, is minimal during the operational cycle (Figure 11A). As a result, the organic matter calculated as COD goes up to 1000 mg/L (Figure 11B), and microorganisms increase close to $6 \log_{10}$ CFU/100 mL (Figure 11C), indicating that the water management system was not adjusted to achieve the fit-for-purpose microbiological quality.
- **Sub-scenario Group 2.2** shows a scenario where disinfectant was continuously added during the operational cycle, and the pH and temperature of the water were adjusted to values favouring the action of the FC. Under these conditions, residual concentrations of FC (and hypochlorous acid as the fraction of FC with the highest antimicrobial efficacy) varied from 20 mg/L to undetected, mostly due to the irregular addition of product during the operational cycle (Figure 11A). The increase of COD was minimised to concentrations close to 500 mg/L (Figure 11B). In most cases, the FC concentration present in the process water managed to control the accumulation of TBCs above $3 \log_{10}$ CFU/100 mL, except at time 50 h (Table 5, Figure 11C). Noteworthy, the considerable addition of product mass at ca 50 h of the operational cycle (Figure 9) introduced organic matter, as shown by the peak in the COD dynamics and although the same flow rate of chlorine dosing was kept, the chlorine demand was higher causing an important reduction of the FC and hypochlorous acid residual concentrations below 5 mg/L. Accordingly, the simulation indicates an accumulation of TBCs in the process water above $3 \log_{10}$ CFU/100 mL. The high peaks of product addition, characteristic of the sector, challenged the management of the microbiological quality of process water.

Scenario Group 3 refers to a handling or processing operation with water replenishment as the only intervention, without the addition of any water disinfection treatment (Figure 11A). The water replenishment is defined in the model by increasing the addition of water from 0.9 L/min (just water refilling) to 50 L/min (Table 5). A water flow rate of 50 L/min was selected as it was considered a flow rate that can reasonably be applied by the industry. Although a higher flow rate than 50 L/min may be possible, this option was not considered in this study. The COD of the process water is kept at low concentrations when compared to SGs 1 and 2, mostly due to the dilution effect of the high addition of water (Figure 11B). According to the model simulations, the application of such an intervention alone is much less efficacious than water disinfection treatments in controlling the accumulation of microorganisms in the water mostly because the concentration of microorganisms accumulate in a logarithmic scale and thus, much higher water addition rate would be needed to observe a dilution effect (Figure 11C). Levels of TBC in SG3 were close to $6 \log_{10}$ CFU/100 mL, while in SG2.2, the highest levels were between 3 and $4 \log_{10}$ CFU/100 mL.

Scenario group 4 represents the situation where both water disinfection and water replenishment (50 L/min) interventions are applied to maintain the microbiological quality of process water. In this case, the simulation includes the same conditions applied in SG 2.2, adjusting both chlorine dosing to a constant addition and the physico-chemical parameters that favour the antimicrobial activity of FC (Figure 10). The water replenishment rate used in this scenario group is the same as in SG 3 (Table 5). Again, a water flow rate of 50 L/min was selected as it was considered a flow rate that can reasonably be applied by the industry. Although a higher flow rate than 50 L/min may be possible, this option was not considered in this study. Based on the output of the model, it is observed that the dilution effect provided by water replenishment, represented by the addition of large volumes of water (50 L/min), has a significant impact in avoiding the accumulation of organic matter (COD) and microorganisms in the process water (Figure 11B,C). As a consequence, compared with SG 2.2, despite the initial higher dilution of the FC residual concentration, it is kept at a slightly higher level since the oxidation of FC by organic matter is lower (Figure 11A). Therefore, the combination of water disinfection (to control the accumulation of microbial load) and water replenishment (to dilute organic matter, determining the FC demand) improves the water management of the system as the microbial load of the process water is well maintained, showing levels below $3 \log_{10}$ CFU/100 mL (Figure 11C).

3.6 | Validation of intervention measures

For the present opinion, the aim and scope (Step 1 in Figure 11, see EFSA BIOHAZ Panel, 2023) of the validation is to demonstrate that a specific processing and/or handling operation for a specific fresh-whole FVH, working under reasonably

foreseeable operating conditions ensures that the microbiological quality of the process water is maintained and, thus, the cross-contamination of the fresh-whole FVH by the process water is minimised. Other validation purposes mentioned in Opinion Part 1 are out of the scope of this opinion (such as mapping the washing equipment to identify the worst-case locations showing the lowest concentration/dose of disinfectant, where the sensors for key monitoring parameters should be placed). The validation allows the definition of the appropriate operational monitoring criteria associated with water management strategies, which will be based on critical limits of certain physico-chemical parameters of the process water. The validation also allows the selection of the most appropriate microbial indicators and their thresholds to be used in the verification of the quality of the process water.

The subsequent steps of the validation (Steps 2 until 5, [Figure 11](#), see EFSA BIOHAZ Panel, [2023](#)) for this sector include:

A) Identification of the handling and processing operation where water is applied in the fresh-whole FVH production

For the specific processing operations applied in fresh-whole FVH, the typical characteristics of the water used need to be set, based on the flow chart in [Figure 1](#) defined for a FBOp where water is applied in the production steps, e.g.:

- Water used in (hydrocooling) cooling units to spray over product: water used to cool product and/or to protect from evaporation.
- Dumping tanks with water for dilution of PPP/additives: water remains on the product and is refilled to compensate for the loss of water with no or limited replenishment (closed system)
- Transport water – water used in pre-sorting, trimming/brushing of FVH (not applied for all commodities; e.g. it is for apples and pears, yet not for tomatoes): unit operations with prolonged use of water with no or limited replenishment (closed system)
- Pre-washing, Washing and Rinsing: recirculation of water; refill to compensate for the loss of water and/or use of replenishment

B) Identification of the fixed and variable process and product-related factors

For each of the specific processing operations applied in fresh-cut FVH, the following operation conditions of the process/es need to be defined to account for the variability of the actual operating conditions of each process line, to be able to test the robustness of the water management strategies:

- (1) Fixed process parameters (remain constant in every processed batch), e.g. equipment dimensions, water source, water filtration and type of intervention measure (if applicable).
- (2) Product-related factors, both
 - Fixed factors that remain constant in every processed batch, such as type(s) of commodity, product feed rate, and
 - Those that may vary considerably from batch to batch include the amount of dust/soil/organic matter. This last factor is particularly important as it may show a range of variability considering the climatic conditions at the moment of harvesting the FVH, e.g. heavy rainfall, as more soil can be present on the FVH and thus in the water.
- (3) Water-related factors, including
 - Fixed factors that remain constant in every processed batch, e.g. water hardness, water temperature, water flow, refreshment or replenishment rate (volume of water replaced in a certain time frame) and the product-to-water ratio.
 - Dynamic factors that vary along the processing cycle, such as relevant physico-chemical parameters, including pH, organic matter measured as COD and UV absorbance, and initial microbial load of the water at the start of the operation (time 0) in relation to the microbial load in the process water.
- (4) Water disinfection treatment-related factors (if applicable), including
 - Fixed factors that remain constant in every processed batch, e.g. type of disinfectant (commercial product and its composition), concentration of application, mode of application (discrete or continuous) and disinfectant dose.
 - Dynamic factors vary along the processing cycle, e.g. the level of disinfectant and the dosing regimen, as well as the temporal and spatial changes of the residual concentration of the disinfectant (in the active form).

Examples of industry data on these factors and their combinations can be retrieved from EFSA's outsourced activities (Gil et al., [2025](#)). However, these activities were not designed to carry out a validation study, and therefore, no scenario could be used as a reference for the validation process.

For a preliminary in-silico study of the validation assay, a decision support tool based on mathematical models can be used to evaluate whether the foreseen conditions of the water treatment (e.g. replenishment rate, concentration of disinfectant, adjustment of pH) could be suitable to maintain the microbiological quality of the process water. Section [3.5](#) of this opinion illustrates the impact of certain conditions in the application of interventions or their combinations on the resulting process water quality using the mathematical model described in the Part 2 Opinion (EFSA BIOHAZ Panel,

2025). The mathematical model has been implemented in a public user-friendly tool (<https://r4eu.efsa.europa.eu/app/WaterManage4You>). A FBOp can introduce the identified fixed and variable product and process-related factors as inputs of the tool to assess the impact of foreseeable ranges of variation in these parameters (e.g. range in pH values, range in product mass, range in temperature) on the resulting microbiological quality and its accumulation during an operational cycle. Based on the outcomes of such simulations, the performance standards to be validated can be set or re-adjusted to achieve the desirable water quality according to the model. The use of the model narrows down the scope of the experimental study in the production plant (see subsections C and D below). However, the currently available models are limited to the behaviour of TBC as potential microbial indicators and chlorine-based disinfectants in combination with or without water replenishment as intervention measures.

C) Selection of indicator parameters and their performance standards

In the validation study, all relevant parameters related to factors 1–4 (indicated above) should be collected for each step of the production where water is involved. In combination with the actual measurement of the physico-chemical parameters and microbiological analysis of the sampled water, it must demonstrate that performance standards of the microbiological load are maintained.

As enteric pathogens (*Salmonella* spp. and pathogenic *E. coli*) were scarcely or never detected in the data set collected through EFSA's outsourced activities for the fresh-whole sector (Gil et al., 2025), no relationship between potential microbial indicator groups and these pathogens could be established (see Section 3.3.1). Despite these outsourced activities were not aimed at performing a validation study, in agreement with the literature (EFSA BIOHAZ Panel, 2023), the collected data suggest TBC, *E. coli* and *Listeria* spp. as possible microbial indicators of the detection of the pathogen *L. monocytogenes* in process water for fresh-whole FVH.

TBC above 10^6 CFU/100 mL, *E. coli* above 100 CFU/100 mL or *Listeria* spp. above 10–100 CFU/mL resulted in odds of detecting *L. monocytogenes* of almost 7-fold, almost 12-fold and about 7-fold the odds of detection when these indicators were below the stated thresholds.

Some recommendations on the microbiological quality of agricultural water used in post-harvest handling and processing operations are already available. For instance, the EU Commission Notice (2017/C 163/01)¹² on guidance document on addressing microbiological risks in fresh fruits and vegetables (FFVs) at primary production (including the associated operations of washing/rinsing, sorting, transport, cooling) through good hygiene, indicates *E. coli* thresholds depending on the intended use of the water, the water source, the characteristic and the nature of the FFVs. The Commission Notice also provides support tools to evaluate the required microbiological quality of the agricultural water (Annex II and III of EU Commission Notice (2017/C 163/01)).

Despite the fact that such indicators and levels could be regarded as possible performance standards to control the detection of pathogens in process water, outputs from EFSA's outsourced activities (Gil et al., 2025) and the scientific literature (EFSA BIOHAZ Panel, 2023) also showed that many features specifically associated with each FVH product, type of processing operations, operational conditions, etc., determine the actual suitability of a bacterial group and its threshold to indicate the possible detection of a pathogen in the process water. Therefore, selecting a specific indicator and its threshold should be done and justified by the FBOps based on the results of its specific validation study. Moreover, setting the thresholds should also be based on the fit-for-purpose water concept depending on the specific handling and processing operation and the intended use of the FVH (RTE or non-RTE FVH, respectively) among others (FAO/WHO, 2021).

In this sector-specific opinion for the fresh-whole FVH industry, based on the fit-for-purpose concept for process water, the available industrial data and the statistical analyses (Section 3.3), it is concluded that each FBOp should generate data to support the selection of the specific microbial indicators and their thresholds (performance standards) depending on the specific handling and processing operation (including the reasonable range of variability of the operating conditions) and the intended use of the FVH among others.

As previously mentioned, the industrial data set collected through EFSA's outsourced activities (Gil et al., 2025) did not identify clear associations between physico-chemical parameters and microbial data (Section 3.3.2), making it difficult to select the most useful physico-chemical indicators and valid performance standards to be validated. This selection needs to be done based on the scientific literature and/or model simulations (see Section 3.7).

D) Data collection in the validation study

Important for a validation study is to take the variability of the processing operation during the production period (e.g. day, week, or month) into account. The variability can be covered by designing and running a validation assay for different independent trials. Ideally, a minimum '3 × 3 approach' should be followed for each processing operation in post-harvest processing where water is applied:

- 3 operational cycles (on different days, weeks or months, e.g. including different seasons or different conditions at harvest (rainy or dry weather)).

¹²European Commission. (2017). Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

- 3 time points within the operational cycle (start, middle and end of operational cycle).

As such, nine data sets should be collected per sampling location where a water sample is taken.

Appendix F shows a recording template table for a validation study of water used in different post-harvest handling and/or processing operations.

Sampling methods, sample treatment (e.g. filtration is only done in case of low contaminated water/ice-making water/cooling water) and analytical procedures should follow standardised protocols (i.e. ISO methods when available, validated tests). Calibrated and verified sensors and devices should be used (see Section 3.8) (EFSA BIOHAZ Panel, 2023). All the conditions used and the results obtained in each validation trial should be systematically recorded (Appendix F) and summarised in the validation report. The information on key parameters should show the actual boundaries of the operating conditions that were validated, which will eventually be considered to set the water treatment conditions for the specific operation, allowing the maintenance of the quality of the process water in routine productions.

3.7 | Operational monitoring of the intervention measures

Operational monitoring is the systematic and continuous observation that allows real-time information from the physico-chemical parameters of the process water identified as critical during the validation study.

As shown in Section 3.3.2, none of the industrial cases included in EFSA's outsourced activities (Gil et al., 2025) could be used as illustrative for operational monitoring put in place. Therefore, recommendations for operational monitoring are based on the following:

- a. The scientific literature, critical physico-chemical parameters that should be used in the operational monitoring, including the residual concentration of the disinfectant, organic matter indicators (e.g. UV absorbance 254 nm), depending on the type of disinfectant and the pH of the process water (e.g. chlorine-based disinfectants) and water temperature. The analytical procedures recommended for each parameter were described in the previous Part 1 EFSA scientific opinion (EFSA BIOHAZ Panel, 2023).
- b. The model simulations included in Section 3.4 showed that, for example, adjustment of specific physico-chemical parameters (e.g. pH and temperature) is necessary for the efficacy of the water disinfection treatment in case of the use of chlorine-based chemicals (SGs 2.2 and 4).

Examples of critical physico-chemical parameters to take into consideration when defining operational monitoring are demonstrated in Section 3.4. However, the specific parameters and thresholds need to be determined as a result of the validation study on a case-by-case basis.

All the results obtained during the operational monitoring should be systematically recorded (Section 3.9) and periodically reviewed as part of the verification procedures (Section 3.8). Appendix G shows an example of a recording template table for operational monitoring.

If the thresholds of the operational monitoring parameters are exceeded, corrective actions to correct the production process need to be taken. Examples of such actions are: (i) complete removal of the water in the tank/processing line, (ii) increase of the water replenishment frequency or rate, (iii) correction of the dose of the disinfectant applied or mode of addition (e.g. continuous vs discrete) and (iv) increase of the dose of the acid (e.g. phosphoric acid) to lower the pH. As the production process is no longer under control, the microbiological quality of the process water can no longer be guaranteed. Therefore, the microbiological quality and the acceptability of the production batch of fresh-whole FVH need to be further evaluated in the frame of the overall food safety management system (EFSA BIOHAZ Panel, 2017).

3.8 | Verification of the water management plan

As described in Opinion Part 1, verification is conducted periodically to check if the microbiological quality of the process water is achieved by the validated and monitored operating conditions. Together with reviewing/checking/auditing the monitoring records and the calibration status of measuring devices, verification typically includes the microbiological testing (for selected microbial indicators after the validation study) of the process water, which can be carried out by the FBOs and/or by the independent authority (e.g. external laboratory).

As a starting point for the verification, a monthly sampling of water applied in each step of the process could be recommended (e.g. 10 process steps where water is applied \times 12 samples/year = 120 outcomes of the microbiological quality of the water per year analysed for the selected microbial indicator group(s)). The frequency of verification for each processing line and/or operation can be decreased with the accumulation of satisfactory results shown by the trend observation and analysis. In the case of seasonal production, e.g. peaches and nectarines, the monthly sampling can be replaced by taking 12 samples over the seasonal production cycle (e.g. 3 months, with a sample every week).

In the EU Commission Notice (2017/C 163/01)¹³ on guidance document on addressing microbiological risks in fresh fruits and vegetables (ffVs) at primary production through good hygiene, including post-harvest operations at the place of such production (Annex II), a sampling frequency scheme for water used in primary agricultural production, i.e. high (one per month), medium (twice a year) and low (once a year) is set as example, which may be modified based on the risk assessment of each farm. Tools for conducting such assessments are also provided in Annexes II and III. However, no recommendations were provided regarding water management plans, and particularly, how to validate, monitor and verify the implemented intervention strategies neither at primary production stage nor at processing facilities.

The same sampling and analytical methods used in the validation study should be used for verification purposes, following standardised protocols (i.e. ISO methods or validated alternatives, when available). Calibrated and verified sensors and devices shall be used.

Apart from the timely intervention when an outcome is not in line with the expected, desired or set parameter (e.g. target pH, target UV absorbance at 254 nm, target residual chlorine concentration) in the operational monitoring, a trend observation (presentation of collected data in a histogram, figures, etc., to retrieve trends) and trend analysis (statistical analysis to detect significant differences) also need to be done to identify potential systemic issues in the frame of the verification. Table 6 gives an overview of the frequency of trend observations and trend analyses based on the data collected for the verification of the water management plan.

TABLE 6 Proposal of frequency for trend observation and trend analysis in the frame of verification of a water management plan for the fresh-whole FVH sector.

| Type of record | Trend observation | Trend analysis |
|--|-------------------------|----------------|
| Operational monitoring data (online, inline and/or offline) | Every month | Twice a year |
| Calibration of analytical sensors, probes, etc. (belonging to preventive measures) | Twice a year | Once a year |
| Validation | In case of revalidation | – |
| Verification | Monthly | Twice a year |

In case the verification outcomes are not performing according to the set performance standards of the microbiological indicators, the quality of the process water is not in compliance with the required standard. In this case, the water management plan needs to be reviewed and revalidated (EFSA BIOHAZ Panel, 2023).

3.9 | Record keeping and review of the water management plan

The outcomes of all records collected for the preventive measures, validation, operational monitoring and verification shall be established in the FBOp documentation systems.

Examples of record-keeping data sheets are provided in Appendixes F and G.

4 | CONCLUSIONS

This opinion only covers TOR 1.1, as sub-TOR from TOR1, because industrial data was unavailable to provide further insights beyond what has been included in Part 1 Opinion on the other sub-TORs from TOR1. However, all the sub-TORs from TOR2 and TOR 3 were addressed.

TOR1 aims to describe the microbiological hazards associated with the use of water in post-harvest handling and processing operations of fffVH and the routes and rates of contamination of the water and the fffVH.

TOR 1.1: Which are the most relevant microbiological hazards associated with the use of water in different post-harvest handling and processing operations for fffVH?

Industrial data covering 29 scenarios for the fresh-whole FVH sector obtained within the framework of EFSA's outsourced activities indicated that:

- Levels of potential microbial indicators in the process water were variable. They ranged from <0 (below LOD) to 9.1 log₁₀ CFU/100 mL for TBC, from <0 (below LOD) to 3.9 log₁₀ CFU/100 mL for *E. coli* and from <0 (below LOD) to 6.5 log₁₀ CFU/100 mL for *Listeria* spp.
- The hazardous events linked to the handling and/or processing operations using water for the fresh-whole FVH sector include: (i) contaminated water source; (ii) the use of the same water to wash large volumes of product during the

¹³European Commission. (2017). Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). OJ C 163, 23.5.2017, p. 1–40.

operational cycle without a well-managed intervention strategy; (iii) incomplete removal of the water used in the water tank; and (iv) uncontrolled water temperature. Water is recommended to be cooled; however, in the case of the production of climacteric commodities, this can provoke a chilling injury.

- Among the bacterial pathogens, *L. monocytogenes* was detected in 22 out of 624 water samples analysed, occurring in 6 out of 29 scenarios. *Salmonella* spp. was only detected in 1 water sample out of 624. STEC was not detected in any of the water samples from the 29 scenarios.
- When aggregating observations within each sampling visit, the levels of TBC, *E. coli* and *Listeria* spp. tended to be higher in process water positive for *L. monocytogenes* than in process water in which *L. monocytogenes* was not detected. When TBC was above 10^6 , *E. coli* above 100 and *Listeria* spp. above 10–100 CFU/100 mL, the odds of detecting *L. monocytogenes* were almost 7-fold, almost 12-fold and about 7-fold, the odds of detection when these indicators were below the thresholds, respectively.
- When applying a multivariable logistic regression model that accounts for the hierarchical structure of the complete data set using random effects, the detection of *L. monocytogenes* in process water is influenced by multiple variables (i.e. specific combination of FVH product, type of operation, operational conditions, etc.). The variability of the detection of *L. monocytogenes* is mainly due to sampling time point, scenario and sampling visit (ordered by relevance) as random effects.
- The suitability of any potential microbial indicator for verification purposes within the water management plan should be validated under the specific operational conditions of each FBOp.

TOR2 aims to describe specific intervention strategies (i.e. water disinfection treatments, water replenishment rates, good hygiene practices, etc.) needed to ensure the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH, taking into account their impact on the physiological state of the microbiological hazards present in the water.

Industrial data covering 29 scenarios for the fresh-whole FVHs sector obtained within the framework of EFSA's outsourced activities showed that:

- Points of attention were identified regarding prerequisite programme practices needed to avoid microbiological contamination and proliferation in process water. The following preventive measures should be prioritised:
 - PRP 1: Infrastructure and fit-for-purpose building and equipment
 - PRP 2: Cleaning and disinfection
 - PRP 4: Technical maintenance and calibration
 - PRP 8: Water and air control
 - PRP 9: Personnel (hygiene, health status and training)
 - PRP 12: Working methodology
- When no water disinfection treatment was applied, the microbiological quality of process water did not achieve the fit-for-purpose microbiological quality. It should be noted that no FBOp applied water replenishment. When water disinfection treatments were applied, physico-chemical parameters indicated that the water management system was not adjusted to achieve a fit-for-purpose microbiological quality. These results stress the need to implement validation, operational monitoring and verification in industrial settings to achieve a proper water management in this sector.
- According to the mathematical model simulations based on chlorine-based disinfectants:
 - Adequate water management requires continuous monitoring and adjustment of physico-chemical parameters such as the residual concentration of disinfectant (e.g. FC concentration > 3–5 mg/L) and pH (6.0–6.5) to maintain the microbiological quality of the process water within established ranges.
 - Disinfection is needed to maintain the fit-for-purpose microbiological quality of the process water, whereas water replenishment applied alone cannot avoid accumulation of microorganisms in process water when applied at realistic feasible rates. Water replenishment combined with water disinfection treatments facilitates the management of the water quality, reducing the impact of the organic matter on the efficacy of chlorine.
- When the VBNC bacterial cells were quantified in process water, it was observed that in the case of TBC, levels of VBNC cells were always higher than the culturable cells, indicating a low efficacy of the intervention strategies to avoid the induction of VBNC cells.

TOR3 aims to describe relevant parameters to assess the appropriate microbiological quality requirements of water used for post-harvest handling and processing operations of fffVH.

- The validation study of the efficacy of the intervention measures should be performed by each FBOp to support the selection of the specific indicators and their thresholds (performance standards), considering the fit-for-purpose water concept depending on the specific handling and processing operation.

- A reasonable range of variability of the operating conditions and the intended use of the FVH among others should be considered.
- FBOps could use the predictive mathematical model made available in a user-friendly tool (<https://r4eu.efsa.europa.eu/app/WaterManage4You>) for a preliminary in-silico study of the validation assay to evaluate whether the foreseen conditions of the water treatment could be suitable to maintain the fit-for-purpose microbiological quality of process water.
- Operational monitoring is the systematic and continuous observation that allows real-time information on the physico-chemical process parameters identified as critical during the validation study.
 - Critical physico-chemical parameters to be monitored should include but are not limited to organic matter (measured as UV absorbance 254 nm), residual concentration of the disinfectant, pH (depending on the type of disinfectant) and temperature.
 - Corrective actions are required when the established thresholds for physico-chemical parameters are not met.
- Verification is conducted periodically to check if the microbiological quality of the process water is achieved by different activities, such as a review of operational monitoring records and the calibration status of measuring devices, in combination with sampling of the process water for analysing the selected microbial indicators (e.g. TBC, TC, *E. coli* and/or *Listeria* spp.).
 - The frequency of verification can be decreased depending on satisfactory results shown by the trend observation and analysis.
 - In case the verification outcomes are not performing according to the set performance standards of the microbiological indicators, the process water does not conform to fit-for-purpose microbiological quality and the water management plan needs to be reviewed and/or revalidated.
- Record keeping and review of the water management plan is critical. The outcomes of all records collected for the preventive measures, validation study, operational monitoring and verification shall be established in the FBOp documentation systems.

5 | RECOMMENDATION

- Relevant stakeholders can use the developed mathematical model for their FVH sector to understand the impact of certain parameters and intervention measures on the process water quality, using specific data generated in their industrial settings.
- To further investigate the efficacy of non-chemical water disinfection methods, either alone or in combination with existing practices, in industrial settings.
- In view of the increasing water scarcity it is recommended to explore the implementation of all possible water treatments to enhance water reuse. To further explore the association between potential microbial indicators and the detection of pathogens in process water.
- To evaluate the chemical hazards linked to the use of water disinfection treatments aiming to maintain the fit-for purpose quality of process water.

ABBREVIATIONS

| | |
|-------------------------------|---|
| AQ | Assessment question |
| BIOHAZ | Biological Hazards |
| CaClO ₂ | calcium hypochlorite |
| CFU | colony forming units |
| COD | chemical oxygen demand |
| EC | electrical conductivity |
| ECDC | European Centre for Disease Prevention and Control |
| FAO | Food and Agriculture Organization of the United Nations |
| FC | free chlorine |
| FBOp(s) | food business operator(s) |
| fffvH | fresh and frozen fruit, vegetables and herbs |
| FVH | fruit, vegetables and herbs |
| GMP | good manufacturing practices |
| GHP | good hygienic practices |
| ISO | International Organization for Standardization |
| H ₂ O ₂ | hydrogen peroxide |
| HACCP | Hazard Analysis Critical Control Point |
| HAV | hepatitis A virus |

| | |
|--------------------|--|
| HOCl | hypochlorous acid |
| LOD | limit of detection |
| MI | microbial indicator |
| NaClO ₂ | sodium hypochlorite |
| OR | odds ratio |
| OPRP | operational pre-requisite program |
| ORP | oxidation–reduction potential |
| PAA | peroxyacetic acid |
| PCA | principal component analysis |
| PCR | polymerase chain reaction |
| pH | potential of hydrogen |
| PRP | pre-requisite programme |
| RTE | ready-to-eat |
| RT-qPCR | quantitative real time polymerase chain reaction |
| SG | scenario group |
| STEC | Shiga toxin-producing <i>Escherichia coli</i> |
| SO | Scientific opinion |
| SOP | standard operating procedures |
| T | temperature |
| TBC | total bacterial count |
| TC | total coliforms |
| TDS | total dissolved solids |
| Th | threshold |
| TOR(s) | Terms of Reference |
| TSS | total soluble solids |
| VBNC | viable but non-culturable |
| WD | water disinfection |
| WHO | World Health Organization |
| WMP | water management plan |
| WR | water replenishment |

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REQUESTOR

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

Uncertainty analysis

TABLE A.1 Potential sources of uncertainty related to the industrial data set collected through EFSA's outsourced activities (Gil et al., 2025) for fresh-whole FVH processing and its use.

| Source or location of the uncertainty | Nature or cause of the uncertainty |
|---|---|
| A limited number of EU FBOs (17) who contributed to EFSA's outsourced activities are active in the fresh-whole FVH sector. | All FBOs belonged to one Member State, resulting in an uncertainty that not all practices in processing fresh-whole FVH across Europe were included in the available data set. |
| A limited number of scenarios (29) in the data set collected through EFSA's outsourced activities for fresh-whole FVH resulted in a limited range of commodities (11 types) and a limited selection of the potential use of water in post-harvest processing activities and water management/treatment possibilities. | This results in an uncertainty that not all practices in the processing of fresh-whole FVH across Europe were included in the available data set. For example, most reported fresh-whole FVH-associated outbreaks were associated with leafy greens, followed by sprouts, but none of these fresh-whole products were included. The scenarios considered also applied a limited number of interventions, i.e. all were based on the use of chemicals to disinfect the water. Therefore, data was lacking for other interventions, such as water replenishment (or refreshment) or physical water disinfection technologies. |
| The data set collected through EFSA's outsourced activities was assembled in a certain time period (4 April 2022 to 4 April 2024) with a fractionated data collection (1 or 2 sampling visits per scenario). | The nature of the collected data can be uncertain as no full picture of the specific situation in a particular company with a certain water management practice was collected. |
| The data set collected through EFSA's outsourced activities harbours an uncertainty related to the distribution of the sampling time points, which do not always represent the full duration of the operational cycle. | For example, 6 sampling time points per sampling visit per scenario set more in the beginning or later in an operational cycle. Therefore, an unequal accumulation of the microbial load of the process water can be expected, or sampling time point 0 may not correspond to the start of the operational cycle. |
| Norovirus and indicator viruses (bacteriophages) were only analysed at the last sampling time point of each sampling visit of an operational cycle. | Furthermore, norovirus was analysed by RT-qPCR, showing the presence of norovirus genomes, and for Norovirus GI, also after the addition of PMAxx, which indicates the presence of intact virions, but not their infectivity. |
| The methods used in EFSA's outsourced activities to confirm presumptive <i>Salmonella</i> spp. and <i>L. monocytogenes</i> colonies were different (PCR vs. latex agglutination (<i>Salmonella</i>) and PCR vs carbohydrate fermentation testing (<i>L. monocytogenes</i>)). These confirmation methods are not reference methods and are considered alternative methods. | The positive samples confirmed using the alternative methods cannot be considered equivalent. For <i>Salmonella</i> , none of the methods used can be considered validated for process water samples, but both methods confirm suspect colonies (no serogroup or serotype identification). Therefore, results presented in the scientific opinion always specify if these were generated by PCR-confirmation, agglutination-confirmation or carbohydrate fermentation testing-confirmation, respectively. |
| There were also sources of uncertainties linked to the predictive model used to illustrate the efficacy of different intervention strategies. | The uncertainty sources associated with the model structure (i.e. suitability of the model to simulate the process) are considered to have a lower impact on the outcome than the uncertainty in the data used to fit the model. This is further elaborated in the Part 2 Opinion (EFSA BIOHAZ Panel, 2025) |

Abbreviations: FBO, food business operator; FVH, fruit, vegetables and herbs; RT-qPCR, quantitative real time polymerase chain reaction; PCR, polymerase chain reaction.

APPENDIX B

Industrial scenarios for the fresh-whole FVH sector in EFSA's outsourced activities (Gil et al., 2025)

TABLE B.1 Overview of the industrial scenarios for the fresh-whole FVH sector (EFSA outsourced activities – Gil et al., 2025).

| Scenario ID | Water source | Handling/processing operation | Disinfectant type | Food product |
|-------------|---------------------|-------------------------------|-------------------------------|--------------------|
| 1 | Municipal tap water | Dumping | None | Apples |
| 2 | Well water | Pre-sorting | None | Apples |
| 3 | Well water | Dumping | None | Peaches/Nectarines |
| 4 | Municipal tap water | Pre-washing | None | Peppers |
| 5 | Surface water | Hydrocooling | None | Carrots |
| 6 | Surface water | Washing | None | Carrots |
| 7 | Municipal tap water | Pre-washing | None | Vegetable mix |
| 8 | Recycled water | Washing | None | Celery |
| 9 | Well water | Dumping | Sodium hypochlorite | Apples |
| 10 | Well water | Pre-sorting | Sodium hypochlorite | Apples |
| 11 | Municipal tap water | Dumping | Calcium hypochlorite | Apples |
| 12 | Municipal tap water | Dumping | H ₂ O ₂ | Apples |
| 13 | Surface water | Dumping | H ₂ O ₂ | Apples |
| 14 | Well water | Dumping | Calcium hypochlorite | Pears |
| 15 | Well water | Pre-sorting | Calcium hypochlorite | Pears |
| 16 | Surface water | Dumping | Sodium hypochlorite | Peaches/Nectarines |
| 17 | Well water | Dumping | Sodium hypochlorite | Peaches/Nectarines |
| 18 | Municipal tap water | Dumping | Sodium hypochlorite | Peaches/Nectarines |
| 19 | Municipal tap water | Dumping | Calcium hypochlorite | Peaches/Nectarines |
| 20 | Surface water | Dumping | Calcium hypochlorite | Peaches/Nectarines |
| 21 | Well water | Dumping | Calcium hypochlorite | Peaches/Nectarines |
| 22 | Well water | Pre-sorting | Calcium hypochlorite | Peaches/Nectarines |
| 23 | Surface water | Dumping | H ₂ O ₂ | Peaches/Nectarines |
| 24 | Surface water | Hydrocooling | Sodium hypochlorite | Cherries |
| 25 | Municipal tap water | Dumping | Calcium hypochlorite | Cherries |
| 26 | Municipal tap water | Pre-washing | PAA | Avocado |
| 27 | Municipal tap water | Pre-washing | PAA | Mango |
| 28 | Municipal tap water | Washing | PAA | Peppers |
| 29 | Municipal tap water | Washing | Sodium hypochlorite | Fruit mix |

Abbreviations: FVH, fruit, vegetables and herbs; PAA, peroxyacetic acid.

APPENDIX C

Pathogen-positive findings in process waters of fresh-whole FVH

TABLE C.1 Pathogen-positive process waters characterised by food product, water source, disinfectant type, sampling visit, sampling time point and mass of fresh-whole FVH processed in the same water (Source: EFSA outsourced activities – Gil et al., 2025).

| Scenario ID | Food product | Handling/processing operation | Water source | Disinfectant type | Sampling visit number | Sampling time point | Sampling time (min) | Product mass processed (kg) | Pathogen findings ¹⁴ |
|-------------|--------------------|-------------------------------|---------------------|-------------------------------|-----------------------|---------------------|---------------------|-----------------------------|--|
| 02 | Apples | Pre-sorting | Well water | No water treatment | 1 | 4 | 2990 | 132,500 | <i>L. monocytogenes</i> |
| 02 | Apples | Pre-sorting | Well water | No water treatment | 1 | 6 | 13,290 | 209,470 | <i>L. monocytogenes</i> |
| 03 | Peaches/Nectarines | Dumping | Well water | No water treatment | 2 | 2 | 95 | 5770 | <i>L. monocytogenes</i> |
| 04 | Peppers | Pre-washing | Municipal tap water | No water treatment | 1 | 6 | 275 | 33,000 | Norovirus |
| 04 | Peppers | Pre-washing | Municipal tap water | No water treatment | 2 | 6 | 255 | 39,000 | Norovirus |
| 12 | Apples | Dumping | Municipal tap water | H ₂ O ₂ | 2 | 4 | 1840 | 4047 | <i>L. monocytogenes</i> |
| 12 | Apples | Dumping | Municipal tap water | H ₂ O ₂ | 2 | 6 | 3310 | 8133 | <i>L. monocytogenes</i> |
| 13 | Apples | Dumping | Surface water | H ₂ O ₂ | 2 | 1 | 60 | 1850 | <i>Salmonella</i> |
| 15 | Pears | Pre-sorting | Well water | Chlorine | 1 | 6 | 3440 | 209,457 | Norovirus ¹⁵ |
| 15 | Pears | Pre-sorting | Well water | Chlorine | 2 | 6 | 3340 | 94,600 | Norovirus ¹¹ |
| 16 | Peaches/Nectarines | Dumping | Surface water | Chlorine | 1 | 6 | 59,290 | 5,746,429 | Norovirus |
| 17 | Peaches/Nectarines | Dumping | Well water | Chlorine | 1 | 4 | 2940 | 58,186 | <i>L. monocytogenes</i> |
| 17 | Peaches/Nectarines | Dumping | Well water | Chlorine | 1 | 5 | 3180 | 67,043 | <i>L. monocytogenes</i> |
| 17 | Peaches/Nectarines | Dumping | Well water | Chlorine | 1 | 6 | 4395 | 82,566 | Norovirus |
| 18 | Peaches/Nectarines | Dumping | Municipal tap water | Chlorine | 1 | 6 | 4425 | 237,763 | Norovirus |
| 22 | Peaches/Nectarines | Pre-sorting | Well water | Chlorine | 2 | 6 | 3370 | 198,593 | Norovirus |
| 23 | Peaches/Nectarines | Dumping | Surface water | H ₂ O ₂ | 2 | 2 | 165 | 9430 | <i>L. monocytogenes</i> |
| 23 | Peaches/Nectarines | Dumping | Surface water | H ₂ O ₂ | 2 | 4 | 1490 | 32,958 | <i>L. monocytogenes</i> |
| 23 | Peaches/Nectarines | Dumping | Surface water | H ₂ O ₂ | 2 | 5 | 1600 | 37,180 | <i>L. monocytogenes</i> |
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 1 | 3 | 100 | 7210 | <i>L. monocytogenes</i> |
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 1 | 5 | 195 | 13,884 | <i>L. monocytogenes</i> |
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 1 | 6 | 225 | 15,992 | <i>L. monocytogenes</i> , Norovirus |
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 2 | 4 | 150 | 12,068 | <i>L. monocytogenes</i> |

(Continues)

¹⁴Confirmation of *L. monocytogenes* and *Salmonella* was performed using PCR. The presence of norovirus GI and GII was detected by RT-qPCR.¹⁵Intact capsid was assessed by RT-qPCR after PMAxx pretreatment and RNA extraction in Norovirus GI-positive samples.

TABLE C.1 (Continued)

| Scenario ID | Food product | Handling/processing operation | Water source | Disinfectant type | Sampling visit number | Sampling time point | Sampling time (min) | Product mass processed (kg) | Pathogen findings ¹⁴ |
|-------------|--------------|-------------------------------|---------------------|-------------------|-----------------------|---------------------|---------------------|-----------------------------|--|
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 2 | 5 | 180 | 14,482 | <i>L. monocytogenes</i> |
| 26 | Avocado | Pre-washing | Municipal tap water | PAA | 2 | 6 | 220 | 17,700 | <i>L. monocytogenes</i> , Norovirus ¹¹ |
| 27 | Mango | Pre-washing | Municipal tap water | PAA | 1 | 6 | 225 | 692 | Norovirus ¹¹ |
| 28 | Peppers | Washing | Municipal tap water | PAA | 2 | 6 | 255 | 39,000 | Norovirus ¹¹ |

Abbreviations: FVH, fruit, vegetables and herbs; PAA, peroxyacetic acid.

APPENDIX D

Relationship between the occurrence of potential microbial indicator groups above a given threshold and the detection of *L. monocytogenes***TABLE D.1** Odds ratio (OR) for the detection of *L. monocytogenes* in process water from the fresh-whole FVH for different thresholds of TBC, TC, *E. coli* and *Listeria* spp. as potential microbial indicator groups (results analysed aggregating by sampling visit). The OR calculated using the complete data set for all sectors (fresh-whole, fresh-cut and frozen FVH) are also included for comparison.

| Bacterial indicator group and its threshold | Data concerning fresh-whole FVH sector | | | Complete data set for all sectors (fresh-whole, fresh-cut and frozen FVH) | | |
|--|--|----------|------------------------|---|-----------|------------------------|
| | OR | 95% CI | Significance level (p) | OR | 95% CI | Significance level (p) |
| Total bacterial count threshold (CFU/100 mL) | | | | | | |
| 10 ⁴ | _ ^a | | | _ ^a | | |
| 10 ⁵ | _ ^a | | | _ ^a | | |
| 10 ⁶ | 6.9 | 1.2–40.3 | <0.001 | 12.2 | 2.6–58.3 | 0.002 |
| Total coliform threshold (CFU/100 mL) | | | | | | |
| 10 ² | 4.0 | 0.4–36.1 | 0.217 | 8.6 | 1.1–68.7 | 0.042 |
| 10 ³ | 5.7 | 0.6–51.6 | 0.119 | 12.7 | 1.6–101.4 | 0.016 |
| 10 ⁴ | 2.4 | 0.5–12.2 | 0.285 | 5.0 | 1.3–19.4 | 0.019 |
| <i>E. coli</i> threshold (CFU/100 mL) | | | | | | |
| 1 ^b | 2.39 | 0.4–13.6 | 0.326 | 7.4 | 1.6–35 | 0.012 |
| 10 | 3.75 | 0.7–21.5 | 0.137 | 11.7 | 2.4–55.7 | 0.002 |
| 100 | 11.56 | 1.9–70.6 | 0.008 | 26.0 | 5.3–127.4 | <0.001 |
| <i>Listeria</i> spp. threshold (CFU/100 mL) | | | | | | |
| 1 ^b | 3.1 | 0.5–17.8 | 0.200 | 5.8 | 1.2–27.6 | 0.026 |
| 10 | 6.9 | 1.2–40.3 | 0.033 | 11.2 | 2.3–53.2 | 0.003 |
| 100 | 7.2 | 1.3–39.6 | 0.023 | 5.2 | 1.6–17.1 | 0.007 |

Note: Bold: indicates statistically significant differences.

Abbreviation: CFU, colony forming unit; CI, confidence interval; FVH, fruit, vegetables and herbs; TBC, total bacterial count; TC, total coliforms.

^aOR could not be calculated because *L. monocytogenes* was not detected in any sample with TBC below the threshold.

^bLimit of detection.

APPENDIX E

Model parameters and variables used to simulate the different scenarios groups (SGs)

TABLE E.1 Input parameter values for model simulations of all the scenario groups (SGs) of the fresh-whole FVH sector.

| Parameter | Value | Units | Description | Source for the value (reference) |
|-----------|-----------------------|-------------------------|---|---|
| α | 7.66 | 1/(mg-FC/L·min) | Inactivation rate coefficient | Estimated value from TBC data collected through EFSA's outsourced activities (Gil et al., 2025) |
| β | 7.81×10^{-4} | 1/(mg-COD/L·min) | Rate constant of reaction FC and COD | Estimated value (Gil et al., 2025) |
| γ | 8.16 | mg-COD/mg-FC | Yield coefficient COD of reaction FC and COD | Estimated value (Gil et al., 2025) |
| λ | 1.70×10^{-3} | 1/min | Natural decay of FC | Retrieved from Abnavi et al. (2021) |
| n | 1 | – | Exponent in Hill equation | Assumed (Gil et al., 2025) |
| K_m | 1.79×10^3 | mg-COD/L | Protective COD effect | Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025) |
| K_x | 1.40×10^7 | CFU/(kg-product·min) | Transfer rate coefficient of bacteria from product to water | Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025) |
| K_{COD} | 3.52×10^2 | mg-COD/(kg-product·min) | Transfer rate coefficient of organic matter from process water to product | Estimated value from data collected through EFSA's outsourced activities (Gil et al., 2025) |

Abbreviations: COD, chemical oxygen demand; CFU, colony forming unit; FVH, fruit, vegetables and herbs.

TABLE E.2 Input data for variables in model simulations of all the scenario groups (SGs) of the fresh-whole FVH sector.

| Variables | Value | Units | Description |
|-----------|--------|------------|--|
| V | 5000 | L | Tank water volume |
| τ | 1 | min | Contact time |
| P | 48,019 | Kg | Total product added |
| COD_i | 0 | mg-COD/L | Initial COD in water (mg/L) |
| X_{wi} | 0 | CFU/100 mL | Initial bacterial contamination level (CFU/mL) |

Abbreviations: CFU, colony forming unit; COD, chemical oxygen demand; FVH, fruit, vegetables and herbs.

APPENDIX F

Recording template table for a validation study for water used in different post-harvest handling and/or processing operations

TABLE F.1 Example of a recording template table for validation of post-harvest handling and/or processing operations using water in the fresh-whole FVH sector (*fixed, ** dynamic, ***in case disinfection is applied).

| Post-harvest handling and/or processing operation | Date | Hour | Product and process-related parameters* (e.g. commodity, equipment information as tank volume) | Water-related parameters* | | Water-related parameters** | | Water disinfection treatment-related parameters*, *** | | Water disinfection treatment-related parameters*** | | Analytical result of indicator in water (CFU/100 mL) | |
|---|----------------|--------------------------------|--|--|-------------|----------------------------|------------------------|---|--|--|--|--|--|
| | | | | (e.g. water hardness, water temperature, water addition) | Measured pH | Measured COD | Measured UV absorbance | Temperature (°C) | e.g. type, mode of dosing (continuous/discontinuous) | Initial concentration (mg/L) | Measured residual concentration (mg/L) | | |
| Transport/sorting water: | Trial 1 | Sampling time point 1 (start) | | | | | | | | | | | |
| | | Sampling time point 2 (middle) | | | | | | | | | | | |
| | | Sampling time point 3 (end) | | | | | | | | | | | |
| | Trial 2 | Sampling time point 1 (start) | | | | | | | | | | | |
| | | Sampling time point 2 (middle) | | | | | | | | | | | |
| | | Sampling time point 3 (end) | | | | | | | | | | | |
| | Trial 3 | Sampling time point 1 (start) | | | | | | | | | | | |
| | | Sampling time point 2 (middle) | | | | | | | | | | | |
| | | Sampling time point 3 (end) | | | | | | | | | | | |
| Wash process: rinsing water | | | | | | | | | | | | | |
| Wash process: prewash water | ... | | | | | | | | | | | | |
| Wash process: washing water | | | | | | | | | | | | | |
| Dumping tank water | ... | | | | | | | | | | | | |

(Continues)

TABLE F.1 (Continued)

| Post-harvest handling and/or processing operation | Date | Hour | Product and process-related parameters* (e.g. commodity, equipment information as tank volume) | Water-related parameters* | | Water-related parameters** | | Water disinfection treatment-related parameters*, *** | | | Water disinfection treatment-related parameters*** | | Analytical result of indicator in water (CFU/100 mL) |
|---|------|------|--|--|-------------|----------------------------|------------------------|---|--|------------------------------|--|--|--|
| | | | | (e.g. water hardness, water temperature, water addition) | Measured pH | Measured COD | Measured UV absorbance | Temperature (°C) | e.g. type, mode of dosing (continuous/discontinuous) | Initial concentration (mg/L) | Measured residual concentration (mg/L) | | |
| Water applied in hydrocooling | ... | | | | | | | | | | | | |

Abbreviations: CFU, colony forming unit; COD, chemical oxygen demand; FVH, fruit, vegetables and herbs.

APPENDIX G

Recording template table for operational monitoring

TABLE G.1 Example of template for recording table for operational monitoring of post-harvest handling and/or processing operations using water in the fresh-whole FVH sector.

| Post-harvest handling and/or processing operation | Food product | Date | Hour | Product mass (kg) being processed | Residual disinfectant concentration (mg/L) | pH | UV absorbance | Temperature (°C) | Water addition (L/min) |
|---|--------------|------|------|-----------------------------------|--|----|---------------|------------------|------------------------|
| | | | | | | | | | |
| | | | | | | | | | |

Abbreviation: FVH: fruit, vegetables and herbs.

ANNEX A

Excel file including the full data set collected during the sampling visits to all FBOs collaborating with the EFSA outsourced activities (Gil et al., 2025).

Annex A is available under the Supporting Information section on the online version of the scientific output.

ANNEX B

Statistical analysis of the relationship between potential microbial indicator groups and pathogen detection in process water of fresh and frozen fruits, vegetables and herbs: a multivariable, logistic mixed-effect regression model.

Annex B is available under the Supporting Information section on the online version of the scientific output.