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European Association of
Fruit and Vegetable Processors

Hygiene guidelines for the control of *Listeria monocytogenes* in the production of quick-frozen vegetables

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Abstract

A multidisciplinary approach is recommended to control the environmental pathogen *Listeria monocytogenes* in the production of quick-frozen vegetables. A food safety management system, based on Pre-Requisite Programs (PRPs, focusing on the hygiene and organization of the production environment) and a HACCP-plan (focusing on the process control), need to have a full focus on *Listeria monocytogenes* in order to prevent the organism from colonizing and persisting in complex biofilm formations, or to prevent contamination with the organism after (thermal) processing during further handling before packaging. Figure 1 illustrates the different PRPs and the HACCP-plan relevant in the prevention and control of *Listeria monocytogenes*. Environmental control needs to be established in order to verify the effectiveness of the implemented PRPs and HACCP-plan and to evaluate the potential accumulation of *Listeria monocytogenes* in the broader production environment. Finally, end product specifications must help Food Business Operators (FBOs) to set intermediate levels towards *L. monocytogenes*, achievable in end products when a proper food safety management system is in place. Risk communication and information sharing towards the users of quick-frozen vegetables must clearly state the proper use of the frozen products to avoid potential abuse. Apart from these techno-managerial activities, an FBO also needs to establish a safety culture and create awareness throughout the whole production organization and all its aspects in the prevention and control of food safety hazards and hygiene disruptions. The presented guidelines cover frozen vegetables, blanched and unblanched, which are considered as Non Ready-To-Eat (nRTE). FBOs intending to market frozen vegetables as Ready-To-Eat (RTE), would also benefit from following these guidelines. Such FBOs, however, should follow additional preventive and control measures to assure the safety of RTE products, but these are not included in the current guidelines.

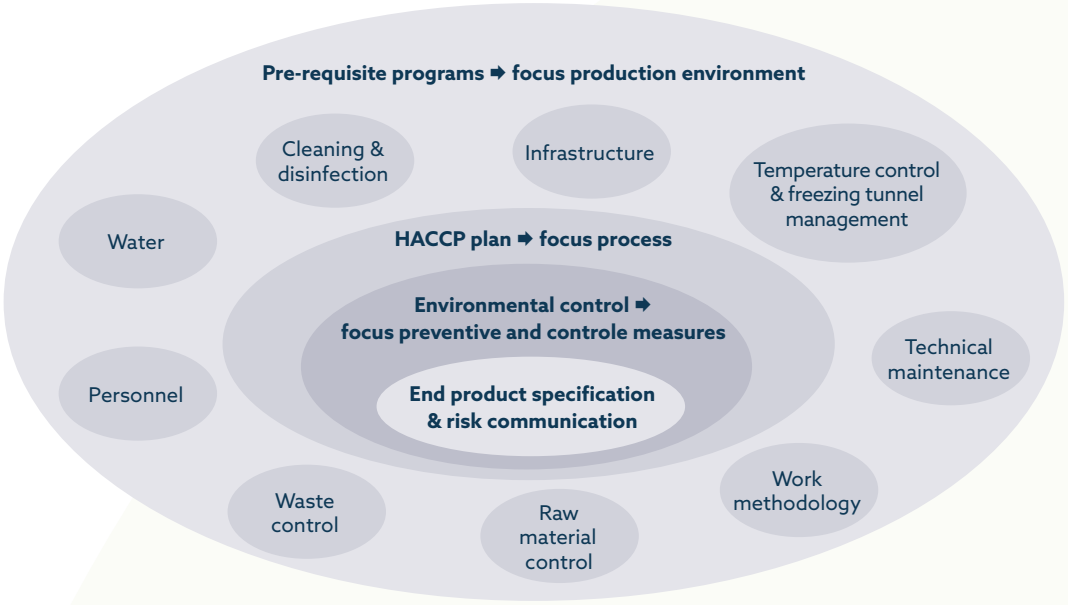

Figure 1. Concept of PRPs (with focus on the broader production environment), HACCP-plan (with focus on the production process and different processing steps), environmental control (as verification of performed preventive and control measures) and finally end product specifications and risk communication towards the users (B2B and B2C) in order to prevent and control potential contamination with L. monocytogenes in the production of quick-frozen vegetables.

Scope

The presented hygiene guidelines including an example of an HACCP-plan refer to the commercial production of quick-frozen (blanched and unblanched) vegetables in accordance with applicable legislation of the European Union. The objective is to set an European guideline in the production and food safety management of quick- frozen vegetables, starting from the reception of raw materials and ending with the packed end products ready for use in the next step in the food supply chain; B2B or B2C. FBOs active in production and/or trade of quick- frozen vegetables may use this document as a starting point for their own food safety management system, elaboration of good practices, PRPs and HACCP-principles. Focus is placed on the control of the hazard, *L. monocytogenes*. Other relevant microbiological hazards for these activities or other hazards (e.g. chemical, physical hazards or allergens) will not be discussed in this document. Next to frozen vegetables, some FBOs also produce frozen herbs and/or fruits, however, these products are out of scope of the presented guidelines. The presented guidelines cover frozen vegetables, blanched or unblanched, which are considered as Non Ready-To-Eat (nRTE). FBOs intending to market frozen vegetables as Ready-To-Eat (RTE), would also benefit from following these guidelines. Such FBOs, however, should follow additional preventive and control measures to assure the safety of RTE products, but these are not included in the current guidelines.

EU legislation applicable to the production of quick-frozen vegetables

The general food safety requirements, including the obligation to only place safe food on the market, are laid down in Regulation (EC) No 178/2002. The hygienic production of foodstuffs in the EU is covered by Regulation (EC) No 852/2004 and in particular by Annex II. The guidelines give practical examples to supplement these general provisions. For this guide, Article 9 for Regulation (EC) No 852/2004 on community guides is respected. EU Commission Notice on Food Safety Management-C278/2016 is applied as basis for good practices, PRPs and HACCP principles. Microbiological criteria on foodstuffs are regulated by Regulation (EC) No 2073/2005. All relevant legal documents are listed in Annex I.

Additional documents beyond the guidelines

Additional guidance is available through relevant publications of the Codex Alimentarius, EFSA Opinions, general hygiene practices developed by different national authorities, scientific papers and books (listed in Annex II).

Consultation of relevant stakeholders

When setting the guidelines, consultation was organized with stakeholder groups: (a) Copa Cogeca (primary production), (b) Hotrec (restaurant activities) and FoodServiceEurope (catering activities), (c) ChilledFOODAssociation (ready-to-eat meals processors), FoodDrinkEurope (processing industry), FRUCOM (fruit and vegetable importers), CULINARIA (sauces, spices and herbs), FRESHFEL (fresh fruits and vegetables including fresh-cut produce "4eme gamme"), (d) EuroCommerce (retail organisations) and (e) BEUC (consumer organization).

Disclaimer

This guideline is a recommendation without legally binding value. It has been established for information purposes only. PROFEL does not guarantee the accuracy of the information provided, nor does it accept responsibility for any use made thereof. Users have therefore to take all necessary precautions before using this information, which they use entirely at their own risk. The duty to enforce European food safety legislation lays with the European Commission and the competent authorities of the EU member states. FBOs in quick-frozen vegetable production and or trade are asked to contact their competent authority to obtain full information about the legal requirements in their EU Member State of establishment.

1 Introduction

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1.1 Industry profile

In Europe, quick-frozen vegetables are produced by +/-145 companies, both large multi-national companies producing in several Member States, as well as many SMEs. PROFEL, the European Association of Fruit and Vegetable Processing Industries, and to some extent AETMD, the European Sweet Corn Processors Association, are the only organisations representing the sector of quick-frozen vegetables. The membership consists of both SMEs and multinational companies, employing more than 80.000 people. The combined annual turnover of PROFEL members amounts to roughly €22 billion, with a production of almost 5.5 million tons only for the vegetable sector (both canned and quick-frozen). EU annual production of quick-frozen vegetables* alone is estimated at 4 million tons. There are approximately 180 production locations in 18 EU Member States. Affiliation to PROFEL is principally via its national associations. Not all countries have national associations for quick-frozen vegetables, and some companies are affiliated directly. While no official figures exist, national associations estimate that PROFEL membership represents 80% of EU production of quick-frozen vegetables.
**excluding potatoes, tomatoes but including quick-frozen sweet corn*

1.2 Product profile

The considered product groups are quick-frozen vegetables, including root and tuber vegetables, bulb vegetables, fruiting vegetables, Brassica vegetables, leafy vegetables, edible flowers, legume vegetables, and stem vegetables. Fruits and herbs are excluded from the presented guidelines.

The included quick-frozen vegetables may be blanched or unblanched. Quick-frozen vegetables can be Individually Quick-frozen (IQF) where the product is free/loose from each other or block quick-frozen. They are packaged as bulk packages for B2B market and subsequent further processing in the food chain (e.g. catering, ready-to-eat meal production) or in small-size consumer packages for B2C markets. The products can be commercialized either as a single product or a mixed product with other quick-frozen vegetables or combined with other food products such as rice, pasta, sauce, quick-frozen fish or meat.

1.3 *Listeria monocytogenes*

Although still considered a zoonotic pathogen, *L. monocytogenes* is widely distributed in nature and food processing environments. It has been isolated from soil, vegetation, sewage, water, animal feed and in the feces of healthy animals including humans. It can enter food-processing settings via incoming raw materials and the movement of personnel and equipment. *L. monocytogenes* can colonize in the form of biofilms on food- processing equipment and (non) food-contact surfaces. Inadequate cleaning and disinfection procedures may lead to persistence of the bacterium for prolonged periods in food-processing environments. *L. monocytogenes* was isolated from a variety of foodstuffs such as fresh and quick-frozen meat, cooked meat products, smoked fish, raw milk, (soft) cheese, ice-cream, deli-salads, fresh or minimally processed vegetables, etc. (Uyttendaele et al., 2018; EFSA and ECDC 2018). *L. monocytogenes* is a Gram-positive non-spore forming bacterium, rod shaped (0.5 µm wide and 1-2 µm long), facultative anaerobic. Although it has an optimal temperature range of 30 to 37°C, it is able to grow over a wide temperature range, between 1 and 45°C. As a psychrotolerant bacterium, it can survive and even grow at refrigeration temperatures. The organism is particularly resistant to environmental stress and is able to survive or multiply under a wide range of unfavourable conditions of pH (4.6-9.4, optimum 7.0) and aw (minimum 0.92) although a 6 log reduction can be achieved by pasteurization (2' at 70°C) or any other equivalent heat treatment (Uyttendaele et al., 2018).

The species *L. monocytogenes* is divided into 13 serovars based on somatic and flagellar antigens. Since 2005, these serovars have been replaced by five genoserogroups determined by PCR: IIa (serovars 1/2a and 3a), IIb (serovars 1/2b and 3b), IIc (serovars 1/2c and 3c), IVb (serovars 4b, 4d and 4e) and L (other serovars). Of these, IVb followed by IIa and IIb are the genoserogroups that are most frequently implicated in human cases (EURL-*L. monocytogenes*, 2019). In recent years, it has been demonstrated that whole genome sequence (WGS)-based subtyping can provide substantial additional discrimination and, consequently, can be of benefit to outbreak investigations. Within the EU, listeriosis is one of the priority diseases for which supranational WGS-enhanced surveillance is being initiated in 2018 (Van Walle et al., 2018).

L. monocytogenes is the only species of *Listeria* that is pathogenic to humans and is the causative agent of listeriosis (McLauchlin et al., 2004). *L. monocytogenes* infection can result in two types of human illness: the non- invasive form of listeriosis affects the digestive system and results in symptoms including fever, muscle aches and sometimes gastrointestinal symptoms (nausea or diarrhoea), whereas the more serious invasive listeriosis is associated with clinical presentations of central nervous system infection, sepsis, and bacteremia. Because of the invasiveness of *L. monocytogenes*, listeriosis fatalities are particularly associated with high-risk populations, e.g. individuals with compromised immune systems such as persons with hematological malignancies (e.g. leukemia), persons suffering from liver cancer, older adults (> 74 years of age), pregnant women, and new born babies (Buchanan et al., 2017; McLauchlin et al., 2004).

An outbreak of invasive *L. monocytogenes* infections confirmed by whole-genome sequencing as serogroup IVb, ST6 (Sequence Type 6) and linked to quick-frozen corn and possibly to other quick-frozen vegetables was reported in five EU Member States (Austria, Denmark, Finland, Sweden and the United Kingdom) in the period 2015 up to June 2018: 47 cases had been reported and nine patients had died due to or with the infection (case fatality rate 19%). *L. monocytogenes* ST6 is a hypervirulent clone of *L. monocytogenes* associated with neurological forms of listeriosis (EFSA, 2018a). However, despite the observed variability in their virulence potential, almost every *L. monocytogenes* strain has the ability to result in human listeriosis because of the complex interaction between the pathogen, food and host. This was the first time that a listeriosis outbreak in EU was linked to quick-frozen vegetables (EFSA, 2018a), and initiated the drafting of this guidance document.

1.4 Definitions

ATP measurement :
ATP (adenosine thriphosphate) detection devices use bioluminescence to indicate the level of residual ATP present on swabbed surfaces (Turner, 2010).

B2B:
business to business, referring to quick-frozen vegetables packed for further processing in food industries or catering activities

B2C:
business to consumers, referring to quick-frozen vegetables packed for final end-consumer (distributed via retailers in small size packages)

Biofilm:
3-dimensional structure on surfaces, containing a high number of micro-organisms which are fixed on the surface via organelles and excreted substances (e.g. extracellular polymeric substances such as glycoproteins) (Devlieghere et al., 2013).

Blanching:
A heat process typically applied to a food for the purpose of inactivating enzymes and/or fixing the product colour (CAC, 1976)

CCP (Critical Control Points):
A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level (1). Most typical CCPs to control microbiological hazards are temperature requirements e.g. the temperature for storage or transport, the time/temperature conditions to reduce or eliminate a hazard (e.g. pasteurization). Other CCPs may include checking that packages are clean and not damaged, checking for physical hazards by sieving or metal detection or checking the time/temperature of frying oil to avoid chemical process contaminants (Commission Notice, C278/2016).

Clean water:
'water that does not compromise food safety in the circumstances of its use'. It is clean seawater (natural, artificial or purified seawater or brackish water that does not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food) and fresh water of a similar quality (Regulation (EC) No 852/2004; Commission Notice C163/2017).

Detergent:
(chemical) product applied for the cleaning of surfaces (removal of organic materials from surfaces) (Devlieghere et al., 2013).

EURL:
European Reference Laboratory

FBO:
Food Business Operator: the natural or legal persons responsible for ensuring that the requirements of food law are met within the food business under their control (Regulation (EC) No 178/2202).

FSMS:
Food Safety Management (or control) system (FSMS): The combination of PRPs as preventive control measures; traceability, recall and communication as preparedness and HACCP plan defining CCPs and/or oPRPs as control measures linked to the production process. The FSMS is also the combination of control measures and assurance activities. The latter aims at providing evidence that control measures are working properly such as validation and verification, documentation and record keeping (Commission Notice, C278/2016).

GHP (Good Hygiene Practices), GMP (Good Manufacturing Practices):
Package of preventive practices and conditions to ensure the safety of the food produced. GHP underline more the need for hygiene, GMP stress correct work methodologies (Commission Notice, C278/2016).

HACCP-based procedures or 'HACCP':
Procedures based on the hazard analysis and critical control points (HACCP) principles i.e. an auto-control system which identifies, evaluates and controls hazards which are significant for food safety consistent with the HACCP principles (Commission Notice, C278/2016).

HACCP-plan:
A document, possibly electronic, fully describing the HACCP-based procedures. The initial HACCP plan shall be updated if there are changes in the production and must be supplemented with records from outcomes of monitoring and verification, and from corrective actions taken (Commission Notice, C278/2016)

Hazard:
means a biological, chemical or physical agent in, or condition of, food or feed with the potential to cause an adverse health effect (Regulation (EC) No 178/2202) (Commission Notice, C278/2016).

HVAC:
Heating, Ventilation and Air Conditioning system

IQF (=individually quick-frozen):
quick-frozen food where the product is free/loose from each other (CAC, 1976).

Niche:
niche is what describes a species' ecology, which may mean its habitat, its role in the ecosystem, etc. (Pocheville, 2015)

NRL:
national reference laboratory

oPRPs (Operational Prerequisite programs):
are points in the production process with a smaller food safety risk or where no measurable limits exist. These points can be controlled via more elaborate general basic control measures belonging to the PRPs e.g. more frequent control, recording etc. Due to a regular control and adaptation of the process/product requirements these risks can be considered as controlled. An immediate corrective action towards the product is not required (Commission Notice, C278/2016).

Prerequisite program(s) (PRP(s)):
Preventive practices and conditions needed prior to and during the implementation of HACCP and which are essential for food safety. The PRPs needed depend on the segment of the food chain in which the sector operates and the type of sector. Examples of equivalent terms are Good Agriculture practice (GAP), Good Veterinarian Practice (GVP), Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP), Good Production Practice (GPP), Good Distribution Practice (GDP) and Good Trading Practice (GTP) (Commission Notice, C278/2016).

Recycled water:
water which is reused in the production process, with or without water treatment (e.g. filtration, disinfection)

RTE:
ready-to-eat food (Regulation (EC) No 2073/2005) : 'ready-to-eat food' means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate micro- organisms of concern or to reduce them to an acceptable level.

nRTE:
non ready-to-eat: refers to foods, opposite to RTE foods, intended by the producer to be cooked or subjected to other processing effective to either eliminate micro-organisms of concern or to reduce them to an acceptable level.

Sanitizer/Disinfectant:
product applies for the disinfection of surfaces after cleaning. A biocidal product should be defined according to the Regulation (EC) No 528/2012.

Quick-frozen:
quick-frozen foodstuffs' means foodstuffs (Directive (EC) 89/108 and CAC, 1976) :

- which have undergone a suitable freezing process known as 'quick-freezing' whereby the zone of maximum crystallization is crossed as rapidly as possible, depending on the type of product, and the resulting temperature of the product (after thermal stabilization) is continuously maintained at a level of -18 °C or lower at all points,and
- which are marketed in such a way as to indicate that they possess this characteristic.

2
Good practices and Pre Requisite Programs (PRPs)

PRPs are important fundamentals in the prevention and control of hygiene and food safety in the frame of a food safety management system implemented in FBOs. PRPs include good hygienic and good manufacturing practices and all measures taken to prevent contamination or outgrowth by microorganisms. These guidelines follow the structure of the EU Commission Notice on Food Safety Management (C278/2016). The role of each PRP in prevention/control of *L. monocytogenes* is described. However, as not all the 12 listed PRPs play a role in the prevention/control of *Listeria monocytogenes*, three are excluded: PRP pest control, PRP allergens, PRP physical and chemical contamination from production environment.

2.1 Cleaning and disinfection

Cleaning and disinfection (C&D) is an important PRP in prevention and control of *Listeria monocytogenes*. FBOs need to have a **C&D plan** to ensure that all relevant areas, machinery and equipment - in direct or indirect contact with the foods - of the facility will be regularly cleaned/disinfected.

The **cleaning plan** includes the area, machinery/equipment/devices (food contact or non-food contact) to be cleaned, disassembly of equipment, method of cleaning (e.g. foam cleaning, cleaning-out of-place (COP), cleaning-in-place (CIP)), types and concentrations of the cleaning compounds, time/temperature (if relevant) of cleaning solutions, flow rate (velocity) or pressure of cleaning solution (if relevant) and frequency at which cleaning takes place. This plan also includes identified areas where moisture, condensation, mould infestation, dirt or bacteria are likely to establish themselves, and describes how to prevent this from happening. In case of COP, e.g. for wash tanks, piping, care needs to be taken to avoid cross-contamination after disassembly of equipment parts (e.g. do not place the equipment directly on the floor or on other unclean surfaces). Water splashing from floors or unclean equipment onto clean equipment needs to be avoided. Therefore, it is preferred not to use high-pressure hoses during cleaning and disinfection.

Besides the cleaning, appropriate **disinfection activities** will avoid and eliminate microbiological accumulation and formation of biofilms. It is recommended to have a disinfection plan similar to the cleaning plan. To disinfect, only authorized biocides are applied according to the technical specifications of the suppliers (e.g. concentration, pH of water, water hardness, efficacy against the target organisms, need for rinsing, allowed to be used in spraying system etc.). Rotating application of disinfectants has been reported to provide a longer effectiveness and prevention of *L. monocytogenes* from niches and biofilms. Hot water or steam can be applied to sanitize racks or equipment that are difficult to access and clean, including potential harborage sites for *L. monocytogenes*.

In case there is **a suspicion of the presence of a biofilm**, specific cleaning and disinfection activities will be needed to remove the biofilm, as standard cleaning and disinfection activities will not be appropriate due to the resistance of the biofilm. However, it is more important to avoid the formation of biofilms and to perform environmental monitoring (see part 4) to pick up in an early stage any environmental contamination.

Validation of cleaning and disinfection plans (= to determine if they are appropriate to remove product leftovers, organic materials and sufficient removal of bacteria) needs to be set up. Therefore, intensive environmental sampling of cleaned areas (e.g. via ATP measurements to evaluate the removal or organic materials) and disinfected areas need to be set up for different targeted groups of bacteria (e.g. removal of Gram negative, Gram positive bacteria, yeasts and/or moulds) to evaluate the effectiveness of applied disinfecting agents, their concentration, contact time, etc. In the cleaning and disinfection plan, FBOs need to consider following a **classification of food contact materials** and related frequency of cleaning and disinfection (Table 1).

Table 1. Example of classification of equipment and devices in the frame of cleaning and disinfection frequency		
Type	**Description**	**Examples of locations**
1	Food contact surfaces in direct contact	Tank interiors, packaging and conveyors, hoppers, pipe interiors
2	Non-food contact surfaces in close proximity to food contact surfaces	Equipment housing framework, floors or drains in direct surrounding of food contact surfaces
3	More remote non-food contact surfaces which could eventually lead to contamination	Forklifts, wheels of garbage bins/devices, footbaths for personnel, walls, floors and drains not in direct contact with food contact surfaces.
4	Non-food contact surfaces and remote areas from processing environment	Hallways outside the production area, areas where raw materials or finished products are stored. Equipment housing framework, walls, floor or drains NOT in direct surrounding of food contact surfaces

In principle, type 1 locations are more frequently cleaned and disinfected compared to type 2, 3 and 4 (type 1 > 2 > 3 and 4) and the frequency can also be determined as a function of hygiene regime of the area in which the equipment and facilities are located/assigned (see part 2.5 on zoning). In principle, the 'safe areas' need higher cleaning/disinfection frequency compared to high hygiene regime and compared to low hygiene regime. A list with all potential food contact surfaces has to be established for each area and the need for cleaning and disinfection (frequency) should be defined.

Equipment and surfaces in DIRECT contact with foods (type 1, table 1)

Equipment and surfaces in direct contact with food (e.g. freezing tunnels, conveyor belts, washing tanks, multi- weigher heads, packaging machines, tank interiors, hoppers, pipe interiors) need to be carefully cleaned and disinfected to avoid cross-contamination and building up of a biofilm. Breakpoints should be organised in continuous production lines for cleaning and disinfection (e.g. washing/blanching equipment and freezing tunnel which runs x consecutive days).

Equipment and surfaces WITHOUT DIRECT contact with foods (type 2 and 3, table 1)

Equipment and surfaces which are not directly in contact with foods can harbour *Listeria monocytogenes* and can be a source of cross-contamination via splashing of water, air, aerosols, materials. Therefore, accumulation of *Listeria monocytogenes* in the whole broader factory environment needs to be avoided. Typical equipment and surfaces without direct contact to food are: air ventilation systems, water tubing systems, waste water drains, wheeled devices etc. These are sensitive to *Listeria monocytogenes* accumulation due to the high moisture content and often non-refrigerated temperatures of the production environment. Based upon company-specific information of the degree of potential accumulation of product leftovers, organic materials, dust and moisture and the potential cross-contamination towards foods or food contact surfaces in direct contact with foods, and the zoning where the equipment/facilities (see part 2.5) belong, a frequency of cleaning and disinfection needs to be established, typically x times per month is recommended.

Periodical cleaning and disinfection (type 4, Table 1)

Bigger infrastructure such as platforms, stairs, ceilings, pipelines, etc. which do not have a direct contact with foods or other food contact materials need periodic cleaning and disinfection to avoid accumulation of dust, product leftovers and organic materials, and to keep the production and storage environment in good condition. Special attention in the control of *Listeria monocytogenes* needs to be given to floor drains to prevent contamination from the drain to other surfaces in the room. Therefore, high-pressure hoses must not be used during processing to clean drains, in order to prevent formation of aerosols; dedicate tools specifically to clean drains and avoid cleaning drains in periods of production. A periodic cleaning plan (x times per x years) is needed to organise this periodic cleaning per area.

Starting up equipment after break period (= pre-operational clean-up)

The production of quick-frozen vegetables is highly seasonal. Several equipment and devices are used when processing a particular commodity and during the rest the of year (off-season) they are stored (e.g. insect removal systems for leafy greens, cutting machines). Before using these equipment/devices again, a thorough cleaning and disinfection is necessary to avoid any cross-contamination. The FBO needs to include the pre- operational clean-up in the cleaning and disinfection planning.

Maintenance of cleaning and disinfection utensils and equipment

Cleaning and disinfection tools (e.g. brushes, mops, water distribution tubes) and equipment (e.g. high-pressure cleaning machine, floor scrubbers) also need maintenance and cleaning to avoid cross-contamination. Hoses and hose nozzles are recommended to be kept off the floor or other unclean surfaces when not in use. Boot cleaning devices or footbaths have to be emptied, cleaned and refilled at least daily to avoid niche formation. It is necessary to dedicate cleaning and disinfection utensils to specific areas (e.g. by colour-coding).

Personnel involved in sanitation

Personnel involved in sanitation activities should be dedicated to these activities, with specific protective gloves, clothing, footwear and protective glasses, that are different from the ones used during regular production activities. They should be trained in sanitation including the application of the chemical products for their cleaning stations. Personnel who handle trash, floor sweepings, drains, production waste should not handle food products or come into contact with food-contact surfaces or packaging materials, unless they first change smocks/uniforms, wash and sanitize hands and sanitize footwear by a footbath or better by boot cleaning devices.

Verification of cleaning and disinfection

After cleaning and disinfection activities of a type of surface and equipment, a careful visual check should be conducted by another person than the one who was responsible for the cleaning and disinfection itself. Such a visual check can be part of a start-up control to the release of production lines for operation. In case a visual organic contamination is detected, cleaning and disinfection have to be redone before operation can start. More difficult-to-reach spots and locations have to be included in this visual check.

Microbiological sampling of contact surfaces and analysis for total plate count or another indicator has to be conducted on a regular basis to verify if cleaning and disinfection activities are still effective and conducted in a proper way. ATP tests or other rapid screening methods can be used for a rapid screening and positive release of a production equipment after cleaning and disinfection. However, this verification of cleaning and disinfection cannot replace the environmental screening for *L. monocytogenes* (see further part 4).

2.2 Water: sources, quality and water distribution network

High volumes of water are applied in the production of quick-frozen vegetables. Water (both its availability and its quality) is becoming more under pressure so care has to be taken by the FBOs that the internal re-use of water is not a source of cross-contamination with *L. monocytogenes* towards food products. FBOs need to address the following points to manage water and its potential contamination with *Listeria monocytogenes* to food products:

a) Identify potential sources of water (e.g. tap water, rain water, ground water, treated recycled water)
b) Identify the quality of the available water by analysis (both microbiological and chemical parameters ➡ does the water meet the requirements of potable water, clean water, non-potable water?)
c) Identify the potential use of recycled/re-used water (e.g. re-using the cooling water after blanching as washwater) in certain production steps ➡ careful evaluation to avoid cross-contamination has to be done in this situation
d) Identify the need for water disinfection (based on physical methods as UV, Reversed Osmosis or chemical disinfection by application of authorized biocides such as chlorine, peracetic acid, ClO2) in case of recycled water, rain water, drain water and/or effluent water to upgrade the water quality
e) Control and validate the applied water disinfection techniques (daily monitoring, check on chemical residues in case of chemical disinfection of the water)
f) Foresee maintenance of storage tanks, tubing systems, filtration systems used in water distribution to avoid biofilm formation and potential *L. monocytogenes* presence ➡ include parts of the water distribution system also in the environmental sampling (as addressed in 4.1)
g) Avoid cross-contamination with drain water/effluent water with other sources of water in the production
h) Avoid stagnant water in machines, tubes, pipelines and on the floors
i) Prevent the accumulation of standing water in and around drains
j) Avoid that drip, condensate from fixtures, ducts and pipes contaminates foods, food contact surfaces or food packaging material
k) Be sure that water applied for glazing is of potable water quality

A **water management plan** needs to be elaborated, including all these elements. An appropriate analytical plan to verify the quality of the applied water based on microbiological and chemical test results needs to be set up, taking into consideration European, national or regional requirements from the competent authorities. EFSA opinion on risk of *Listeria monocytogenes* in this type of production also identifies water applied during washing, cooling etc. as an important source of contamination (further reading in EFSA, 2020).

2.3 Temperature control of production & storage environment including freezing tunnel management

Temperature control of production and storage environment

L. monocytogenes is a cold-tolerant environmental pathogen and is able to proliferate even at temperatures of 0°C. In cold conditions, their growth rate will slow down, so maintenance of a cold chain will avoid (fast) growth of the pathogen. Typically, in a production environment of quick-frozen vegetables, not all areas are under temperature-controlled conditions. As mentioned in section 2.1 (cleaning and disinfection), these areas need careful consideration for cleaning and disinfection activities of both direct and indirect equipment in contact with foods. High moisture conditions (=relative humidity), aerosols formation and/or drip from higher constructions (e.g. ceiling, piping systems) can be triggered by fluctuating temperatures. Once the product is quick-frozen, the freezing temperature of -18°C or less need to be guaranteed by quick-frozen storage and transport conditions. In case quick-frozen products need to be handled again (e.g. mixing, packaging) cold environmental temperatures are recommended. Unless otherwise feasible, quick-frozen products have to stay (very) shortly in ambient temperature conditions to avoid defrosting. The allocated time needs to be verified by the FBO and will depend on the particular product and surrounding temperature.

Freezing tunnel management

Freezing tunnels are crucial devices in the production of quick-frozen vegetables, and will have, depending on the applied technology (air blast or cryogenic freezers) and their design, a fluctuation in low and higher temperature cycles. Temperature cycles between -30/-40°C are followed by short defrosting cycles towards 30/50°C to avoid excessive ice in the tunnel. In case food products remain or pile up in the tunnel, these can become a harbor for *Listeria monocytogenes*. Therefore, the freezing tunnels need to get periodic technical maintenance (section 2.6), a proper follow-up and temperature control of the cycles (this section), be part of the cleaning and disinfection plan (section 2.1) and regular visual checks to avoid excessive product build-up as part of working methodology (section 2.9) in order to avoid accumulation of *Listeria monocytogenes* and/or biofilm formation in the tunnel.

Two types of tunnel defrosting are distinguished:

1. **Complete tunnel defrosts.** Which depends upon the type and freezing capacity of the tunnel. Deep cleaning has to be done during each complete defrost (see section 2.1).
2. **Partial/Sequential defrosts during production.** This only exists in certain brands of freezing tunnels and comes as an add-on option. Evaporators never defrost all at the same time during production. Sections of evaporators being defrosted are completely closed and pasteurized with hot water/gas or steam. During the defrosting cycle of an evaporator section, air flow is diverted towards other sets of evaporators, which are in the freezing mode.

Heating, ventilation and air conditioning system (HVAC)

A temperature and humidity gradient can occur in the quick-frozen processing plants, due to areas with high (ambient) temperatures, low temperatures and the air which moves in between. Typically, in areas between the outlet of freezing tunnels and the collection of intermediate quick-frozen vegetables in big bags/recipients (bulk), or in areas between blanching and cooling of the blanched product, temperature gradients can be present. The temperature gradient may provoke condensation and water drip. A professionally installed and maintained heating, ventilation and air conditioning system (HVAC) is a PRP in these plants.

2.4 Personnel: awareness, training and behavior

Personnel hygiene is important in the prevention/control of *L. monocytogenes* mainly by correct behaviour of operators and their risk awareness towards the pathogen. Therefore, (repeated) training and communication (e.g. results of hygiene-inspections, results of cleaning and disinfection screening) to feed the awareness is relevant. An important factor associated with personnel is the potential source of cross-contamination by footwear, hands and gloves and smocks (or uniforms) when crossing from one production location or area to another. Moving from 'lower hygiene' regime towards 'higher hygiene' regime areas is critical in in the potential spread of *Listeria monocytogenes* as an environmental pathogen. Therefore, clear instructions need to be set and communicated towards the operators on how to cross these borders in a production zone. Eventually, hygiene buffers can be built in, such as a hygienic lock, boot cleaning devices, specific boots dedicated to the zone, hand disinfection posts to facilitate crossing areas and to prevent *Listeria monocytogenes* to migrate from one area to another one (see also for 2.5). These facilities need to be included in the cleaning and disinfection program, e.g. footbaths to avoid niche formation. Smocks or uniforms are distinguished according to the task that the personnel perform (e.g. production in low hygiene regime area, high regime areas and technical maintenance). In case temporary staff are working in the production and trading facilities, a tailored training and agreements on do's and don'ts need to be set up. Consideration of minimizing the use of temporary staff at more critical activities regarding control of *Listeria monocytogenes* is recommended as good practice.

2.5 Infrastructure, equipment and devices

Infrastructure and organization of the production and storage facilities will be of utmost importance in the prevention and control of *Listeria monocytogenes* in the quick-frozen vegetables production.

Zoning

A differentiation between 'low hygiene' regime areas and 'high hygiene'regime areas is recommended. This should be organized across production and storage facilities. These different zones are also indicated on the flowcharts (see Figures 2-4). Different areas can be identified:

Zone 1: Low hygiene regime area

➡ Characterized by:

- Areas with direct connection to the outside
- Outside reception areas of raw materials
- Production steps before washing and/or blanching
- Technical areas

➡ Control measures:

- Presence of wood, carton and/or soil is possible
- No need to access via hygienic lock
- No temperature control, no controlled ventilation/air flow

Zone 2: High hygiene regime area

➡ Characterized by:

- No direct contact with outside
- Production steps from washing and blanching till quick-frozen vegetables
- Handling of open quick-frozen products such as during glazing, mixing or packaging

➡ Control measures:

- Need to access via hygienic lock (= controlled access to the outside)
- Controlled ventilation/air flow
- Advisable to have temperature control
- Controlled and presence of clean wood or cartons (e.g. octabins)

Zone 3: Safe area

➡ Characterized by:

- Storage of packed bulk or end products (quick-frozen)
- Freezing temperatures of product

➡ Control measures:

- Only closed packages/recipients
- Temperature control (freezing temperature)

Linked to the segregation of the areas in the production and storage facilities, another hygiene regime will be required per area as control measures such as:

- higher frequency of cleaning and disinfection activities
- more restrictions in personal hygiene for the operators
- dedicated use of materials for production (surely mobile devices such as containers, waste bins) and/or materials for cleaning and disinfection in a certain zone
- avoid cross-contaminations between areas in different hygiene regime: think on the organisation of connections between the hygiene zones for operators, materials, food products, (mobile) equipment and devices, air and water flow ➡ flow from 'safe areas' and 'high hygiene' regime areas to 'low hygiene' regime areas and NOT the other way around.

Food contact materials and hygienic design of equipment, devices and infrastructure in general

Food contact materials and those equipment and infrastructures not in direct contact with foods should be constructed with appropriate materials (such as stainless steel or food-approved plastic materials) which are sustainable in use, are not made from porous or absorbent materials and are not sensitive to corrosion to avoid creation of niches. In those niches (such as small incisions or cracks), *L. monocytogenes* may accumulate, making the affected part a harbor site for the pathogen. During infrastructure and plant design, care must be taken for hygienic design: e.g. smooth surfaces, avoid sharp junctures, no dead ends in piping, no cross-connections between conveyance of food products, sufficiently elevated equipment and devices above the floors to facilitate sanitation and avoid floor splash, easy-to-clean equipment (after dismantling). Cabling and tubing systems are sensitive to dust accumulation and, in combination with high humidity conditions, niches for environmental pathogens may be created on/around them. It should be ensured that catwalk framework and stairs with open grating are not positioned over exposed foods and/or water. Non-food contact surfaces need to be included in periodic cleaning and disinfection activities (see section 2.1) and as much as possible, horizontal constructions need to be avoided.

Air flow/ventilation systems

Air flow between high hygiene regime zones and low hygiene regime zones is advisable to be controlled: air should flow from clean to dirty areas and so, a positive air stream from high hygiene to low hygiene regime is recommended. The ventilation systems, including evaporators in freezing tunnels, must be maintained and cleaned according to their needs. It has to be evaluated if filters are needed to purify air. The **source of air** applied as inlet may be a potential contamination source, therefore, FBOs have to check where the air is coming from (e.g. avoid intake from technical areas, dirty areas as waste disposal areas). In case **compressed** air is applied (e.g. for optical sorting), filters are necessary to avoid oil droplets from pumping systems, and circulation of micro-organisms. The filters need to be included in the periodic maintenance program (see 2.6) to avoid niche formation with *L. monocytogenes*.

Mobile equipment

Some parts of the equipment is designed to be mobile and can by switched in/out of processing lines in function of type of produce (e.g. leafy versus tuber), filth grade of raw materials (e.g. presence of soil, sand), need for additional sorting or insect removal, cutting devices (e.g. sticks versus slices) etc. In case parts of the equipment are switched in or are removed to another zone of the factory, its status of cleanness and potential for cross- contamination has to be evaluated (e.g. crossing low and high hygiene areas, circulation of persons, materials) and a pre-operational check-up is needed. Smaller (monitoring) devices (e.g. thermometers, ATP meters) moving around in a facility may provoke a cross-contamination and need to be handled in a dedicated manner e.g. not from low hygiene regime towards high hygiene regime or, as recommendation for good practice, be dedicated to a particular zone/area in the factory.

2.6 Technical maintenance

Preventive technical maintenance as a planned revision and check-up of equipment and infrastructure is of utmost importance in the prevention and control of *L. monocytogenes*. FBOs need to roll out a preventive maintenance plan including:

- Detailed description of type of technical maintenance
- Planned in function of the production activities (do not organize any technical maintenance during production activities to avoid product contamination)
- Need for pre-operational check-up in case of machines and equipment which are not frequently used (i.e. in case of seasonal production)
- All machines and equipment, including also larger installations (water tubing and pumping systems, freezing tunnels, etc.) in direct or indirect contact with food products, need to be included in the maintenance plan
- Replacement of air and water filters and biofilm control of those filters
- Water management equipment and effluent removal systems
- Organisation of start-up cleaning after technical interventions
- Dedicated uniforms and footwear for internal and external technicians to different zones in the plant
- Dedicated maintenance equipment and trolleys or mobile equipment with utensils for technicians restricted to different zones and hygiene regimes in the production plant

Periodical hygiene inspections (e.g. 3-4 times a year) need to be organized in order to identify additional contamination spots such as cracks, incisions, corrosion where a technical intervention is necessary.

2.7 Waste control

Food waste has different gradations, and as long as food streams are part of the food/feed chain, the appropriate hygiene and safety regime and restrictions need to be followed. Throughout the whole production and storage activities, cross-contaminations between 'foods' and 'waste' need to be avoided. It has to be determined by the FBO what to do in case food products are on the floor (e.g. overloaded conveyor belts and products falling on the ground), to prevent *L. monocytogenes*, housing in drains or on the floor, to cross-contaminate the food. It is strongly recommended that the latter is applied for feed production and are not used anymore as 'food', unless at the very start of the production process, where products from the field are entering the production facilities (in low hygiene regime area).

Waste bins, waste containers and rolling collection systems have to be in good condition (see section 2.5 and 2.6) and part of the cleaning and disinfection plan (see section 2.1). They are included in the requirements for the operators in the frame of working methodology to avoid that waste bins cross different areas and by doing so spread *L. monocytogenes* in the production environment (see section 2.9). Containers need to be dedicated by function (e.g. accepted product, rework, feed removal, waste) and clearly distinguished from each other (e.g. colour coding, labeling, tags).

2.8 Raw material control and supplier selection

Minimizing the probability that raw materials (such as vegetables from the field), semi-finished products (such as precleaned, prewashed vegetables) and ingredients (e.g. precooked rice, fish or meat products, spices etc.) are contaminated upon delivery is a preventive measure to decrease the presence of *Listeria monocytogenes* in the production of quick-frozen vegetables.

Several types of contamination can take place depending on the nature of the incoming products:

- **Raw materials from the field such as raw vegetables,** may contain *L. monocytogenes* upon arrival to the factory ➡ presence of soil and containers used to transport may be risk factors for contamination. In case products are cooled on the field or farm, moisture related contamination can potentially occur (e.g. application of cooling water, spraying droplets of cold water to reduce the temperature of the products)
- **Semi-finished products (e.g. pre-cleaned raw materials which are washed, peeled, shredded such as carrots and onions)** ➡ these products are coming from other processing facilities and may be contaminated during processing or cross-contaminated from the containers in which they are transported. Temperature abuse may stimulate growth of *L. monocytogenes*.
- **Ingredients** (e.g. quick-frozen vegetables, fish, meat, rice, dried products etc.) ➡ may be contaminated by the supplier and are introduced in the production process of the FBO
- **Packaging materials** (e.g. primary materials, material used during storage (such as big bags, containers for bulk materials) ➡ less sensitive to contamination with *Listeria monocytogenes*, but need to be clean, no dust and need to be protected from cross-contamination upon their arrival.
- **Technical aids** (e.g. water disinfection agents, anti-foaming agents applied in washing tanks, etc.) or additives ➡ less sensitive to contamination with *L. monocytogenes* but need to be stored/distributed in clean tanks/recipients to avoid cross-contamination towards the factory environment.
- **Water** ➡ see section 2.2

Selection of suppliers and communication to suppliers on the presence of *L. monocytogenes* related to the specific raw material is an important step to avoid potential contamination. However, due to the nature of different raw materials, it will be impossible to have '*Listeria*-free' raw materials, as most raw materials applied in this sector do not receive a listericidal control measure during their production or manufacturing process (such as pasteurization, sterilization). Therefore, it is necessary to have a thorough supplier selection including following control measures:

- develop procedures for selecting and approving suppliers,
- establish (long-term) relationships with the suppliers,
- conduct regular on-site audits to ensure the suppliers have a robust FSMS, implement good practices and general hygiene rules to avoid contamination with *L. monocytogenes*.
- consider EU and non-EU suppliers (in non-EU countries other legislation may be enforced)

In case of raw vegetables coming from the field, environmental contamination with *Listeria* spp. or eventually *L. monocytogenes* can be expected. However, these (primary production) suppliers must control potential additional contamination by avoiding the use of unclean containers/boxes/recipients, unclean harvesting materials and equipment, contaminated water sources, avoid biofilm formation in refrigerated storage and humidifying areas, when appropriate. All these measures have to be part of their good agricultural practices and be focused on minimizing microbiological contamination at primary production. Farmers are recommended to work according to the document 'Commission notice on guidance document on addressing microbiological risks in fresh vegetables at primary production through good hygiene' (Commission Notice, C163/2017) in which a range of good agricultural and good hygienic practices are presented to avoid or minimise microbiological contamination at farm level and during first post-harvest activities.

Testing a single batch of raw materials for the presence of *L. monocytogenes* (=batch sampling) is of limited value in establishing the acceptability of that batch and cannot substitute further PRPs and HACCP to control *Listeria monocytogenes* in the production process of the FBO (see further in section 5.1). The primary value of raw material testing is part of a history built up, and allows the follow up of suppliers as part of the supplier evaluation/verification. Therefore, raw material testing and batch control is NOT an appropriate control measure for *L. monocytogenes*.

2.9 Working methodology

Finally, the working methodology, the organization of the production process and management system implemented in the plant will be of utmost importance in the daily prevention and control of spread of *Listeria monocytogenes* and potential biofilm/niche formation in the production environment. The following aspects are of particular concern regarding the prevention and control of *L. monocytogenes*:

Neatness

A factory and its surroundings need to be neat and clean. Products piling up along the process line (e.g. conveyor belts, freezing tunnel) can be removed immediately, and they do not need to accumulate till the periodic cleaning and disinfection. It is important to have the principle of "visual cleaning as you go", by removing material frequently from conveyors, processing equipment, floors etc. as this will lower the overall burden across the site.

Commitment and awareness of management and personnel

The management of the plant needs to identify **key positions for personnel** in the different areas/zones to daily implement and control the needed pre-requisite, oPRP and CCPs (see section 3, based upon the HACCP plan). All personnel (including technical persons, temporary personnel) needs to **be aware and trained** in the control of *Listeria monocytogenes* (as stated in 2.4). The management has to **allocate resources** (i.e. money, time, personnel, expertise) for environmental sampling, investments in infrastructure and maintenance, water treatment etc. needed to be able to prevent and control *Listeria monocytogenes*.

Organisation of the production process of quick-frozen vegetables

The production of quick-frozen vegetables is heavily dependent on the seasonal availability of the raw produce. Peaks in production coincide with harvesting season of the processed commodity. The plant needs to be organized for this in terms of:

- availability of devices and equipment (to have all needed equipment and processing lines ready and installed),
- allocated time for breakpoints in the production for cleaning and disinfection activities (see section 2.1)
- availability of personnel
- availability of water
- etc.

Several processing lines and products can be in operation at the same time, which can contribute to a higher potential of cross-contamination between lines, personnel and products. It is recommended to have the production well organized so that movement of personnel and devices between different areas and lines is minimized. Intermediate breaks in full-time running production lines have to be introduced to allow intermediate cleaning and sanitation activities, freezing tunnel emptying by air to remove pilling up of products, to remove remaining products etc.

The production process is mostly a continuous process, starting from raw materials until bulk quick-frozen products. **The principle of forward movement** of products is normally not a problem. However, the movement of rolling devices, personnel, movable equipment needs to be controlled and especially when shifts are made from low hygiene regime towards high hygiene regime areas.

All steps in the production process need to have their instructions for the personnel regarding do's and don'ts in terms of production activities, hygiene rules, food safety steps, quality controls to be made, etc. Therefore, an appropriate **documentation system** with easy-to-understand and easy-to-access instructions and procedures has to be in place.

3
HACCP (=Hazard Analysis Critical Control Plan)

PROFEL

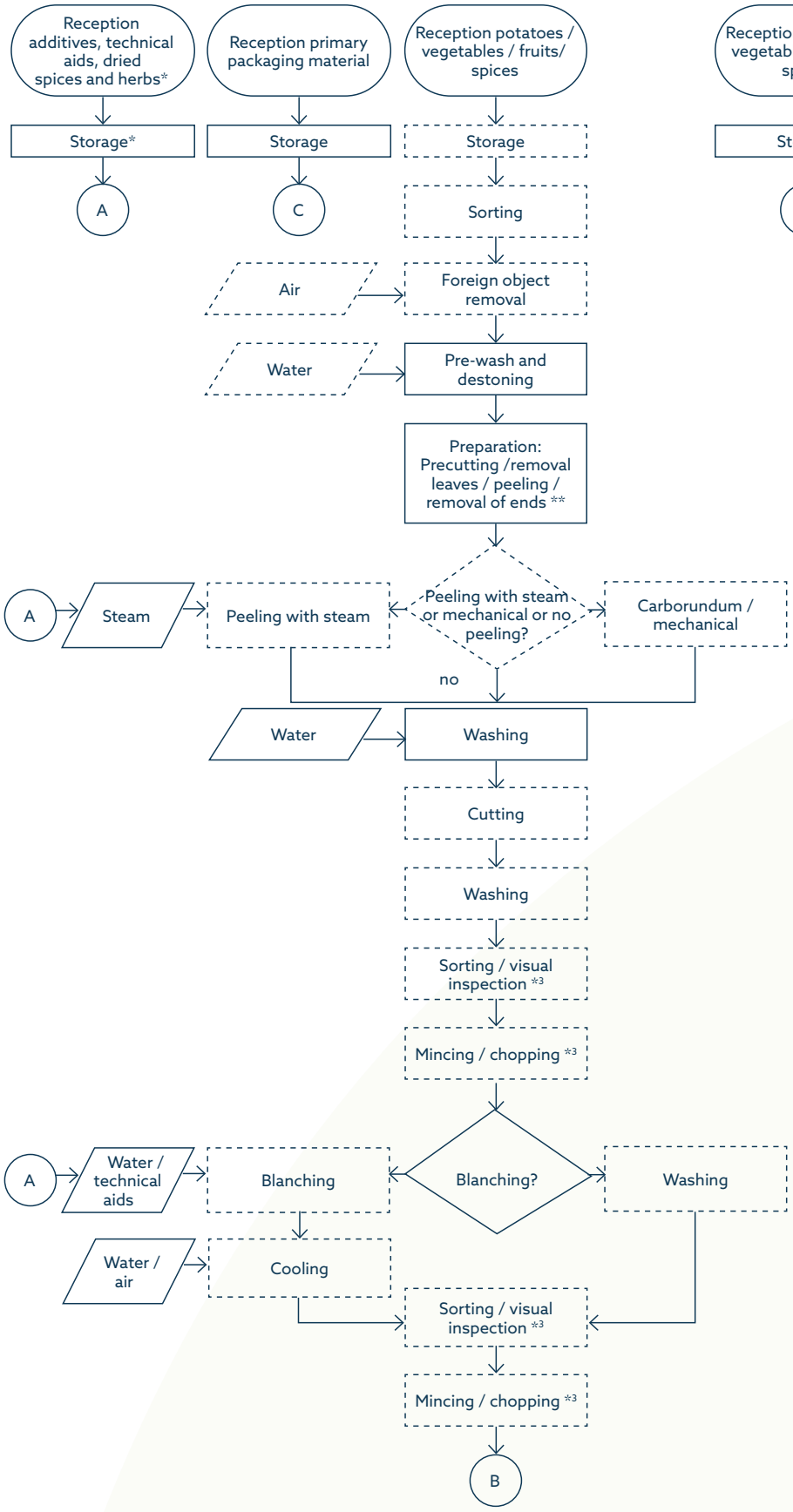
Not only the PRPs, but also the HACCP plan in quick-frozen vegetables production and storage plants need to address *L. monocytogenes* to identify where in the process potential occurrence, accumulation, growth or reduction of the hazard is feasible. For the HACCP plan, the structure and methodology of EU Commission Notice on Food Safety Management (C278/2016) is followed. A flow chart describing the different steps in the process is given in Figure 2, 3 and 4.

Remark 1 : This HACCP plan can be used as a starting point for a company's own HACCP plan, or for revision of the current plan. It is important to make the tailoring towards a company-specific plan by adaptation of production steps, specific equipment, information on validation and measurements of production lines, etc.

Remark 2 : Focus is made on hazard identification, preventive measures, hazard evaluation (PxE=R) and also defining potential CCP, oPRP or PRPs and on the hazard *Listeria monocytogenes*. All the other parts of the HACCP-plan (i.e. validation, verification, documentation) are not further elaborated in this guide. Also, other hazards (i.e. other microbiological, chemical and physical hazards) are not included and need to be further analysed by the FBO. Therefore, EU Commission Notice on Food Safety Management (C278/2016) can be followed.

Remark 3 : These guidelines cover frozen vegetables, which are considered as Non Ready-To-Eat (nRTE). Food Business Operators (FBOs) intending to market frozen vegetables as Ready-To-Eat (RTE) should follow additional preventive and control measures to assure the safety of RTE products, but these are not included in the presented HACCP-plan.

In Table 2, the hazard identification per process step is conducted, identified control measures are added, Probability (P) and Effect (E) on human health is estimated and a Risk (R) is attributed. Finally, depending on the attributed risk level, a PRP, oPRP or CCP is allocated. Table 3 represents examples of monitoring tables, inserting the monitoring and corrective actions to be taken.



Zone 1: Low hygiene regime area

Zone 2: High hygiene regime area

* not relevant with regard to survival/growth *L. monocytogenes*
** flexibel, depending on work methodology of company
*3 sorting/visual inspection and mincing chopping before or after blanching

Figure 2. Flow chart of production of quick-frozen vegetables – part 1

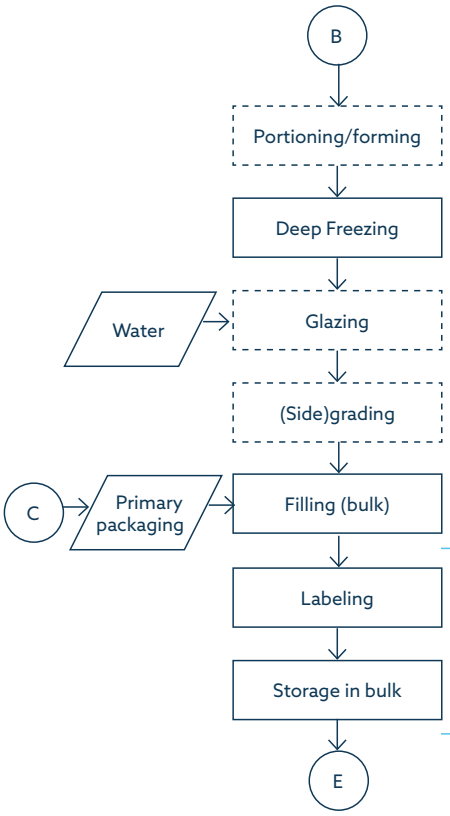


Figure 3. Flow chart of production of quick-frozen vegetables – part 2

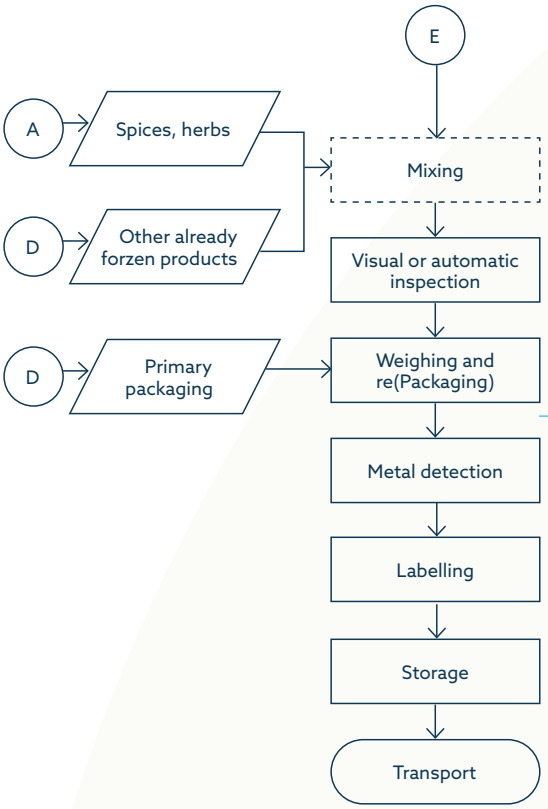


Table 2 : Hazard identification, preventive/control measures, Probability (P), Effect (E), Risk (R) and attribution of PRP, oPRP or CCP

Hazard identification	Preventive/control measure	P	E	R	Motivation	PRP/oPRP/CCP
Reception and storage of raw materials, semi-finished products, quick-frozen ingredients and water (zone 1: Low hygiene regime area) – fig. 2						
L. monocytogenes present in raw materials coming from primary production (field) (vegetables)	Supplier selection/purchase policy : • Good agricultural practices • Cleaning and disinfection of equipment/recipients • Biofilm control in case of refrigerated storage/ humidification of products	1	3	3		PRP raw material (section 2.8)
L. monocytogenes present in semi-finished products (pre-cleaned vegetables) and ingredients (quick-frozen products)	Supplier selection/purchase policy: • Good hygienic practices and HACCP • Elaborated *Listeria monocytogenes* control plan in place with supplier • Clean recipients upon arrival • Proper refrigerated temperature upon arrival	2	3	4		PRP raw material (section 2.8) PRP work methodology (section 2.9) : incoming check upon arrival
Contaminated water with *L. monocytogenes*	Selection of proper water sources, regular checks on water quality	1	3	3		PRP water (section 2.2)
Contamination from reception and storage areas with *L. monocytogenes*	• Temperature control in case of refrigerated or quick- frozen storage • Time control and FIFO principles for (refrigerated) products • Cleaning and disinfection of storage areas/ equipment Technical maintenance of storage areas	2	3	4		PRP Temperature control (section 2.3) PRP work methodology (section 2.9) PRP cleaning and disinfection (section 2.1) PRP infrastructure (section 2.5)
Additives, technical aids, dried spices and herbs, packaging materials ➡ no relevant sources for *L. monocytogenes*						

Sorting, foreign object removal, prewash/destoning, preparation, washing steps, cutting, sorting/visual inspection, peeling, mincing and chopping (zone 1: Low hygiene regime area) – fig.2						
Contamination via production environment, equipment, utensils	• Cleaning and disinfection program • Technical maintenance including pre-operational checks in case of seasonal use ofdevices/equipment • Infrastructure	1	3	3	Biofilms can be formed in/on equipment; P=1: zone 1	PRP Cleaning and disinfection (section 2.1) PRP technical maintenance (section 2.6) PRP infrastructure (section 2.5)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	1	3	3	P=1, still in low hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Contaminated air used for foreign object removal and/or in washing tanks to create 'jacuzzi' washing systems	• Appropriate filters and cleaning of filters and evaporators • Check where air is coming from	1	3	3	P=1, still in low hygiene regime area	PRP infrastructure –air control (section 2.5)
Contaminated water and formation of biofilms in washing tank (for washing steps)	Water management: • Cleaning and disinfection of tubing system and washing tank (and other washing equipment as paddles, rotating drums) • Frequent refreshing of water and/or refilling of water tanks • Water recycling and/or water treatment when needed	1 2*	3 3	3 4	P=1, still in low hygiene regime area *P = 2, in case of non blanched product (e.g. leek, onions)	PRP cleaning and disinfection (section 2.1) PRP water management (section 2.2) oPRP 1: water contamination in washing tanks in case of unblanched products
Use of contaminated water for steam preparation in case of peeling with steam	Proper water treatment to avoid contamination	1	3	3	P=1, still in low hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP water management (section 2.2)

Blanching (zone 1-2) –fig.2 Remark : in the case of vegetables which are not subjected to blanching, this production step will be an additional washing step, as products follow the same processing lines.	Blanching is a thermal treatment and a technological step aiming at enzyme de-activation to stabilise quick-frozen vegetables during prolonged storage in freezing conditions. Some commodities are blanched while others are not; this will highly depend on the decisions in the FBO, requirements from customers etc. Blanching is mainly conducted by immersion of the products in hot water or steam. Temperatures can vary between 65 and 110°C and are maintained for a specified time (1-10 minutes, depending on the commodity, size of pieces of the vegetables, seasonal variability, etc.) ➡ time/temperature combinations depend on the time required for inactivation of polyphenol oxidase (POD) and peroxidase (PPO) enzymes. Some products cannot be blanched because of detrimental effects on the product quality (e.g. onions or leek). [a] : Blanching will have a reducing impact on the microbial flora (nowadays also referred to as 'microbiota') of the vegetables, although it does not aim at elimination of pathogens as *L. monocytogenes* or reduction to an acceptable number, according to the definitions of a CCP in HACCP. Therefore, blanching is NOT considered as a CCP in the elimination of *Listeria monocytogenes* and a full pasteurization (i.e. 6 log reduction of *L. monocytogenes*)					
Blanching time/ temperature too short/ low so that *Listeria monocytogenes* can proliferate in the water/ product	• Monitoring time and temperature of blanching step • Check destruction of enzymes via enzymatic tests	2	3	4	See [a]	oPRP 2: blanching process, time/ temperature
Contamination via production environment, equipment, utensils	• Cleaning and disinfection program • Technical maintenance • Infrastructure	2	3	4	P = 2, because of shift towards high hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP technical maintenance (section 2.6) PRP infrastructure (section 2.5)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	2	3	4	P = 2, because of shift towards high hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Use of contaminated water/steam – recycling of water	• Cleaning and disinfection of the tubing system • Decision on recycling water in blanching steps • Follow up of potential contamination in water and need for water disinfection	2	3	4	P = 2, because of shift towards high hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP water management (section 2.2)

Cooling (zone 2 : high hygiene regime) – fig.2						
Growth of *L. monocytogenes* when cooling is too slow (in case of survival after	• Monitoring cooling time /temperature	2	3	4	See [b]	oPRP 3: temperature follow up of cooling water
Blanching or post-contamination after blanching)	• Respect cooling capacity – volume of products which can pass by the cooling step • Investigate need for water disinfection to avoid accumulation of bacterial growth in the cooling water					
Contamination via production environment, equipment, utensils	• Cleaning and disinfection program • Technical maintenance • Infrastructure	2	3	4	P = 2, high hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP technical maintenance (section 2.6) PRP infrastructure (section 2.5)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	2	3	4	P = 2, high hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Cross-contamination via contaminated water	• Cleaning and disinfection of the water tubing system • Evaluation of need to add disinfectant as technological aid to maintain water quality • Evaluation of the volume of water added in the cooling tanks to refresh the cooling water	2	3	4	P=2, because zone 2	PRP cleaning and disinfection (section 2.1) PRP water management (section 2.2)
[b] Usually reduce the temperature of the product below 10°C in 1 min, with a maximum of 2 min. (EFSA, 2018b) Avoid stay in temperature range between 50°C and 10°C						

Sorting/visual inspection, mincing/chopping, portioning/forming – size grading after freezing (zone 2 : high hygiene regime) – fig.2/3						
Contamination via production environment, equipment, utensils and food products which are sorted out after optical/ visual sorting to be treated as waste	• Cleaning and disinfection program • Technical maintenance • Infrastructure • Correct collection of waste or removal of products whichare sorted out	2	3	4	P = 2, high hygiene regime area	PRP Cleaning and disinfection (section 2.1) PRP Technical maintenance (section 2.6) PRP infrastructure (section 2.5) PRP Waste (section 2.7)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	2	3	4	P = 2, high hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Cross-contamination from zone 1	Work methodology to avoid cross- contamination: • Separate utensils/equip-ment for different zones • Waste bins to collect food products which are sorted out	2	3	4	P=2, high hygiene regime area	PRP work methodology (section 2.9)
Deep freezing / glazing (zone 2 : high hygiene regime) – fig.3						
Freezing too slowly or temperature fluctuations in freezer, resulting in growth/contamination of *L. monocytogenes* – to temperatures < -18°C	• Validation and monitoring of freezing time and temperature • Freezing time temperature /cycles to be defined per product group (depending on the nature of the vegetables, their size, etc.)	2	3	4	See [c]	oPRP 4: freezing time/temperature
Contamination from inside freezing tunnel/freezer (e.g. biofilm formation, drip)	• Cleaning and disinfection of the freezing tunnel, conveyor belts, installations • Hygienic design (including air flow)	2	3	4	P=2, high hygiene regime	PRP Cleaning and disinfection (section 2.1) PRP infrastructure (section 2.5)
Contaminated air (e.g. air blast freezer)	• Check origin of air • Cleaning and disinfection, appropriate filters and cleaning of filters and evaporators	2	3	4	P=2, high hygiene regime	PRP Cleaning and disinfection (section 2.1) PRP infrastructure (section 2.5)
Contaminated water used for glazing.	• Cleaning and disinfection of the tubing system/ evaporator/nozzle • Use water of potable quality	2	3	4	P=2, high hygiene regime	PRP Cleaning and disinfection (section 2.1) PRP water management (section 2.2)

[c] As L. monocytogenes is not fully eliminated during blanching and potential cross-contamination may occur, it is of utmost importance that no growth occurs in the freezer

Filling in bulk (zone 2-1: high hygiene regime toward low regime when closed packages) – fig.2						
Contaminated packaging material	• Purchase policy • Clean and dry storage environment • In case of re-usable materials: proper sanitation	2	3	4	P=2, high hygiene regime due to direct contact of packaging material with quick-frozen product	PRP raw material control (section 2.8) PRP infrastructure (section 2.5) PRP cleaning and disinfection in case of re-usable materials (section 2.1)
Contamination via production environment, equipment, utensils ➡ use of bulk containers which are transported around and introduced in high hygiene regime	• Cleaning and disinfection program • Infrastructure • Work methodology for bulk containers (instruction of use)	2	3	4	P = 2, low hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP infrastructure (section 2.5) PRP work methodology (section 2.9)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	2	3	4	P = 2, high hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Potential growth of *L. monocytogenes* in case of temperature fluctuations of products, or in case of disruption of flow towards storage in freezer due to no temperature control follow-up in the part of the plant	• Work methodology: instruction on continuous transport from bulk recipients filled with quick-frozen vegetables towards the freezer; tight closing of the bulk containers • In case of work interruptions ➡ corrective actions to be taken towards products (T measurements, sampling of products for batch release)	2	3	4	P = 2, important to avoid growth and proliferation	PRP work methodology (section 2.9)

Labeling and storage in bulk (zone 3: safe area) –fig.2						
Incorrect shelf life date/ product coding	• Work methodology : shelf life will be important in the frame of identification and traceability	1	3	3	Temperatures are too low for growth of *L. monocytogenes* (quick-frozen)	PRP work methodology (section 2.9)
Damaging and contamination of product with *L. monocytogenes* during storage (e.g. drip)	• Keep storage room in good condition • Work methodology: No packages on the floor, no packages open • Regular cleaning and disinfection	1	3	3	Product is packaged and quick- frozen. (zone 3)	PRP infrastructure (section 2.5) PRP work methodology (section 2.9) PRP cleaning and disinfection (section 2.1)
Growth of *L. monocytogenes* in case of temperature abuse	• Monitoring of storage temperature and storage time • Work methodology : FIFO principle	2	3	4		PRP temperature control (section 2.4) PRP work methodology (section 2.9)

Mixing with spices/herbs/other quick-frozen products and (visual/automatic) inspection and reweighing/ repackaging (zone 2 : high hygiene regime) – fig.4						
Contamination during opening bulk packages	• Work methodology: to open in a hygienic way packages (bulk recipients with quick-frozen vegetables, or recipients from suppliers with ingredients) to avoid dust or outer layers of packaging materials from coming into contact with quick-frozen vegetables	2	3	4	P=2, high hygiene regime and packages are opened	PRP work methodology (section 2.9)
Contaminated packaging material	• Purchase policy • Clean and dry storage environment • In case of re-usable materials: proper sanitation	2	3	4	P=2, high hygiene regime due to direct contact of packaging material with quick-frozen product	PRP raw material control (section 2.8) PRP infrastructure (section 2.5) PRP cleaning and disinfection in case of re-usable materials (section 2.1)
Contamination via operators	• Training and awareness of personnel • Infrastructure: hygiene locks between different areas	2	3	4	P = 2, high hygiene regime area	PRP personnel (section 2.3) PRP infrastructure (section 2.5)
Contamination via production environment, equipment, utensils	• Cleaning and disinfection program • Technical maintenance • Infrastructure	2	3	4	P = 2, high hygiene regime area	PRP cleaning and disinfection (section 2.1) PRP technical maintenance (section 2.6) PRP infrastructure (section 2.5)
Time out of freezer too long, product temperature too high allowing growth of *L. monocytogenes*	• Monitor time and temperature of this area • Work methodology : only take restricted number of containers from freezing storage to avoid temperature increase of products • In case of production interruptions, take corrective actions towards product e.g. temperature measurements and decision what need to be done with products (e.g. batch sampling and positive release)	2	3	4	P = 2, high hygiene regime area	PRP temperature control (section 2.4) PRP work methodology (section 2.9)

Metal detection/ labelling/ storage/transport (zone 3 : safe area closed packages) – fig.4						
Incorrect shelf life date/ product coding	Work methodology: shelf life will be important in the frame of identification and traceability	1	3	3	Temperatures are too low for growth of *L. monocytogenes* (quick-frozen)	PRP work methodology (section 2.9)
Damaging and contamination of product with *L. monocytogenes* during storage (e.g. drip)	• Keep storage room and transport modes in good condition • Work methodology: No packages on the floor, no packages open • Regular sanitation	1	3	3	Product is packaged and quick- frozen. (zone 3)	PRP infrastructure (section 2.5) PRP work methodology (section 2.9) PRP cleaning and disinfection (section 2.1)
Growth of *L. monocytogenes* in case of temperature abuse	• Monitoring of storage temperature and storage time (also during transport) • Work methodology : FIFO principle	2	3	4		PRP temperature control (section 2.4) PRP work methodology (section 2.9)

Table 3. Monitoring and corrective actions for oPRPs identified in the HACCP plan (Table 2)
Remark : each FBO needs to validate if these oPRPs are fit-for-purpose and tailor them further towards company-specific mode of production

oPRP or CCP	Step in production process	Objective	Validation	Monitoring	Corrective Actions
oPRP 1	Contaminated water and formation of biofilms in washing tank (for washing steps) – in case of non blanched products	Avoid accumulation of *L. Monocytogenes* in washing tanks	- Evaluate and define how frequent and/or volume of water in the washing tanks needs to be refreshed - Evaluate and define the conditions for water recycling and if water treatment is necessary	- Follow up the defined frequency of refreshing of water and/or refilling of water tanks - Follow up the defined conditions for water recycling and/or water treatment (including water disinfection) when needed	- Refresh and refill water tanks - Revise the water recycling and/or water treatment conditions
oPRP 2	Blanching of vegetables	Blanching time/ temperature too short/low so that *Listeria Monocytogenes* can proliferate in the water/product	- Evaluate and define if temperature/time could allow growth or proliferation of *L. monocytogenes* during blanching process for the different products, cuts, seasons, etc.	Follow up blanching time/ temperature according to validated time/temperature for the different products, cuts, seasons, etc.	- If blanching time and temperature are not meeting the set criteria, an evaluation towards potential growth of *L. monocytogenes* need to be made - In case there is a potential growth, refreshment or treatment of the blanching water has to be done and product sampling needs to be conducted to evaluate potential product contamination

oPRP 3	Cooling after blanching	Growth of *L. Monocytogenes* when cooling is too slow (in case of survival after blanching or post-contamination after blanching)	- Evaluate and define if cooling temperature/time could allow growth or proliferation of *L. monocytogenes* during cooling after blanching - Avoid stay in temperature range between 50°C and 10°C by follow up of water temperature and/or the flow of the product - Evaluate and define if disinfection of the cooling water is needed	- Follow up the cooling time and temperature as set in the validation - Usually reduce the temperature of the product below 10°C in 1 min, with a maximum of 2 min. (EFSA, 2018b)	- If cooling time and temperature are not meeting the set criteria, an evaluation towards potentia growth of *L. monocytogenes* need to be made - In case there is a potential growth, refreshment or treatment of the cooling water has to be done and product sampling needs to be conducted to evaluate potential product contamination
oPRP 4	Freezing of vegetables in freezing tunnel	Freezing too slowly or temperature fluctuations in freezer, resulting in growth/ contamination of *L. monocytogenes* – to temperatures < -18°C	- Freezing time/temperature/ cycles to be evaluated and defined per product group (depending on the nature of the vegetables, their size, etc.)	- Follow up of time/temperature of freezing tunnel and product temperature - Follow up that no accumulation of products in freezing tunnel is occurring	- If the validated time/temperature of a particular product during freezing is not respected, it must be checked if no accumulation of product is occurring in the freezing tunnel. - Evaluate if product temperature is above -4°C (at such temperatures, microbiological activity and potential growth of *Listeria monocytogenes* can recur) - If this is the case, a cleaning/disinfection of the freezing tunnel must be organized

4 Environmental sampling for verification control of *Listeria monocytogenes* as environmental pathogen and verification of conducted preventive/control measures

PROFEL

As *Listeria monocytogenes* is an environmental pathogen and microbiological accumulation is not visually detectable, environmental sampling is necessary to screen the production environment for the potential harbourage of *L. monocytogenes*. The goal of environmental monitoring is threefold:

(1) to verify the effectiveness of your preventive and control measures (Pre-Requisites and HACCP-plan),
(2) to detect *L. monocytogenes* and harbourage sites if present in the plant, and
(3) to ensure that corrective actions have eliminated *L. monocytogenes* when detected in your plant.

Important references on environmental sampling are :

a) Principles on sampling procedure for environmental sampling are described in EN ISO 18593:2018
b) Detection analysis of environmental samples for *L. monocytogenes* are included in EN ISO standard method 11290 part 1
c) EURL *L. monocytogenes* has provided a document giving specific guidance on sampling processing areas and equipment for the detection of *Listeria monocytogenes* (EURL *L. monocytogenes*, 2012).
a) Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of quick-frozen vegetables aiming at detecting *L. monocytogenes* (EFSA, 2018b)
b) References to environmental screening protocols are also available in Lakshmikanta (2013) and CAC (2007).
c) An interesting modelling approach to determine the most appropriate sampling location and sampling time in a frozen production plant is presented by Zoellner et al. (2019)

4.1 *Listeria* spp. or *L. monocytogenes* ?

It should be noted that on some occasions, food manufacturers prefer environmental monitoring for non- pathogenic *Listeria* spp. as an indicator for *L. monocytogenes*. Targeting this broader group *Listeria* spp. as indicator organisms could lead to more robust verification of adequate sanitation of environmental conditions (and thus a good indicator of adequate process hygiene) and allow correction of situations that potentially lead to contamination with *L. monocytogenes* (CAC, 2007). But the use of *Listeria* spp. as marker/indicator organism for *L. monocytogenes* is debatable. *Listeria* spp. also includes other species that are non-pathogenic and which are also ubiquitous micro-organisms and occasionally encountered in foods or a food production environment. Thus, mere presence of *Listeria* spp. does not necessarily indicate the presence of the pathogen *L. monocytogenes*. According to EFSA (2018b), it is recommended to test directly for *L. monocytogenes* following the EN ISO11290 part 1 protocol (detection) and in case of a positive result, it is highly recommended that isolates which are confirmed to be *L. monocytogenes* are sent to an NRL or the EURL for further characterization (typing). Surely, in case of listeriosis outbreak investigations aiming to track the potential source of *Listeria monocytogenes*, testing for *L. monocytogenes* is needed (EFSA, 2018b).

4.2 Sampling locations

The environmental monitoring plan needs to include selected sampling locations based on the potential for the site to be contaminated with *Listeria monocytogenes*. It is up to the FBO to develop historical information on the testing results of this screening, so that critical areas in the production environment can be identified e.g. certain equipment (in direct or indirect contact), certain periods of the year, the production of certain commodities, etc. An FBO may generate a long list of sampling sites (including food contact and non-food contact surfaces) from which samples are taken randomly), however, it is recommended that over a certain period of time all these sampling locations are tested. It is recommended to make a differentiation in sampling locations according to their potential of *Listeria monocytogenes* cross-contamination towards food products and harbourage of the organism. An example of differentiation is made in Table 4. A long list of potential sampling locations is represented in EFSA (2018b). It is advised to have fixed sampling locations and rotating sampling locations, which change with each sampling time in a 70/30 proportion, meaning that 70% of the locations are fixed and 30% of the locations are alternated for each sampling round.

Table 4. Overview of types of food contact and non-food contact surfaces, potential sampling locations and proposed frequency of sampling (based upon Table 1)

Type	Description	Examples of locations	Proposed frequency of sampling
1	Food contact surfaces in direct contact	Tank interiors, packaging and conveyors, hoppers, pipe interiors	Every week
2	Non-food contact surfaces in close proximity to food contact surfaces	Equipment housing framework, floors or drains in direct surrounding of food contact surfaces	Every month
3	More remote non-food contact surfaces which could eventually lead to contamination	Forklifts, wheels of garbage bins/ devices, footbaths for personnel, walls, floors and drains not in direct contact with food contact surfaces.	Every 6 months
4	Non-food contact surfaces and remote areas from processing environment	Hallways outside the production area, areas where raw materials or finished products are stored. Equipment housing framework, walls, floor or drains NOT in direct surrounding of food contact surfaces	Every 6 months

4.3 Sampling frequency, sampling time, sampling area and sampling techniques

The **frequency of sampling** needs to be more intensive in areas where a high hygiene regime is required (see section infrastructure 2.5) and for those sampling locations belonging to type 1 > 2 > 3 and 4. In case of outbreak investigation, a more concise sampling plan as presented by EFSA needs to be followed (EFSA, 2018b).

Apart from the sampling locations and frequencies of sampling, the appropriate **sampling time** at which the environmental samples will be collected can also be specified. The most important time to collect these samples is several hours into production (e.g. 3 to 4 hours) or preferably just before cleaning, because it allows *L. monocytogenes* (if detected) to work its way out of harbourage sites and contaminate the production environment. Rotation needs to be included in sampling day and time to get a full scatter plot of potential contaminations. If samples are taken too close in time after disinfection, the disinfection agent may not be adequately neutralised and could interfere with the analytical test. More information is available in EFSA (2018b). As mentioned in section 3.1, the objective of this environmental sampling is not to verify if the performed cleaning and disinfection activities are effective, but to get a complete verification of performed preventive and corrective measures in the control of *L. monocytogenes*.

Various sampling techniques and sampling areas are generally described in EN ISO 18593:2018 and specified in the EURL Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes* (EURL for *Listeria monocytogenes*, 2012). In summary:

- For the sampling **of hard-to-reach, small/narrow areas and cracks,** stick swabs are used to sample; typically ≤ 100 cm2is sampled (e.g. narrow cracks sampled over multiple meters)
- For the sampling **of large surfaces,** sterile cloths or sponges are applied; typically > 100 cm^2 - total sampled area as large as possible to increase the probability to detect *Listeria monocytogenes*. Recommended to sample between 1000 and 3000 cm^2

4.4 Data processing and trend analysis/observation

Based on the outcomes of the analysis, a database and historical knowledge can be built up. The following information can be retrieved to gain insight into potential contamination routes as part of the trend observation: sensitivity of sampling location, commodity involved, time of the year (seasonal variability), personnel involved and other issues which may impact the contamination e.g. technical maintenance, change in staff, change in equipment, seasonal use of equipment etc. This trend observation can help in the learning path of the FBO to understand when their factory environment can be more sensitive towards contamination with *L. monocytogenes*. A plant map with identification of hot spots sensitive to contamination may facilitate the communication. Environmental monitoring programs shall be adapted to capture new insights following the review of trends and data.

4.5 Corrective actions

In case a monitoring test is positive for *L. monocytogenes*, a more dedicated screening on the positive sampling locations and its broader surrounding is necessary, and additional corrective actions need to be taken. The following actions need to be taken for root-cause analysis and to avoid future problems:

a) Upon detection of *L. monocytogenes* in environmental testing, it is highly recommended to keep isolates in case further (internal) investigation is required by the FBO. Further investigation can be strain characterization e.g. genotyping to enable microbial source tracking. For example, in case of recurring positive *L. monocytogenes* test results, genotyping of the collected isolates can provide information on whether the recurring *L. monocytogenes* is linked to one particular persistent 'in-house' strain of *Listeria monocytogenes* or not. This recommendation is also important in case of a positive detection of the product sample (see section 5.1).

b) An intensive cleaning and disinfection of the sampling area is needed, followed by a more intensive follow-up monitoring until the contamination is solved.

c) A link to the batches of quick-frozen products produced in the same time frame of the positive environmental contamination needs to be made to evaluate potential contamination of the processed foods:

   c1) A well-documented risk evaluation and trend observation/analysis needs to be made towards the batches that were processed during the contamination event, taking into account any other historical data of *L. monocytogenes* contamination on product batches or environmental testing that had been noted before the latest *L. monocytogenes* incidence present in the company. This risk evaluation can include e.g. identification of potential contamination routes from the production environment towards the food, type of raw material or ingredients used and their supplier information, any unusual activities in the company (e.g. change of staff, on-going construction, change in cleaning & disinfection procedures, use of seasonal equipment, different process parameters etc.) and needs to be backed by historical test data of products and environment.

   c2) In the absence of end product testing results (historical data), and where a risk evaluation indicates increased likelihood of contamination of batches frozen during the timeframe of detected environmental contamination, sampling of the involved batches is recommended to confirm whether the produced batch(es) of end products have been found contaminated and should be deemed unacceptable (at least n = 5 sample units per batch analysed for detection of *L. monocytogenes* in 25g is recommended).

In summary, available historical data (see c1) along with data from temporarily intensified end product sampling (if needed, see c2) on the batches of quick-frozen products produced in the same time frame of the environmental monitoring tests having been found positive for *L. monocytogenes*, need to demonstrate that the end product complies to the set intermediate limit for the product (preferably *L. monocytogenes* not detected in 25g and at all times < 10 CFU/g or any other intermediate limit set, see section 5.1). Therefore, a well- documented risk evaluation is necessary, and a detailed trend observation/analysis that enables linking of environmental sampling results with product samples needs to be established to build up historical data.

d) Evaluation of possible biofilm formation, identify the source of the contamination and consider specific biofilm removal actions.
e) The monitoring program needs to be adapted (i.e. other sampling sites, change of frequency) for better follow up in future.
f) Organise a clear communication to the involved and responsible persons for cleaning and disinfection, maintenance and operational activities of the found contamination.

4.6 Procedure of environmental screening for *L. monocytogenes*

A procedure for environmental screening needs to be established by the FBO and should include the following:

1) identification of sampling locations
2) determination of sampling surface area (cm^2 to be swabbed)
3) definition of frequency of sampling (taking different hygiene regimes into consideration and type of sampling locations, see Table 4), and sampling time
4) protocol used for the detection of *Listeria* spp. or *L. monocytogenes* in environmental samples in a quality control lab (see EFSA, 2018b)
5) method of sampling (swab, or others) and transport of samples to the lab
6) trend analysis of obtained results to be able to identify potential additional corrective or preventive measures which need to be taken as corrective action
7) to foresee an annual review of the procedure of environmental screening for updates according to new evolutions in the production areas (e.g. new equipment, other zoning, etc.), new elements in production methods, etc. to keep the procedure up-to-date
8) to indicate a responsible person to work out this procedure, to follow up and to take action in case of any potential contamination
9) to define a communication channel in the organisation in case a positive result is detected and corrective actions need to be taken.

5
End product specifications and risk communication towards users of quick-frozen vegetables

PROFEL

It is clear that although PRPs, HACCP and a well implemented FSMS may be in place, it cannot be excluded that some quick-frozen products on some occasions can be contaminated with low levels of *L. monocytogenes* (detected per 25 g but usually <10 CFU/g). The presence of *L. monocytogenes* can occur as no full thermal inactivation step is included in the production process (blanching is designed as a technological heat treatment and not necessarily validated to provide a 6 log reduction of *L. monocytogenes* – see HACCP-plan section 3). Moreover, the quick-freezing process is situated post-blanching and is an open process and thus, even upon adherence to a stringent PRP, contamination with *L. monocytogenes* cannot be completely avoided in the typical production processes and infrastructure applied in this quick-frozen vegetable industry (see HACCP-plan section 3). These guidelines cover frozen vegetables, which are considered as Non Ready-To-Eat (nRTE).

It is therefore important that a **clear communication strategy** is used to inform users about the quick-frozen vegetables being either B2B (as food industry, institutional catering or horeca-activities) or B2C (quick-frozen vegetables further distributed to consumers via retail activities). This should be done not only via labelling or technical end product specifications, but also via other communication channels such as websites, recipes, information brochures, social media etc. The communication needs to be consistent to avoid misunderstanding on how to store, defrost, and prepare or use these frozen vegetables in an appropriate manner.

In this section, principles of sampling plan for end products testing, end product specifications and risk communication strategies are further proposed, based upon the conducted challenge testing (Annex III) and EFSA opinion (EFSA, 2020).

5.1 Testing towards an intermediate limit set for *L. monocytogenes* to verify the food safety management system (FSMS)

Different sampling strategies of **end products (B2B or B2C)** can be identified (i.e. batch sampling for batch release, monitoring aiming at detection of a prevalence rate of pathogens in foods based on statistical approaches).

However, sampling is a tool in the **verification of the FSMS**, to gain information on the safety of the produced foods with the current production process and food safety management system implemented. End product testing reflects the effective integration of all preventive and control steps in the formulation and manufacturing of the food being placed on the market (Zwietering et al., 2016). The **year-round sampling** in the frame of verification will allow the FBO to gain insight in variability of the contamination and will allow trend observation/trend analysis (e.g. which period of the year or for which type of quick-frozen vegetables do we find more problems, and what could be a potential reason for this?). The actual sample size (or number of samples) for end product testing in an FSMS verification is often determined from the point of view of what is economically feasible and/or requirements from customers. These **random convenience sampling plans** are also known as pragmatic or empirical sampling plans (CAC, 2004). The number and type of samples are mostly selected intuitively, based on the experience and knowledge of the quality or operations manager at the production site about the sampling locations and sampling times (Uyttendaele et al., 2018).

In the production and trading of quick-frozen vegetables, a **year-round end product sampling plan in the frame of verification of the FSMS** needs to be designed by the FBOs active in the production of quick-frozen vegetables to verify the preventive and control measures implemented in the control of *L. monocytogenes* (Figure 1). In the sampling plan, the number of end product samples taken on a yearly basis for different quick-frozen vegetables as end products (B2B or B2C) and the sampling frequency (or interval in between sampling), need to be established, considering:

- different categories of quick-frozen products (i.e. type of vegetable, single or mixed products);
- type of production process (blanched/unblanched);
- production volume;
- potential sensitivity for presence of *L. monocytogenes*;
- seasonality of production;
- potential for supporting growth or no growth (see 4.1)
- etc.

For a verification year-round sampling plan, samples are analysed for detection/non-detection of *L. monocytogenes* in 25 g. If *L. monocytogenes* is detected in 25 g, further enumeration needs to be conducted, on the same samples, to verify if intermediate set limits are reached (< 10 CFU/g) or not. However, it is likely that due to the heterogeneous distribution of a *Listeria monocytogenes* contamination in a batch, some products are contaminated and others not. Therefore, it is possible that re-analysing the same sample, other results are obtained. In the year-round sampling plan, it is therefore recommended to perform direct enumeration of *Listeria monocytogenes* from time to time (e.g. every x samples direct enumeration of *L. monocytogenes* to demonstrate that the intermediate limit of < 10 CFU/g is not exceeded). Analytical protocols are EN ISO 11290 part 1 (detection of *L. monocytogenes* in foods) and part 2 (enumeration of *L. monocytogenes* in foods) or equivalent rapid methods (validated according to ISO 16140). In case of positive detection, it can be helpful to further characterize isolates (typing) by a recognized National Reference Laboratory (NRL) or by the European Reference Laboratory for *L. monocytogenes* (EURL *L. monocytogenes*) (EFSA, 2018b). For example, in case of recurring positive *L. monocytogenes* test results, genotyping of the collected isolates can provide information on whether the recurring *L. monocytogenes* is linked to one particular persistent 'in-house' strain of *Listeria monocytogenes* or not (see also corrective actions for environmental monitoring, part 4.5). Further, based on the end product sampling, **trend analysis/observation** can be elaborated to gain insight into the potential contaminations of products and sources of the contamination to the above indicated factors.

5.2 End product specification and risk communication

The presented guidelines cover frozen vegetables, which are considered as Non Ready-To-Eat (nRTE). However, PROFEL does realize that some consumers (professional or not) may not read the label and takes into account 'reasonably foreseen abuse' i.e. that some of these frozen vegetables are used as ready-to-eat and not cooked before consumption. Moreover, the sector does take into account 'reasonably foreseen abuse' about some consumers not adequately defrosting the food or not heating thoroughly (less than 2 minutes at 70°C).

The sector therefore aims to prevent by best practices as stipulated in the guideline contamination of frozen foods with *L. monocytogenes* (i.e. target value is not detected in 25g) and based upon challenge testing (see Annex III) including reasonably foreseen abuse in defrosting under refrigeration (challenge tests performed in refrigerator at 9±1°C i.e. higher than recommended temperature on the label – and using a fast growing *L. monocytogenes* isolate retrieved from the 2018 frozen sweet corn outbreak event) has set an intermediate limit at < 10 CFU/g.

It should be noted that the potential for false-positive results is low with both the *L. monocytogenes* method for detection (ISO 11290-1) or the *L. monocytogenes* method for enumeration (ISO 11290-2) or equivalent ISO16140 validated rapid detection methods but that indeed, a non-homogenous bacterial distribution might well account for discordance between results if performing enumeration and detection on a different subsample of the batch, particularly for low counts. Further, it should be noted that sampling and testing have restrictions for ensuring food safety of a batch food (more information : ICMSF-website, http://www.icmsf.org/).

On the basis of challenge testing (refer to Annex III), EFSA (2020) opinion and expert discussion in the frame of elaborating these hygiene guidelines, the following **end product specifications combined with product label and risk communication are proposed for quick-frozen vegetables (as non-ready-to-eat products) :**

	Target value – after production	**Intermediate limit – after production**	**Throughout their shelf-life during both frozen storage and defrosting/ refrigerator storage[1]**
L. monocytogenes	Not detected in 25 g (a)	< 10 CFU/g (b)	< 100 CFU/g (c)

[1] Note frozen vegetables are assumed to be a non-RTE food.

a) the target if the proposed sector-wide hygiene guidelines for the production of quick-frozen vegetables in control of *L. monocytogenes* are respected
b) but although PRPs, HACCP and a well implemented FSMS is in place, it cannot be excluded, that occasionally quick-frozen vegetables are contaminated with low levels of *L. monocytogenes*, therefore the intermediate limit can be set at < 10 CFU/g.
c) the *L. monocytogenes* food safety objective to ensure safe food to consumers (for the non-susceptible population group: for definition refer to section 5.2.2)

5.2.1 Risk communication by product label

Taking into account the outcome of the *L. monocytogenes* challenge testing to assess the pathogen's behaviour during defrosting/refrigerated storage of frozen vegetables under reasonably foreseen conditions at consumer's home (refer to Annex III), further risk communication and information to users is recommended on the product label, technical specifications, website information, social media etc. Based upon the outcomes and different growth potential of *L. monocytogenes* from the performed challenge testing (Annex III) and the growth modelling performed by EFSA (EFSA, 2020), it is recommended to use different risk communication for frozen sweet corn and frozen sweet potatoes than for other frozen vegetables.

1) For frozen sweet corn and sweet potatoes:
Given the established *L. monocytogenes* growth potential of more than 1 log10 within the 24h defrosting/refrigerated storage time, frozen sweet corn and sweet potatoes should be regarded as non-ready- to-eat frozen foods.

Thus, consistent and sector-wide communication to the consumer via the retail pack label is recommended. The label of the packaged end products in case of B2B or B2C packages should clearly mention:

(1) Conditions for appropriate frozen storage (time/temperature) at -18°C and -12°C
(2) Advice on the use of the products:
*a. Need of cooking (product nRTE) and cooking instructions (e.g. mode, time and temperature)**
b. 'Cook from frozen' (no prior defrosting and refrigerated storage recommended/no consumption without thorough heating i.e. at least 2 minutes above 70°C)

*Furthermore, consumption of frozen vegetables as RTE by the end-users can be discouraged by making reference to instructions for preparation (various suggestions of heat treatment) on the label.

2) For other frozen vegetables:

Given the established *L. monocytogenes* growth potential is less than 1 log10 within the 24h defrosting/refrigerated storage time, the other frozen vegetables subjected to challenge testing (peas, parsnips, white cabbage) and those other frozen vegetables that were taken up in the frozen vegetables categorization and scored as being less at risk than the five selected frozen vegetables that were part of the *L. monocytogenes* challenge testing (see Annex III) are not to be defrosted or subjected to refrigerated storage for more than 24h. They are also used as non-ready-to-eat.

The following consistent and sector-wide communication to the consumer via the retail pack label is recommended. The label of the packaged end products in case of B2B or B2C packages should clearly mention:

(2) Conditions for appropriate frozen storage (time/temperature) at -18°C and -12°C
(3) Advise on the use of the products:
*a. Need of cooking (product nRTE) and cooking instructions (e.g. mode, time and temperature)**
b. Thawing instructions (if required)
*c. Defrosting and refrigerated storage to be restricted for up to maximum 24h at 5-7°C***

* Furthermore consumption of frozen vegetables as RTE by the end-users can be discouraged by making reference to instructions for preparation (various suggestions of heat treatment) on the label.
** Refrigerated temperature between 5-7°C, or upon specification of national competent authority, as national legislation on product temperature may differ amongst EC member states.

5.2.2 Risk communication to susceptible groups

In case frozen vegetables are intended for catering or servings directed towards susceptible consumers, these frozen vegetables need to be considered as non-RTE and thus adequate heat treatment is mandatory during preparation and must be clearly communicated to the caterer or susceptible group for listeriosis, in particular pregnant women, elderly over 74 years old and immunocompromised patients i.e. those with recognized underlying diseases such as liver disease, cancer, and diabetes or organ transplant. These groups of persons whose underlying conditions were associated with the highest incidence of listeriosis represent ca. 1% of the total population (in France) but accounted for 43% of cases and 55% of deaths (Goulet et al., 2012). Instead of addressing these persons directly, it might also be useful to clearly inform their medical staff, healthcare professionals, caregivers or those who provide dietary guidance to these persons about the need for appropriate defrosting conditions *('Defrosting and refrigerated storage to be restricted for up to maximum 24h at 5-7°C')* and for all quick-frozen vegetables to stress the *'need to cook thoroughly and achieve at least 2 minutes above 70°C'* prior to consumption.

The communication to these susceptible consumer groups is an initiative also to be taken by public health agencies, food safety authorities or non-governmental organisations active in this domain of the health care sector. However, it is also a shared responsibility with other stakeholders in the food chain: in particular, when quick-frozen vegetables are sold in B2B setting to institutional catering serving hospitals or residential care facilities this type of risk communication should be taken into account.

Still, quick-frozen vegetables remain the best (and only) alternative, if properly heat treated before consumption, for those persons with recognized underlying conditions or diseases that impair cell-mediated immunity, and for pregnant women to eat vegetables (as part of a healthy diet) as consumption of fresh produce for these type of susceptible groups in need of a low microbial (neutropenic) diet is not recommended.

Some guidance to these persons/healthcare professionals is available via the following links :

- https://www.health.belgium.be/nl/advies-9311-listeriose and the annex in this (Dutch/French) downloadable document which contains several links to recommendations in various countries
- https://www.food.gov.uk/research/research-projects/development-of-an-initial-report-for-reducing- the-risk-of-vulnerable-groups-contracting-listeriosis or https://www.food.gov.uk/sites/default/files/media/document/listeria-guidance-june2016-rev.pdf

Annex I: legal references

Commission Notice C278/2016. Commission notice on the implementation of food safety management systems covering prerequisite programs (PRPs) and procedures based on the HACCP principles, including the facilitation/ flexibility of the implementation in certain food businesses (2016/C 278/01). Official Journal of the European Union: C 278/271-C 278/232.

Commission Notice C163/2017. Commission notice on guidance document on addressing microbiological risks in fresh fruits and vegetables at primary production through good hygiene (2017/C 163/01). Official Journal of the European Union: C 163/1

Directive (EC) 89/109. Council Directive 89/108/EEC of 21 December 1988 on the approximation of the laws of the Member States relating to quick-frozen foodstuffs for human consumption

Regulation (EC) No 178/2002 Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24

Regulation (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. 1–54

Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26 Regulation (EC) No 528/2012 of 22 May 2012 concerning the making available on the market and use of biocidal products.

Annex II: other references

AFFI (American frozen food institute). *Listeria* control Plan. www.affifoodsafety.org.

Buchanan, R. L., Gorris, L. G. M., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: an update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control, 75, 1–13.

CAC (1976). Code of Practice for the processing and handling of quick-frozen foods (CAC/RCP 8-1976).

CAC (2004). CAC/GL 50-2004 General guidelines on sampling.

CAC (2007). Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods. CAC/GL 61 - 2007

CAC (2015). Standard for quick-frozen vegetables CXS 320-2015

Devlieghere, F., Rajkovic, A., Samapundo, S., Uyttendaele, M., Vermeulen, A., Jacxsens, L. Debevere, J. (2013). Food microbiology and analysis. Laboratory of Food Microbiology and Food Preservation, Faculty of Bioscience Engineering, Ghent University.

ECDC (2016). https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2016-listeriosis.pdf

EFSA (2018a). Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. doi:10.2903/sp.efsa.2018.EN-1448

EFSA (2018b). Urgent scientific and technical assistance to provide recommendations for sampling and testing in the processing plants of frozen vegetables aiming at detecting *Listeria monocytogenes*. EFSA-2018-0141. EFSA Journal.

EFSA and ECDC (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food- borne outbreaks in 2017. EFSA Journal 2018;16(12):5500, 262 pp

EFSA (2020). The public health risk posed by *Listeria monocytogenes* in frozen fruit and vegetables including herbs, blanched during processing. EFSA Journal 2020;18(4):6092. doi: 10.2903/j.efsa.2020.6092

EN ISO 11290 part 1 (2017). Microbiology of the food chain – horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – part 1: detection method. International organization for standardization, Geneva.

EN ISO 11290 part 2 (2017). Microbiology of the food chain – horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – part 2 : enumeration method. International organization for standardization, Geneva.

EN ISO 18593 (2018). Microbiology of the food chain – Horizontal methods for surface sampling. International organization for standardization, Geneva.

EURL-*L. monocytogenes* (2012). Guidelines on sampling the food processing area and equipment for the detection of *L. monocytogenes*. Version 3 – 20/08/2002.

EURL – *L. monocytogenes* (2019). Technical guidance document for conducting shelf-life studies on *Listeria monocytogenes*
in ready-to-eat foods. Version 3 of 6 June 2015 - Amendment 1 of 21 February 2019.

Goulet V, Hebert M, Hedberg C, Laurent E, Vaillant V, De Valk H, Desenclos JC. (2012). Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. Clin Infect Dis. 1;54(5):652-60.

ICMSF : http://www.icmsf.org/

Lakshmikantha, C. (2013). Environmental Monitoring Program: An Early Warning System for Microbiological Hazards. Quality Assurance and Food Safety. https://www.qualityassurancemag.com/article/aib1213-environmental-monitoring-program

McLauchlin, J., Mitchell, R. T., Smerdon, W. J., & Jewell, K. (2004). *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. International Journal of Food Microbiology, 92(1), 15– 33.

Pocheville, A.(2015).The Ecological Niche: History and Recent Controversies. In Heams, Thomas; Huneman, Philippe; Lecointre, Guillaume; et al. (eds.). Handbook of Evolutionary Thinking in the Sciences. Dordrecht: Springer. pp. 547–586. ISBN 978-94-017-9014-7.

Turner, D.E., Daugherity,E.K. ,Altier,C. and Maurer K.J.(2010). Efficacy and Limitations of an ATP-Based Monitoring System. J Am Assoc Lab Anim Sci. 2010 Mar; 49(2): 190–195.

Uyttendaele, M., De Loy-Hendrickx, A., Vermeulen, A., Jacxsens, L., Debevere, J. en Devlieghere, F. (2018). Microbiological guidelines : support for interpretation of microbiological test results of foods. Die Keure, ISBN978 2 87403 503 6.

Van Walle I., Björkman J.T., Cormican M., Dallman T., Mossong J., Moura A., Pietzka A., Ruppitsch W., Takkinen J., European *Listeria* WGS typing group. Retrospective validation of whole genome sequencing-enhanced surveillance of listeriosis in Europe, 2010 to 2015. Euro Surveill. 2018;23(33) via https://ecdc.europa.eu/en/listeriosis/microbiology

Zoellner, C., Jennings, R., Wiedmann, M. and Ivanek, R. (2019). EnABLe: an agent-based model to understand *Listeria* dynamics in food processing facilities. Nature Scientific reports (www.nature.com/scientificreports), https://www.ncbi.nlm.nih.gov/pubmed/30679513

Zwietering, M.H., Jacxsens, L., Membre, J.M., Nauta, M. and Peterz, M. (2016). Relevance of microbial finished product testing in food safety management. Food Control, 60, 31-43.

Annex III: Technical report on challenge testing to asses behaviour of *Listeria monocytogenes* during defrosting/refrigerated storage of frozen vegetables under reasonably foreseen conditions at consumer's home

1) Set-up of the *L. monocytogenes* challenge testing

i. Categorization of vegetables:
To identify the most relevant products for the challenge tests, a categorization of frozen vegetables was made based on characteristics such as pH, sugar content, anti-bacterial compounds, nutrient level, structure/texture of the product.

ii. Refrigeration time:
after discussion, it was agreed that tests should not be performed in ambient temperature; this falls out of the responsibility of the producer. The tests should focus on growth potential during shelf life (meaning up to 24h in the fridge). In order to evaluate one step further, it was agreed to make an analysis also after 48h in the fridge.

iii. Refrigeration temperature:
It was agreed to use a temperature of 9°C (as accepted/recommended temperature for *L. monocytogenes* challenge testing in Belgium (by FASFC) & the Netherlands (NVWA) and supported by the data presented by Roccato et al. (2017) as published in the peer reviewed journal of Food Research International (2017: 96, 171–181) to mimic reasonably foreseen abuse both for countries of the South and North of EU.

iv. Batches:
it was agreed to work with 3 batches of the selected frozen vegetable from 3 different producers, if possible. The first batch was delivered to the lab/subjected to testing in March, the 2nd batch was delivered to the lab/subjected to testing in April-May; the 3rd batch was delivered to the lab/subjected to testing in July-August 2019;

v. Sample size:
it was agreed to use samples of 200g, the equivalent of a consumer portion of frozen vegetables (per sampling time a single pack of 200g was prepared and inoculated; a minimum of 150g is required for all the analyses scheduled).

vi. *L. monocytogenes* strains:
The challenge test was performed by the academic service laboratory of the Food Microbiology and Food Preservation research unit at Ghent University (FMFP-UGent) which has a track record of elaborating challenge testing using a cocktail of 3 *L. monocytogenes* strains (LMG 23194, LMG 23192, LMG 26484; for more information on the strains refer to www.bccm.belspo.be/catalogues/lmg-catalogue- search). In addition to these 3 strains, a fourth *L. monocytogenes* strain was added to the cocktail: *L. monocytogenes* ST6 strain, isolated from frozen vegetables/production environment related to the outbreak as described in EFSA/ECDC (2018) (Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update. EFSA supporting publication 2018:EN-1448. 19 pp. doi:10.2903/sp.efsa.2018.EN-1448)

vii. Inoculum level:
in accordance to the Technical guidance document on shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods" (EU-RL *Listeria*, June 2014) an inoculum of ca. 100 CFU/g was used (inoculum range from 30-300 CFU/g).

viii. Inoculation procedure:
frozen vegetables (large packs) were delivered by the frozen vegetable company to the lab and stored at -18°C. Shortly after arrival, from the large frozen packs, (without defrosting) individual frozen packs of 200g were pre-weighted and packed under air in a high barrier foil and stored frozen for maximum 2 weeks before inoculation. Next, these individual pre-weighed (200 g) frozen packs were thawed overnight (in a refrigerator of 4°C) and were inoculated with 400 µl of an inoculum (ca. 1 x 105 CFU/ml) of a cocktail of the 4 selected *Listeria monocytogenes* strains (LMG 23194, LMG 23192, LMG 26484 and LFMFP 1049) to obtain an inoculum of approximately 100 CFU/g. Strains were separately cultured: first 24 hours at 37°C followed by a subculture in fresh medium incubated for 3 days at 7°C for strain LFMFP 1049 (the ST 6 strain isolated from frozen vegetables/production environment during the 2018 EU outbreak) and for 4 days at 7°C for the other 3 strains (during prior trial characterizing growth characteristics of the ST6 outbreak strain, it was shown to grow faster than the other 3 strains). Inoculation was performed by dripping the culture suspension on the semi-thawed (overnight at 4°C) vegetable packs. Immediately after inoculation, the inoculated 200g semi- thawed vegetable packs were closed/ sealed and again put at -18°C for 14 days.

ix. Sampling and testing:
The frozen packages were taken from the freezer and put in a refrigerator at 9°C for 24 hours to defrost (refer to temperature profile in results section). Three replicate samples were tested in parallel (test 1, test 2 and test 3). For all replicate samples (test 1, 2, 3) enumeration of *L. monocytogenes* was performed after 14 days at -18°C (day 0) and after 1 and 2 days of defrosting (24 and 48h storage in a 9°± 1°C refrigerator). The enumeration of *L. monocytogenes* was performed under ISO 17025 accreditation.
Note: For one of the replicate samples (test 1) the total aerobic count, lactic acid bacteria and pH were determined before inoculation, after 14 days storage at -18°C and after 1 and 2 days of defrosting (24 and 48 hours at 9°C). For all replicate samples (test 1, 2, 3) *Listeria monocytogenes* detection (presence or absence per 25g) and pH and aw was measured on the blank sample before inoculation. The blank samples were inoculated with 400 µl diluent (Physiological saline solution).

2) Results on the categorization of vegetables

The following food characteristics were taken into account:

- Specific vegetable category
- pH (minimum and maximum)
- sugar and starch content
- Presence of anti-*Listeria* component
- Blanching
- Cut surface

pH, sugar and starch content were used to group the various specific vegetable categories in main groups. Furthermore, all products containing anti-*Listeria* components were classified in a separate group. The other characteristics such as blanching and cut surface were used to determine which vegetable type will be selected in due time for challenge testing to assess the growth potential of *L. monocytogenes* within these (main) groups.

i. Specific vegetable categories
Products were classified in eleven different categories based on the EFSA Scientific Opinion on the risk posed by pathogens in food of non-animal origin Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations) (EFSA Journal 2013, 11, 3025). Products were classified according to the 'General commodity category'. Only in a few cases was this category further split into the mentioned specific categories.

ii. Classification according to pH
pH values were obtained from a list published on PickYourOwn.org and uses following references:

a. Anon. 1962. pH values of food products. Food Eng. 34(3): 98-99.
b. Bridges, M. A., and Mattice, M.R. 1939. Over two thousand estimations of the pH of representative foods, American J. Digestive Diseases, 9:440-449.
c. Warren L. Landry and et al. 1995. Examination of canned foods. FDA Bacteriological Analytical Manual, 8th Ed. Chapter 21, Table 11, AOAC International, Gaithersburg, MD 20877
d. Grahn M.A. 1984. Acidified and low acid foods from Southeast Asia. FDA-LIB

Based on the reported maximum pH of the vegetable, they were classified as follows:

- pH < 4.4: not relevant as pH is lower than the pHmin for challenge test according to EU Reg. 2073/2005
- 4.4 < pH < 5.0: low risk
- 5.0 < pH < 6.0: medium risk
- pH > 6.0: high risk

iii. Classification according to sugar and starch content
Sugar and starch content were based on the Belgian nutrition table (Nubel). All values were based on fresh products since for most of the vegetables, no data on nutritional composition of their frozen forms were present. Products were classified for sugar and starch content in three categories:

- Low content: < 1%
- Medium content: between 1 and 4% for sugar; between 1 and 5% for starch
- High content: more than 4% for sugar; more than 5% for starch

iv. Classification according to presence of anti-*Listeria* component
It has been reported that Allium species from the Alliaceae family contain allicin derivative products and sulfur components which have shown antimicrobial activity (Mnayer et al., 2014). Also, carrots are reported to contain anti-*Listeria* components which have shown reduction of *L. monocytogenes* in ready-to-eat carrots during refrigerated storage (Sant' Ana et al. 2012). Products were only divided into either "no reports found" or "reports published on presence of anti-*Listeria* components" (no detailed information on the concentration of these components is known).

v. Blanching as a risk factor
Products are classified in three groups: blanched (yes), not blanched (no) or both (multiple).
Note that blanching is a technological heat treatment, the main objective being to inactivate enzymes that cause product degradation with quality loss. However, blanching can also accomplish some microbiological inactivation. The exact level of *Listeria monocytogenes* reduction will depend on the process conditions applied (time/temperature). Although blanching may cause inactivation of the pathogen, as a technological treatment, it may cause loss of texture and soften the vegetable which might facilitate growth of *L. monocytogenes* (if only mild heat treatment was used and/or the blanched product was prone to post-contamination). After discussion with the expert group, 'blanching' was not taken into account to classify the products in the different main categories because the use of a blanching step might vary for the same vegetable type across product varieties batches/producing companies

vi. Cut surface as a risk factor Products were classified in different groups:
- Absent: intact
- Low: only one cut surface
- Medium: more than one cut surface (e.g. after peeling)
- High: shredded

If the vegetable food type appeared in more than one variety, the cut surface was classified as 'multiple'. After discussion with the expert group, these differences in cut surface were not taken into account to classify the products in the different main categories because they might vary for the same vegetable type across product varieties batches/producing companies, but this factor was used to define within one (main) group which product type to be used to perform the challenge test.

Conclusion: 4 main risk groups and selection of frozen vegetables subjected to L. monocytogenes challenge testing
Based on the attribution of risk classification (based upon pH, sugar & starch content and presence of anti-*Listeria* components) to the various specific categories; four main risk groups could be established

4 main groups

1.	Score 0 (contain anti-Listeria component)
2.	Score < 0.2
3.	Score < 0.2 to < 0.35
4.	Score ≥ 0.35

The result of the scoring for the main frozen vegetables being set to the EU market is as follows.

Based on the scoring, the following frozen vegetables which belonged to the main category with the highest score (> 0.35) were selected for further *L. monocytogenes* challenge testing:
- Sweet corn Kernels
- Sweet Potatoes
- Peas
- Parsnips

In addition
- white cabbage

was taken up for *L. monocytogenes* challenge testing. White cabbage was added to include a frozen vegetable in the 'leafy green' group and also considering the history of implication of cabbage in a *L. monocytogenes* outbreak. (Cabbage also belonged to the one but highest scoring group (Score 0.2 to < 0.35).

3) Results of growth potential of *L. monocytogenes* in frozen vegetables: the EU-RL Guideline interpretation

The growth potential of *L. monocytogenes* in three batches of the five selected vegetables defrosted at 24h & 48h at 9°C after freezing at -18oC for 14 days is shown in Table 1. It is to be noted that Day 0 is not the day of *L. monocytogenes* inoculation (this was done at day -14). Day 0 rather represents the start of defrosting, when the 200 g packs of prior *L. monocytogenes*-inoculated frozen vegetables were transferred to the refrigerator. For temperature profile during defrosting/refrigerated storage, refer to section 4.

Calculating the growth potential

According to the EU RL technical guidance document (EURL, 2019) the growth potential (log CFU/g) is defined as the difference between the median of results (three replicates) at the end of the challenge test and the median of the results at the beginning of the challenge test (three replicates). It should be noted that in some EU Member States, the national competent authorities (e.g. NVWA in the Netherlands) have decided that if the maximum difference between the three replicates at the end of shelf life is higher than 0.5 log CFU/g, not the median but the highest value of the three replicates should be taken.

Interpretation of the test results of a challenge test to assess growth potential

According to the EU RL technical guidance document (EURL, 2019), a growth potential higher than 0.5 log CFU/g indicates that the food is able to support the growth of *L. monocytogenes* during the shelf-life according to used time-temperature profile. The target value at the end of the manufacturing process should always remain 'absence in 25 g'. Depending on the growth potential that was established during challenge testing, a certain intermediate limit can be obtained (Table 2).

Table 2 Intermediate limit at the end of the manufacturing process in relation to the calculated growth potential.

Growth potential (log CFU/g) during shelf life, when products are set to the market, as determined by challenge testing	Intermediate limit at the end of the manufacturing process to prevent the pathogen exceeding 100 CFU/g at the end of shelf life
Negative or Between 0.00 and 0.49	< 100 CFU/g
Between 0.50 and 0.99	< 10 CFU/g
Between 1.00 and 1.99	< 1 CFU/g or absence per g
Between 2.00 and 2.99	Absence in 10 g
More than 3.00	Absence per 25 g

Table 1 *L. monocytogenes* growth potential after 24h & 48h defrosting in a refrigerator at 9°C

Batch 1							Growth potential Day 1		Growth potential Day 2	
vegetable	**Batch**	**replicate**	**pH**	**Day 0**	**Day 1**	**Day 2**	**EU**	**NVWA**	**EU**	**NVWA**
Garden peas	1	1	6,42	1,78	2,52	3,28	**0,62**	0,62	**1,26**	1,26
		2	6,44	2,18	2,4	3,04				
		3	6,48	1,6	2,2	2,88				
Parsnip	1	1	6,2	2,15	2,28	2,98	**0,13**	0,13	**0,96**	0,96
		2	6,11	2,28	2,23	3,11				
		3	6,12	1,7	2,32	3,11				
Sweetcorn	1	1	6,69	2	2,74	3,43	**0,69**	0,69	1,37	**1,89**
		2	6,76	2,08	2,88	3,45				
		3	6,76	2,46	2,77	3,97				
Sweet potatoes	1	1	6,09	1,6	2	3	0,89	**0,97**	1,76	**1,97**
		2	6,09	1	2,49	3,36				
		3	5,88	2,15	2,57	3,57				
White cabbage	1	1	6,04	1,6	2,04	1,6	**0,44**	0,44	**0**	0
		2	6,01	1,95	2	1,7				
		3	6,01	1,48	2,08	1,6				

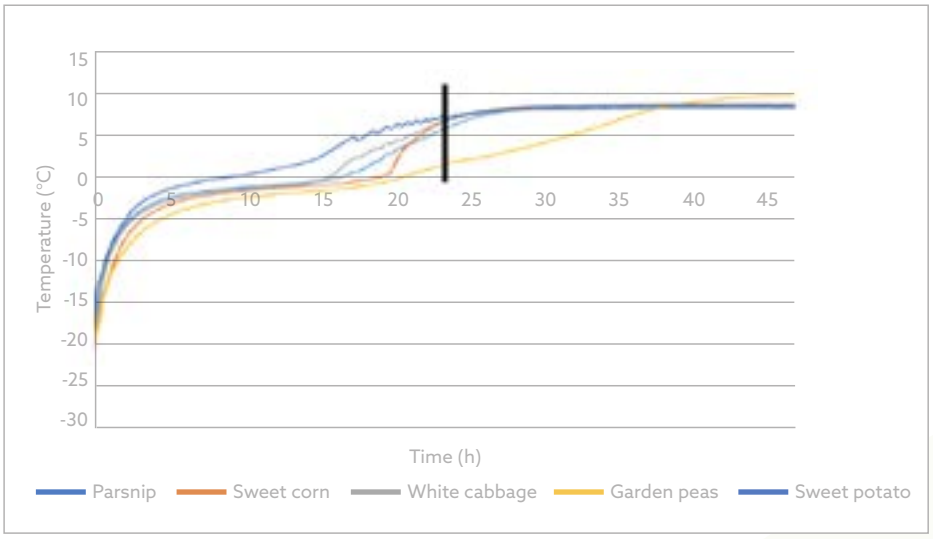
Batch 2							Growth potential Day 1		Growth potential Day 2	
vegetable	**Batch**	**replicate**	**pH**	**Day 0**	**Day 1**	**Day 2**	**EU**	**NVWA**	**EU**	**NVWA**
Garden peas	2	1	6,86	1,70	3,04	2,97	**0,70**	0,70	**0,85**	0,85
		2	6,91	2,11	2,81	3,04				
		3	6,95	2,34	2,76	2,87				
Parsnip	2	1	6,27	1,60	2,76	3,71	**0,85**	0,85	**1,71**	1,71
		2	6,16	1,90	2,83	3,61				
		3	6,16	2,15	2,62	3,53				
Sweetcorn	2	1	7,4	1,60	2,88	4,20	**1,10**	1,10	2,35	**2,38**
		2	7,4	1,32	3,04	4,00				
		3	7,49	1,85	2,95	4,23				
Sweet potatoes	2	1	6,2	2,00	3,34	3,63	0,71	**1,26**	1,58	**1,61**
		2	6,21	2,20	2,78	3,69				
		3	6,14	2,08	2,79	3,66				
White cabbage	2	1	6,4	1,48	2,54	3,53	**0,59**	0,59	**1,79**	1,79
		2	6,46	2,04	2,57	4,00				
		3	6,5	1,95	2,52	3,74				

Batch 3							Growth potential Day 1		Growth potential Day 2	
vegetable	**Batch**	**replicate**	**pH**	**Day 0**	**Day 1**	**Day 2**	**EU**	**NVWA**	**EU**	**NVWA**
Garden peas	3	1	6,85	1,60	2,43	3,66	**0,73**	0,73	**2,16**	2,16
		2	6,86	1,90	2,67	3,86				
		3	6,83	1,70	2,40	3,86				
Parsnip	3	1	6,31	2,26	2,68	3,81	**0,33**	0,33	**1,73**	1,73
		2	6,3	1,85	2,41	3,99				
		3	6,33	2,08	2,41	3,81				
Sweetcorn	3	1	7,22	1,70	3,00	3,57	**1,28**	1,28	**1,87**	2,02
		2	7,25	1,70	2,98	3,51				
		3	7,23	1,85	2,94	3,72				
Sweet potatoes	3	1	6,08	1,85	2,73	3,23	**0,23**	0,55	**1,08**	1,12
		2	6,19	2,34	2,41	3,26				
		3	6,08	2,18	2,40	3,30				
White cabbage	3	1	6,41	2,04	2,54	2,93	**0,50**	0,55	**0,80**	0,80
		2	6,38	2,04	2,65	2,84				
		3	6,41	2,41	2,36	2,84				

4) Time-Temperature profiles of frozen vegetables during defrosting

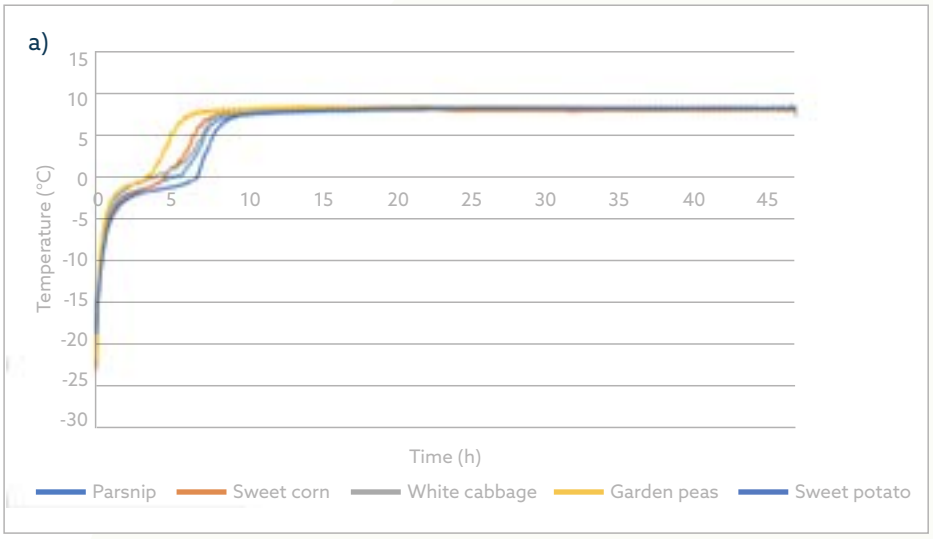
Batch 1: temperature profile (high volume loading : 11-7 kg; 5 frozen vegetables in 1 set-up)

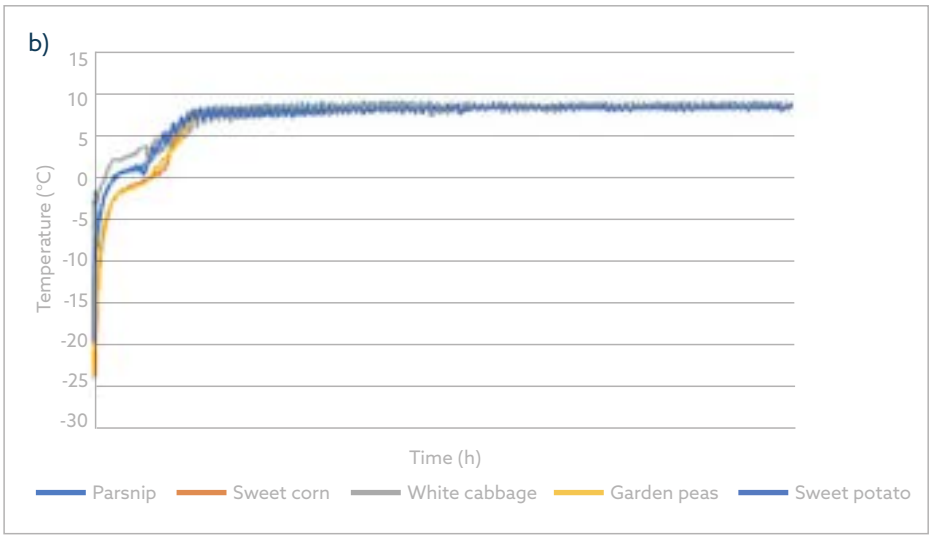
Figure 1.
Measured temperature profile of a 1 x 200g pack for each type of frozen pre-cut vegetable transferred from the freezer (-18°C) to a refrigerator at 9°C during 48h residence time (Temperature recorded with i-button temperature loggers (Maxim Integrated, California, USA) (Refrigerator 331 L volume – holding in total 35-55 x 200 g packs of frozen pre-cut vegetables) = scenario 1 (high volume loading simulating defrosting in catering or business to business refrigerator scenario)



Batch 2 and 3 : temperature profile (low volume loading : 1,4-2,2 kg; 1 set-up per frozen vegetable type)

Figure 2.
Measured temperature profile of a 1 x 200g pack for each type of frozen pre-cut vegetable transferred from the freezer (-18°C) to a refrigerator at 9°C during 48h residence time (Temperature recorded with ibutton temperature loggers (Maxim Integrated, California, USA) a) Batch 2 temperature profiles and b) Batch 3 temperature profiles (Refrigerator 331 L volume – holding in total 7-11 x 200 g packs of frozen pre-cut vegetables) = scenario 2 (low volume loading simulating defrosting in household refrigeratorscenario)





Focus on temperature profile (time (t)-temperature (T) recordings for (uninoculated) sweet corn in two conditions of defrosting (high volume loading versus low volume loading))

As it was noted that it took a prolonged time to defrost the frozen vegetable packs upon high volume loading (batch 1) (temperatures > 0°C achieved after > 18h) the temperature profile of an alternative scenario of defrosting (low volume loading) was explored, which was considered more representative of 'household' defrosting/refrigeration condition. In this alternative scenario, a total 10 frozen (-18°C) packs of 200g were taken from the freezer and put into a hitherto empty refrigerator at 9°C (2 pack per refrigerator 'level' i.e. top, intermediate-above, middle, intermediate-under, under). A 10-pack loading in one refrigerator allowed individual packs of all replicates (and blanks) that were part of a one batch *L. monocytogenes* challenge test of one selected food category to be put together. The recorded temperature profile for 9 of these 10 packs (200g each = 2 kg of defrosting frozen sweet corn) in the refrigerator is shown in Figure3.

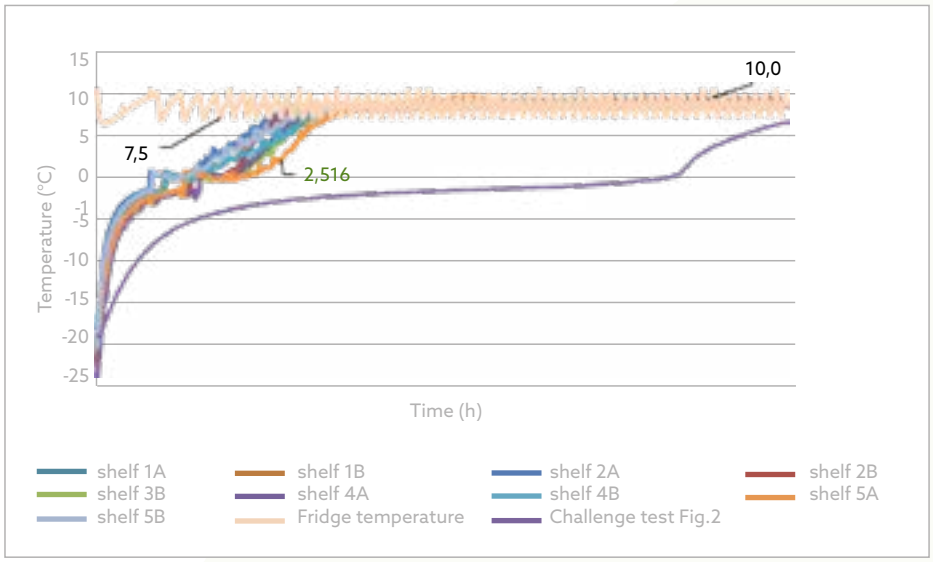


Figure 3. Measured temperature profile of 9 out of 10 x 200g defrosting frozen packs of sweet corn (2 kg or 2000 g of defrosting frozen sweet corn in total) transferred from the freezer (-18°C) into an (otherwise empty) refrigerator at 9°C during 48h residence time (Temperature recorded with i-button temperature loggers (Maxim Integrated, California, USA) (Refrigerator 331 L volume – holding in total 10 x 200 g packs of frozen sweet corn)
(the yellow line labelled 'Challenge test' refers to Batch 1 scenario 1 high volume loading temperature profile)

5) Discussion of *L. monocytogenes* growth potential during defrosting/refrigerated storage of frozen vegetables

It is clear that although PRPs, HACCP and a well implemented FSMS is in place – as stipulated by the PROFEL hygiene guidelines – it can be expected that for this type of production process of quick-frozen vegetables an occasional (post-) contamination can still occur and thus it cannot be excluded, and it has been noted from sector-wide microbiological analysis of quick-frozen vegetables, that some quick-frozen products set to the retail market as frozen foods might be occasionally contaminated with low levels of *L. monocytogenes* (< 10 CFU/g).

Although the majority of the frozen vegetables is not meant to and is not used as ready-to-eat (RTE), in order to ensure the *L. monocytogenes* safety limit of max. 100 CFU/g at the time of consumption for (RTE) foods on the market, the time for defrosting (in a refrigerator) or refrigerated storage of frozen vegetable packs should not support more than 1 log10 unit as otherwise an accidental low level *L. monocytogenes* contamination (of < 10 CFU/g) could exceed 100 CFU/g at the time of use and consumption of these frozen vegetables by the consumer.

Overall the *L. monocytogenes* growth potential observed after 24h is restricted to less than 1 log10 , except for frozen corn (Batch 2 and Batch 3) and except for one of the replicates (of Batch 2) of frozen sweet potatoes.

If refrigerated storage is prolonged with an additional 24h (up to 48h thus), often the outgrowth of *L. monocytogenes* on the defrosted refrigerated vegetables exceeds more than 1 log10 and quite some variability in the extent of *L. monocytogenes* is observed between the batch. This observed inter-batch variability (and also noted intra-batch variability) can be attributed to several factors. Indeed, they were different batches (derived from different producing companies as well) from the same type of frozen vegetable which can differ slightly in product characteristics. Furthermore, variability was noted in the measured 'temperature profile recorded' (e.g. Figure 3 in multiple blank samples of sweet corn) and hence some variable temperature profile between packs simultaneously defrosting in a single refrigerator was expected to occur as well. This might also affect to some extent the outgrowth and thus the observed growth potential of *L. monocytogenes* inter-batch andintra-batch.
As mentioned above, from the results of the *L. monocytogenes* section shown in Table 1 (section 3) it became clear that sweet corn is the most susceptible to support growth of *L. monocytogenes*, and also may support outgrowth of more than 1 log10 within the 24h defrosting/storage time in the most facilitating conditions (reaching temperatures > 0°C in 2-5h) as was observed in Batch 2 and 3 (refer to Table 3 for a summary of *L. monocytogenes* growth potential on sweet corn). It was noted in a preliminary trial to characterise the growth of LFMFP 1049 (the ST 6 strain isolated from frozen vegetables/production environment during the 2018 EU outbreak) that this latter strain grew faster than the other 3 strains at 7°C. Therefore, an extra challenge test was performed for Batch 3 of sweet corn using now a cocktail of the standard three *L. monocytogenes* strains (and thus without the expected faster growing ST6 strain). It was noted (refer to Table 3) that the *L. monocytogenes* growth potential as determined in the latter case was indeed restricted to less than 1 log10 unit within the first 24h storage at 9°C. Thus, the inclusion of the ST 6 strain isolated from frozen vegetables/ production environment during the 2018 EU outbreak might also explain to some extent the noted increased (more than 1 log10 within the 24h defrosting/storage time) growth of *L. monocytogenes* in the sweet corn.

Table 3: Summarized results of of *L. monocytogenes* growth potential on sweet corn

vegetable	Batch	EU	NVWA	EU	NVWA
Sweetcorn	1**	**0,69**	0,69	1,37	**1,89**
Sweetcorn	2*	**1,10**	1,10	2,35	**2,38**
Sweetcorn	3*	**1,28**	1,28	**1,87**	2,02

* challenge test performed with 4 *L. monocytogenes* strains (in batch 1-2-3)
i.e. including the *L. monocytogenes* ST6 strains isolated from the EU 2018 frozen corn outbreak

° temperature profile in Batch 1 deviated (during defrosting longer time to reach > 0°C)

vegetable	Batch	EU	NVWA	EU	NVWA
Sweetcorn	3*	**0,62**	0,62	**1,33**	1,33

In conclusion, the knowledge established by challenge testing as described above on the behaviour and growth potential of *L. monocytogenes* during defrosting/refrigerated storage of frozen vegetables was used as an input to 1) establish *L. monocytogenes* end product specification and 2) develop appropriate risk communication to consumers via the label as described in the hygiene guidance in Section 5.2.

References for Annex III :

Mnayer, D., Fabiano-Tixier, A.-S., Petitcola, E., Hamieh, T., Nehme, N., Ferrant, C., Fernandez, X. and Chemat, F. (2014). Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae Family. Molecules, 19, 20034-20053.

Noriega, E., Newman, J., Saggers, E., Robertson, J., Laca, A., Diaz, M., Brocklehurst, T.F. (2010). Anti-Listerial activity of carrots: effect of temperature and properties of different carrot fractions. Food Research International, 43, 2425-2431.

Sant'Ana, A.S., Barbosa, M.S., Destro, M.T., Landgraf; M., Franco, B.D.G.M. (2012). Growth potential of Salmonella spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. International Journal of Food Microbiology 157,52-58.

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