Validation, verification and monitoring of food safety and quality parameters in ice cream production

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Abstract

Over the past 50 years, the food industry has seen significant changes: globalisation of supply chains, evolution of consumer eating habits, and agricultural impacts from climate change. Such transformations require manufacturers to continually update their quality control systems. This dissertation has reviewed food safety standards at an ice cream factory in Portugal through a practical and case study approach, focused on the three essential elements of food production: equipment, personnel and processing environment. Firstly, cleaning-in-place systems for ageing tanks and continuous freezers were assessed and successfully validated. Secondly, the role of personnel as a potential vector for contamination was studied. Uniforms were not recognised as a relevant source of allergen cross-contamination and hand sanitation protocols were found to effectively eradicate enteric bacteria. Conversely, significant prevalence rates of *Listeria spp.* in footwear (27%) were discovered. Lastly, the presence of *Listeria* in floor drains of the processing environment was investigated. In total, 55% of the drains were contaminated with *Listeria spp.* and 26% tested positive for pathogenic *L. monocytogenes*. A deep cleaning treatment reduced contamination by 25%, but two niches of persistent *L. monocytogenes* remained. Overall, the results of this dissertation show that hygiene standards at this production unit are generally satisfactory and adequate to the manufacturing of safe and high-quality products. The principles described and solutions proposed may both help to further enhance conditions in this plant and be used as a benchmark for future projects pertaining to the production of safe foods.

Keywords: Ice cream; Food safety; HACCP; *Listeria*; CIP Validation; Monitoring

1 Introduction

Over the past 50 years the food industry has seen significant changes. International trade and travel have transformed the way we approach sourcing and distribution of food, which now reaches consumers through a global supply chain. Eating habits have evolved drastically and new products have emerged to reflect this trend. Climate change has greatly impacted agriculture and water availability, both of which considerably influence food production [1;2]. The most important task of food scientists, and the food industry as a whole, is to provide consumers with safe and high-quality food products [3]. As such, this evolution calls for an increased emphasis on manufacturing practices and quality standards to ensure a safe global food supply [1].

The design and implementation of food safety management systems have come a long way since the very early iterations of the 1960's [4]. Nowadays, most countries and government agencies endorse the use of proactive programs such as HACCP (Hazard Analysis and Critical Control Points) to reduce the risk of contamination and its consequences: product recalls, damage to the brand, loss of market share and, most importantly, endangering the health and safety of consumers [2;4–7]. Several authors have reported an improvement in conditions, costs and overall quality from the application of this program [1;4;6–9]. However, rapid changes in the socio-economic climate and consumer trends require that manufacturers think strategically and continuously improve their food safety systems. HACCP implementation should hence not be viewed as an isolated event, but rather an ongoing process which must be re-evaluated and adapted as new challenges arise [1;10].

Frozen dairy desserts have a strong record of safety, but recent outbreaks of foodborne disease linked to ice cream have brought new attention to this industry (11;12). With sales over 20 billion US dollars, Western Europe is the biggest ice cream and frozen desserts market in the world, and the company that owns this factory one of its most distinctive players (13). In light of these considerations, assessment of food safety standards and revision of HACCP elements in this factory has never been more relevant.

This summary reviews food safety standards at an ice cream factory in Portugal through a practical case study approach, focused on the three essential elements of food production: equipment, personnel and the processing environment. Firstly, the performance of cleaning-in-place (CIP) systems for ageing tanks and continuous freezers is assessed. Secondly, the role of personnel as a potential vector in the transmission of allergens and microorganisms is investigated. Lastly, the colonisation patterns of *Listeria spp.* and *Listeria monocytogenes* in floor drains of the production environment is studied. In each case corrective actions and solutions are proposed for the continuous improvement of sanitary conditions.

2 Practical case studies

2.1 Case I : Validation and monitoring of CIP in ageing tanks and freezers

2.1.1 Background and motivation

Cleaning and disinfection (C&D) is vital in any manufacturing operation to ensure the quality and safety of final products. As such, it is indispensable to periodically review cleaning programs and ensure they remain effective and compliant with the established HACCP plan [2;14;15]. The processed food industry has seen a major shift towards CIP over the past decades, and with it increased demands from customers and regulatory agencies in regard to its monitoring, verification, validation, and attendant improvements in plant hygiene and efficiency [16;17]. Assessment of CIP processes has hence become an essential part of C&D operations [15;18]. Monitoring refers to the routine measurements performed after C&D that serve as indicators that these processes are in a state of control [14–17]. The purpose of recording this data is not only to oversee the effectiveness of these procedures, but also to develop a database over time that allows swift identification of unhygienic equipment, maintenance problems, and opportunities to optimise the cleaning program [14;15]. Validation studies assess C&D operations and provide evidence that the protocols in place are effective against relevant hazards (microbiological, chemical, physical and allergens) [1;16;19]. A comprehensive validation study will typically consist of the following steps [14]:

Step 1. Production process review
Step 2. Production process verification
Step 3. Cleaning and disinfection review
Step 4. Cleaning and disinfection verification
Step 5. Selection of most difficult to clean product
Step 6. Identification of relevant contaminants
Step 7. Selection of sampling sources
Step 8. Selection of sampling locations
Step 9. Selection of analytical method
Step 10. Determination of acceptance limits
Step 11. Sampling and analysis
Step 12. Evaluation of the results

Cleaning and disinfection is considered successful if all the samples have contamination levels within the acceptance range and validated after three consecutive successful repetitions.

2.1.2 Objectives

This case study aims to collect samples from ageing tanks and freezers after a complete CIP cycle to serve as (i) performance indicators in the routine monitoring of cleaning and disinfection operations (ii) data for the mandatory annual cleaning and disinfection validation study of these pieces of equipment.

2.1.3 Materials and methods

Validation of the CIP system in ageing tanks and freezers was performed using the steps outlined in subsection 2.1.1. In ageing tanks, C&D is performed using two CIP independent circuits (CIP A for the tanks in lines 600, 500 and 400; CIP B for the tanks in lines 300, 200 and 100), thus each circuit was validated separately. Sampling protocols and validation criteria are summarised in Table 1. The monitoring protocol was identical to the one used for validation, excluding allergen testing. In freezers, C&D is performed using three CIP circuits (C, D or E). Each machine is usually connected to more than one circuit, thus validation was performed on the three circuits combined. Sampling protocols and validation criteria are summarised in Table 2. The monitoring protocol was identical to the one used for validation.

Table 1: Sampling protocol for monitoring and validation of CIP A , B

Contaminant	Sampling source	Analytical method	Acceptance limit
Product residue	Surface	Sensorial inspection	Visually clean Absence of scent
Microorganisms	Surface Rinse water	Microbiological analysis (TVC, *Enterobacteriaceae*)	TVC: <100 c.f.u / 100 cm^2 <100 c.f.u / 100 mL Entero: <5 c.f.u / 100 cm^2 <1 c.f.u / 100 mL
Chemicals	Rinse water	Chemical analysis (pH, Conductivity)	pH: <8.764 Cond: <1.006 mS/cm
Allergens	Surface Rinse water	Fast detection allergen kits	Negative
Sampling location : CIP A , CIP B (each tank line) Product : Mix containing allergens (worst-case)			

Table 2: Sampling protocol for monitoring and validation of CIP C/D/E

Contaminant	Sampling source	Analytical method	Acceptance limit
Microorganisms	Rinse water	Microbiological analysis (TVC, *Enterobacteriaceae*)	TVC: <100 c.f.u / 100 mL Entero: <1 c.f.u / 100 mL
Chemicals	Rinse water	Chemical analysis (pH, Conductivity)	pH:<8.764 Cond: <1.006 mS/cm
Sampling location : CIP C/D/E (each processing line) Product : Aged mix			

2.1.4 Results and discussion

CIP Monitoring For the mix ageing tanks (CIP A and CIP B), product residue was never detected and microbiological analysis showed very promising results. CIP A (Figure 1, a) had generally good results, with only two samples outside of the acceptable range. In this case, both samples were not rinse medium but rather swabs from the inside of the tank vent*, which suggests that the CIP program is effective overall but can struggle to clean areas of difficult access. This specific fault in hygienic design had already been registered as an improvement opportunity by plant managers, and over the past years ageing tanks in this factory have been progressively altered or replaced by newer versions with better vent placement. CIP B (Figure 1, b) had the best outcome of all circuits, as every sample was within the acceptable range. Overall, the fact that no enteric bacteria was detected in either circuit, and that the only instances of high TVC were from difficult to reach areas*, indicates that the CIP program is successful at eliminating microbiological contaminants.

By contrast, chemical analysis showed less favourable results. CIP A had the worst chemical outcome of all circuits, with 23% of samples outside of the acceptable range. Most of these samples were collected from the same line (CIP A - 400), with tank 432 being especially problematic. CIP B had five instances of inadequate samples, two of each from the same ageing tank (312) and three others from the same line (CIP B - 200). High pH and conductivity levels indicate presence of a chemical contaminant, most likely detergent residue which was not completely removed during the final rinsing stage. This can be caused by several different factors. On one hand, where inadequate samples are repeatedly isolated from the same unit, such as tank 312, the cause is most likely related to the unit itself. For instance, a partially obstructed or defective spray ball would fail to produce the necessary pressure required for complete rinsing. Disassembly of moving parts is impractical and exposes the pieces to microbiological contamination. The best course of action in this case would be to monitor the CIP program through an endoscope to verify that these parts are working as expected. On the other hand, inadequate samples which are part of a pattern, such as those from lines 200 and 400, tend to indicate a more general problem. For instance, spatial arrangement of equipment and piping design have a great impact on fluid pressure, which directly affects rinsing efficiency. In the studied plant, tanks with samples outside of the acceptable range are concentrated towards central lines (200, 400), which are more difficult to access and thus fluids lose more pressure. The piping network for these machines is extremely complex, so this information alone is insufficient to perform a substantial diagnosis. However, it provides valuable insight into the overall performance of the system and serves as an important first step in the development of an appropriate course of action. In this case, the piping and instrumentation diagram (P&ID) must be studied to confirm which tanks are more vulnerable to pressure loss and define strategic positions in the piping network to measure flow rate.

For freezers (CIP C/D/E) microbiological analysis results were identically encouraging, as 98% of samples were within the acceptance range (Figure 1, c). In total, only three contaminated samples were found, and each one was isolated from a different processing line (Line A, Line G and Line W). Moreover, only one of these samples tested positive for enteric bacteria*. These results confirm that the CIP protocol effectively eliminates bacteria from freezers and any contamination that may occur is sporadic. Chemical analysis results were generally better than those of ageing tanks, with 89% of

*Data not shown in this summary. Available in full-version dissertation.

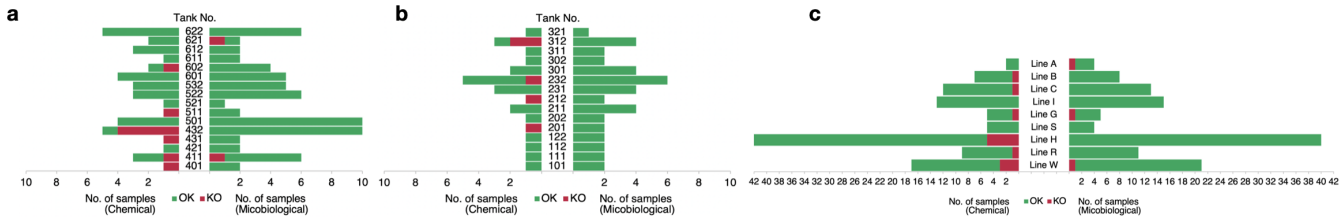

Figure 1: Chemical and microbiological analysis of samples from CIP A (**a**), CIP B (**b**) and CIP C/D/E (**c**). OK (■) = samples within the range of acceptance; KO (■) = samples beyond the range of acceptance. Ranges of acceptance: (Chemical) pH <8.764, conductivity <1.006 mS/cm; (Microbiological) TVC <100 c.f.u / 100 cm^2 or 100 mL, Entero <5 c.f.u / 100 cm^2 or <1 c.f.u / 100 mL

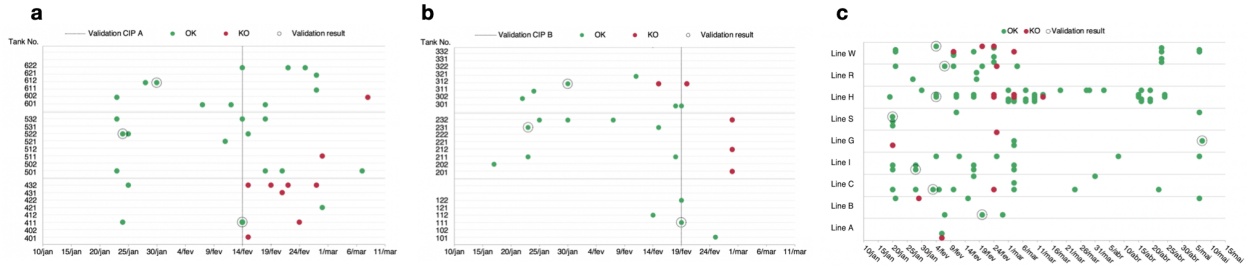

Figure 2: Validation timeline for CIP A (**a**), CIP B (**b**) and CIP C/D/E (**c**). OK (■) = samples within the range of acceptance; KO (■) = samples beyond the range of acceptance. Validation result = Third consecutive green sample for the same line. Ranges of acceptance: (Product residue) Visually clean, Absence of scent; (Allergens) Negative; (Chemical) pH <8.764, conductivity <1.006 mS/cm; (Microbiological) TVC <100 c.f.u / 100 cm^2 or 100 mL, Entero <5 c.f.u / 100 cm^2 or <1 c.f.u / 100 mL

satisfactory samples, but some cases of contamination were still discovered. Samples beyond the range of acceptance were found across two-thirds of all production lines, with an average prevalence of 11%. Since all freezers share the same make and model, these contaminations are likely to stem from a more general issue, but the underlying cause is not entirely clear. Very few researchers have investigated CIP parameters in continuous freezers, and the existent studies focus solely on microbiological contaminants[11]. In other types of heat exchanger, fouling and corrosion have been shown to increase the conductivity of rinse water during CIP[20]. The use of drinking water is generally adequate to prevent either condition, but the manufacturer has advised that sensitivity may vary with operating parameters[21]. While it is possible that this system is more prone to fouling or corrosion, some of these freezers had been upgraded to the newer model only a few months prior, so it is unlikely the equipment would show significant signs of use in such a short period of time. Another possibility is that the design of the equipment itself does not allow adequate rinsing of every part of the freezer, and thus some detergent residue may remain. Alternatively, cleaning parameters may need to be adjusted. Increasing rinsing time at the end of the CIP program may lead to a more thorough detergent removal and thus fewer residue inside the machine. Finally, incorrect sampling practices also need to be considered as a possible cause. Since samples from the production hall are collected by personnel with no formal laboratory training, it is possible that collection is performed prematurely (i.e. before the last 30 seconds of rinsing), which would produce misleading results. All of these possibilities must be investigated to ensure the CIP protocol is fully effective and the risk of contamination with chemical hazards as low as possible.

CIP Validation Monitoring results showed several opportunities for improving CIP operations, yet expecting any process to have a flawless performance is highly unrealistic and thus should never be the standard for C&D. Instead, food manufactures must endorse a system which consistently reduces contaminants to a point where food is considered safe. Validation is used to ensure that the program in place has this ability.

For ageing tanks, a preliminary review and verification confirmed that production and C&D were compliant with the documentation and working as expected. Since each line (100-600) had three consecutive samples within the ranges of acceptance established (see 2.1.1), validation of CIP A and B was successfully completed within the first trimester of production (Figure 2, a,b). For freezers, preliminary review and verification are performed with the rest of the production line, which could not be completed at the time of this dissertation's internship. Similarly to ageing tanks, three successful rounds of sampling and analysis are required for each line. For Line A, only two samples of rinse water were collected and only one was within the criteria established (Figure 2, c). As such, CIP could not be validated. For all other lines, three consecutive samples within the range of acceptance were obtained and thus validation was successfully completed within the second trimester of production. It should be noted that validation of the freezer CIP system does not translate into a successful C&D validation for the whole line, as results from other sampling locations must also be considered. Overall, these observations demonstrate that the CIP system for either type of equipment is well-suited to the removal of relevant contaminants, and thus contributes to the production of safer food.

2.2 Case II : Personnel as a potential vector for allergen and microbiological contamination

2.2.1 Background and motivation

Ready-to-eat (RTE) food products that have been submitted to an adequate heat-treatment are free of vegetative pathogens. Nevertheless, RTE items have been implicated in various food-borne illnesses, caused by recontamination during subsequent production steps which unintentionally expose the product to bacteria, allergens, chemicals, and foreign bodies[10;22;23]. Food handlers are a well recognised route of post-pasteurisation recontamination in ice cream production, namely for microbiological and allergen contaminants[1;7;10;22]. As such, it is essential to regularly review the procedures in place for the hygiene of personnel and assess the need for additional policies.

2.2.2 Objectives

This case study aims to investigate the role of personnel as a vector in the transmission of allergens, enteric bacteria and *Listeria monocytogenes* in the food processing environment. To this end, samples were collected from clothing, hands, and shoe soles, respectively. The hand driers and boot scrubber used by personnel in regular hygiene protocols were also sampled to verify that these pieces of equipment (i) are not prone to the accumulation of bacteria and (ii) are effective in the removal of microbial contaminants.

2.2.3 Materials and methods

To investigate the transmission of allergens through personnel's clothing, 60 uniforms were swabbed in the chest area (30 x 20 cm) and tested over the course of a month (2 almond, 4 peanut, 9 hazelnut, 10 gluten and 35 total protein). Specific allergens were detected using Reveal 3-D Food Allergen Kits from Neogen[24]: Almond (902086G), Peanut (901041L), Hazelnut (90208E), and Gluten (8505). Total protein was detected using Clean-Trace™ Surface Protein Plus Test Swabs from 3M (PRO100)[25]. To investigate the transmission of *Enterobacteriaceae* through food handlers, 86 hands were swabbed over the course of a month (74 bare, 12 wearing gloves). Samples were collected with sterile cotton wool swabs (COPAN 150C) as directed by the manufacturer. Detection of *Enterobacteriaceae* was performed by plant analysts using a plate count method. To investigate the transmission of *Listeria* through personnel's shoes, 28 soles were swabbed over the course of 15 days, before and after cleaning in an industrial boot scrubber, also swabbed. Samples were collected with sterile cotton wool swabs (COPAN 150C) as directed by the manufacturer and stored at 4℃. Detection of *L. monocytogenes* and *Listeria spp.* was performed by Silliker Portugal, S.A., using the ALOA® One Day qualitative method (internal protocols PAM.16.4 and PAM.09.0, respectively)[26].

2.2.4 Results and discussion

Personnel clothing as potential vectors for cross-contamination with allergens The first part of this study aimed to assess the role of personnel as a carrier of allergens and the associated risk of cross-contamination in the production hall. To this end, uniforms were swabbed and tested for specific allergens among the most commonly known to cause adverse reactions. Tests for total protein were also performed to account for allergens excluded from this group. Results are shown in Table 3.

Table 3: Detection of allergens in personnel's clothing

	Positive	Inconclusive	Negative	Total
Almond			2	2
Peanut			4	4
Hazelnut			9	9
Gluten			10	10
Total Protein	1	2	32	35
Grand Total	1	2	57	60

Of the 60 uniforms swabbed, no trace of food allergens was discovered in 57 (95 %). Moreover, the only 3 swabs with non-negative results were total protein tests, which are non-specific and thus able to detect any protein present in a sample, rather than just allergenic proteins[25]. Several publications have recognised work wear as a source of cross-contamination with food allergens[27;28], but little is known about the exact conditions in which it happens. In this factory, uniforms worn by personnel do not seem to be a relevant vector in the transmission of allergens, and thus cross-contamination through garments is unlikely to occur.

Personnel hands as potential vectors for transmission of *Enterobacteriaceae* The second part of this study aimed to assess the efficacy of hand sanitation protocols and whether foodhandlers are a relevant source of contamination with *Enterobacteriaceae*. Swabs were collected from bare and gloved hands and tested for *Enterobacteriaceae*. Results are shown in Table 4.

Table 4: Detection of *Enterobacteriaceae* in personnel's hands and hand dryers

	<10 c.f.u / 100 cm^2	Total
Hands	86	86
Bare	74	74
Gloved	12	12
Hand dryers	4	4
Grand Total	90	90

All hand swabs tested negative for contamination with enteric bacteria and none was found in any of the jet dryers. These results suggest that the hand sanitation protocol implemented in this factory is highly effective and strictly enforced by food handlers. It should also be noted that the use of gloves did not seem to increase the microbial load on personnel's hands, contrary to findings from other studies[29;30]. The absence of elevated counts of enteric bacteria is a strong indicator that high standards of hygiene are maintained in this factory. Nevertheless, the fact that the same result was obtained for all swabs warrants further testing. One possible variation would be to use an alternative sampling method.A similar study using contact plates was performed in 2017 at another food factory, located in the same industrial site. It would be interesting to organise a second round of sampling at the present site using this method, even if some authors have reported a lower recovery efficiency with contact plates when compared to swabs[31;32]. Additionally, Scott and Bloomfield[32] studied the performance of different sampling methods in stainless steel surfaces and found electrostatic wipes to have the best results overall. Though this strategy has not been reported in literature for collecting bacteria from hands, materials per sample have a very low cost and thus might be worth considering. Alternatively, the same method could be used with an adjusted technique. In experiments with cotton wool swabs, Chamberlain et al.[33] reported a consistent ten-fold increase in the amount of bacteria recovered from hands by going over the same area five times.

Personnel shoes as potential vectors for transmission of *Listeria* The third part of this study aimed to assess the efficacy of an industrial boot scrubber and whether the shoes of personnel increase the transmission and propagation of *Listeria* in the production hall. Swabs were collected during the morning and evening shifts, before and after shoes were cleaned in the boot scrubber. Samples were tested for *Listeria monocytogenes* and *Listeria spp.*. *Listeria monocytogenes* could not be isolated from the boot scrubber or any of the shoes tested, suggesting that personnel is not the main entry route for this pathogen in the food processing environment. In spite of this, workers may still contribute to the dissemination of *Listeria* after entering the production hall, as evidenced by samples collected from the drains of Line S. These findings are discussed in further detail in another case study (2.3). Although

no contamination with *L. monocytogenes* was discovered, several swabs tested positive for *Listeria spp.*, as shown in Figure 3.

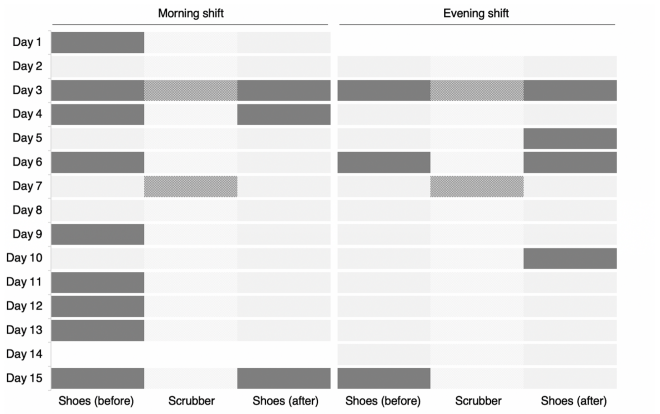

Figure 3: Detection of *Listeria spp.* in personnel's shoes and boot scrubber (84 samples), in the morning shift (left) and evening shift (right). For each shift, the first column represents shoes sampled before scrubbing, the second samples collected from the scrubber and the third shoes sampled after scrubbing.
Colour code: *Listeria spp.* (shoes) *Listeria spp.* (scrubber), No contamination (shoes), No contamination (scrubber)

From a total of 84 swabs, 23 (27%) tested positive for *Listeria spp.*, consistent with literature's reports of footwear as a meaningful source of bacteria in industrial settings[34–37]. When comparing the results from the two shifts, significant differences were discovered in the samples taken before scrubbing. In these conditions, the number of positive results in the morning was threefold higher when compared to the same samples from the evening shift. It seems unlikely that this difference was caused by elements external to the plant, since employees are required to wear work boots provided by the company and these are kept in personal lockers inside the factory when not in use. One possibility is that the results were influenced by the different work schedules followed by personnel and quality assurance. Office hours start later in the day than morning production, so samples taken in the morning were collected in the middle of the shift (most likely from employees coming back from a break) while samples taken in the afternoon were collected at the beginning of the shift (most likely from employees who had just put their shoes on). While workers are required to change shoes before going home, they are not required to do so when taking a short break or going outside to smoke. Under these circumstances one could argue that swabs obtained before scrubbing are more likely to test positive if collected during the morning shift than if collected during the evening shift. Otherwise, results from the two shifts showed no notable differences. To better assess the efficiency of the boot scrubber, each group of three samples (shoes before, scrubber, and shoes after) was analysed as a whole. Possible scenarios for the cleaning sequence were hypothesised and the number of occurrences of each one registered, as shown in Table 5.

Table 5: Scenarios compiled from the detection of *Listeria spp.* in personnel's shoes and boot scrubber

Scenario	Shoe	>	Scrubber	>	Shoe	No. of occurrences	Ref.
	Negative	>	Negative	>	Negative	12	A
	Listeria	>	Negative	>	Negative	7	B
	Listeria	>	Negative	>	Listeria	3	C
	Negative	>	Negative	>	Listeria	2	D
	Negative	>	Listeria	>	Negative	2	E
	Listeria	>	Listeria	>	Listeria	2	F

For the most part, events were consistent with what was expected: employees arrive at the entrance of the production hall, wearing shoes which may or may not be contaminated with *Listeria*. These are cleaned in a uncontaminated scrubber, which effectively eliminates bacteria when present. This sequence describes scenarios A and B, which account for 68% of cases.

In other occurrences, the uncontaminated scrubber failed to eliminate *Listeria* from shoes, which continued to test positive after cleaning (scenario C). Several uncontrolled variables could be responsible for this difference in performance. The microbiological tests performed were only meant to determine the presence of *Listeria*, so the bacteria found was not quantified. It is possible that the scrubber is successful at eliminating *Listeria* up to a certain concentration, but fails to fully eradicate bacteria in more contaminated shoes. Further testing using quantitative methods would be required to confirm this hypothesis. Contact time is also a decisive parameter of cleaning and disinfection. Scrubbing duration is defined by equipment programming to ensure optimal results, but instances of incorrect use have been observed. To correct this, personnel should be advised on the importance of contact time during the use of the scrubber machine.

In two other cases, *Listeria* was isolated from clean shoes where none had been previously detected nor found in the scrubber. While they reflect a small percentage of the overall results (7%), these findings are extremely significant, as they demonstrate the potential for contamination despite the implementation of sanitation procedures. The floor space between the scrubber and entry door is cleaned thoroughly and frequently, so it is unlikely that it could harbour enough bacteria to be the source of contamination. By contrast, rubber floor mats placed after the scrubber to keep employees from slipping have a honeycomb pattern which allows water to be retained, making them a possible offender. Reviews targeted at industrial audiences have identified porous floor mats as potential harbourage sites for *Listeria*[38]. A study by Lappi et al.[39] found these to be the motive for persistence of a particular *L. monocytogenes* subtype in a fish processing plant, which was eradicated after the mats were removed. Growth and formation of *Listeria* biofilms in other rubber objects have also been described[38;40;41]. In light of this evidence, additional sampling to assess the potential of these mats as a source of bacteria is advised.

Listeria was isolated from the scrubber itself in four different cases. In scenario E, uncontaminated shoes were cleaned using the contaminated scrubber, but no bacteria was detected afterwards. While the presence of *Listeria* in cleaning equipment is always undesired, these results confirm that it does not transfer easily to footwear. Results for scenario F show that a contaminated scrub is unable to remove *Listeria* from contaminated shoes, as expected, but very few other conclusions can be drawn. If sampling is repeated for future studies, quantification of bacteria would be helpful in this scenario to confirm that the scrubber does not increase the load of bacteria in shoes, despite testing positive for *Listeria*

Overall, the boot scrubber appears to adequately remove *Listeria* from personnel's shoes without accumulating significant amounts of bacteria throughout the day. However, results from this case study show that prevalence of *Listeria spp.* in footwear is still considerable. This type of equipment is considered the industry gold-standard for cleaning and disinfection of shoes[42], but in this plant it seems lacking as a standalone measure for adequate control of *Listeria*. Other authors have also noted this trend, but alternatives have yet to be proposed. Rashid et al.[34] reviewed decontamination techniques for shoe soles in food production and healthcare facilities and found most currently used methods to have variable success. Jordan et al.[43] has

described the use of overshoes for additional contamination prevention during reconstruction phases, but extrapolating this measure to daily operations would have substantial economic costs and environmental impact. Novel strategies for cleaning and disinfection of shoe soles would help to prevent colonisation of the processing environment with *Listeria*, recognised as one of the main concerns in the ice cream industry.

2.3 Case III : Presence and persistence of *Listeria monocytogenes* in drains of the production hall

2.3.1 Background and motivation

The processing environment has been well established as a source of contamination in the production of RTE foods[38;44–46]. Surfaces like floors and walls act as indirect sources of bacteria, which are then carried by air, staff and cleaning systems[40]. Some pathogens become established and find niches where they can survive for long periods of time[22;46]. *Listeria monocytogenes* is an opportunistic pathogen and one of the main causes of foodborne illness[37;40;43;44]. It is the microorganism responsible for listeriosis, a bacterial infection with fatality rates of 15-20% in high-risk groups (pregnant, newborns, immunocompromised and elderly)[44;47]. A wide range of growth conditions and potential to adapt under stress allow this species to survive and persist in processing plants for years or decades, with specific strains isolated repeatedly over time[37;38;40;44;45;47;48]. A variety of studies have described this trend, notably in refrigerated premises[38;49;50]. While elimination of *L. monocytogenes* is a priority in the ice cream industry, its resilience to environmental factors makes it a difficult challenge[43–45;47]. Routine environmental sampling is indispensable to reduce the spread of *L. monocytogenes*. A thorough analysis of the processing environment can serve as an early warning and trigger (i) elimination of the bacteria through appropriate deep cleaning measures and (ii) prevention of recolonization though improved procedures and sanitary redesign[22;38;43–45;47].

2.3.2 Objectives

This case study aims to investigate the presence and persistence of *Listeria* species in floor drains of the nine processing lines in the production hall. Results were used to assess the overall hygiene level of each line, detect niches of persistent *L. monocytogenes*, gain insight into colonisation patterns specific to this plant and appraise the effectiveness of an annual deep cleaning treatment.

2.3.3 Materials and methods

To investigate the presence and persistence of *L. monocytogenes* in the production hall, 69 drains were sampled across the nine processing lines: 2 Line R, 4 Line I, 7 Line G/Line C, 3 Line W, 11 Line A/Line H, 8 Line B, 34 Line S (Figure 4). Samples of water (100 mL) were collected with a sterile pipette, before and after an annual deep cleaning treatment, and stored in sterile cups at 4 ℃. Detection of *L. monocytogenes* and *Listeria spp.* was performed by Silliker Portugal, S.A., using the ALOA® One Day qualitative method (internal protocols PAM.16.4 and PAM.09.0, respectively).

2.3.4 Results and discussion

Routine environmental sampling of the processing environment in this plant had revealed several drains contaminated with *L. monocytogenes* and *Listeria spp.*. During the annual shutdown period, a deep cleaning treatment was performed to remedy this. New samples were taken from the same drains after manufacturing was resumed and three performance metrics were determined from these findings to ascertain the efficacy of the cleaning treatment:

- Prevalence of *L. monocytogenes* and *Listeria spp.* before and after treatment (percentage of drains from where the respective species was isolated);
- Eradication of *L. monocytogenes* (net difference in number of drains contaminated with *L. monocytogenes*);
- Overall hygiene (net difference in number of negative drains).

Overall, results show that the application of this treatment considerably reduced contamination with *L. monocytogenes* and *Listeria spp.* (Table 6). Before treatment 38 of the 69 (55%) drains sampled were contaminated and 18 (26%) tested positive for *L. monocytogenes*. This species was found in all production lines, with the exception of Line G and Line C. After treatment results improved significantly, both in eradication of *L. monocytogenes* (14%) and overall hygiene (25%). The majority (70%) of samples tested negative for either form of *Listeria* and prevalence of *L. monocytogenes* was reduced to 12%. All lines except Line I showed an increase in overall hygiene and four lines were completely rid of contamination with *L. monocytogenes* (Line R, Line G, Line C and Line B).

Line R showed the biggest improvement in both indexes. Before treatment, all drains tested in this line were contaminated with *L. monocytogenes*. After treatment, *L. monocytogenes* was completely eliminated and all samples tested negative. This is a great achievement considering the position of this line within the production hall. As shown in Figure 4, Line R is both easily accessible from the main entrance and the only way for employees and materials to easily reach other processing lines (access through Line I is possible, but difficult). The elevated traffic makes drains in this area more susceptible to contamination, so hygiene practices must be strictly followed.

Line G and Line C were the most hygienic lines overall, since contamination was limited to a single drain and no *L. monocytogenes* was detected. Treatment successfully eliminated *Listeria spp.* from this site while all others remained negative. The lower levels of contamination in these lines may be attributed to the type of items produced. In this factory, almost all water ice desserts are manufactured in Line C, so drains from this line accumulate less organic waste and thus sustain less bacterial growth.

Line B was the most hygienic line after Line G and Line C, with only one contamination in total (Figure 4, drain 72). Bacteria seems to have been transferred from Line H, namely from drain 71, which is directly upstream and also tested positive for *L. monocytogenes**. Drains 73, 59 and 76 seem to have remained uncontaminated by drains 71, 56 and 57 respectively, presumably from being farther away than drain 72. The cleaning treatment successfully eradicated *L. monocytogenes* from this drain, but not from the ones in Line H, which increases the risk of re-contamination. Several measures can be implemented to reduce this risk. Firstly, cone boxes usually placed near this site should be moved to a different location. Secondly, cleaning and disinfection of Line H during production on Line B should be avoided at all costs. This will prevent contamination from the flushing of drains and the formation of aerosols, as both practices aggravate the dissemination of *L. monocytogenes*[22;38;40;51]. Lastly, workers from this line can be advised to reinforce cleaning efforts for this particular drain, ideally before and after production.

Line I revealed no change in eradication of *L. monocytogenes* nor overall hygiene. As shown in Figure 4, samples from this line were collected from two separate zones: freezing (drains 6 and 9) and coating (drains 83 and 86). Contamination was found only in the freezing zone, where both drains tested positive for *L. monocytogenes*, before and after treatment.

These findings are consistent with presence of persistent

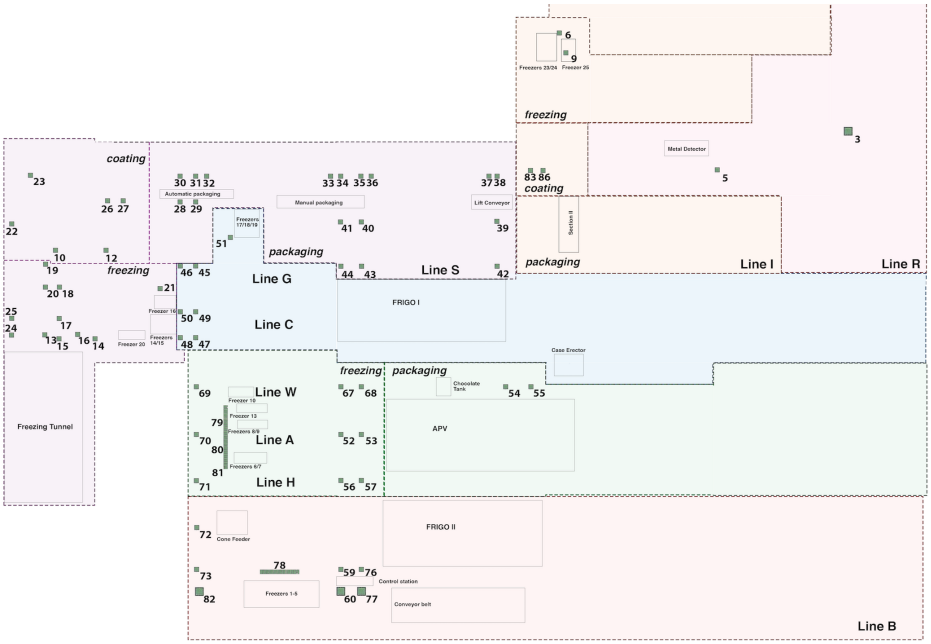

Figure 4: Sampling plan used to investigate the presence and persistence of *Listeria* in floor drains

Table 6: Performance indicators for the application of a deep cleaning treatment in drains of the production hall

		Before treatment (%)			After treatment (%)			Improvement	
		L	LM	Neg	L	LM	Neg	Eradication LM	Overall hygiene
Line R	$n = 2$	0	100	0	0	0	100	2	2
Line I	$n = 4$	0	50	50	0	50	50	0	0
Line B	$n = 8$	0	12	88	0	0	100	1	1
Line S	$n = 34$	39	26	35	29	3	68	8	11
Line G & Line C	$n = 7$	14	0	86	0	0	100	0	1
Line A, Line H & Line W	$n = 14$	42	29	29	21	36	43	-1	2
Grand Total	$n = 69$	29	26	45	18	12	70	10	17

L = Contaminated with *Listeria spp.*; LM = Contaminated with *Listeria monocytogenes*; Neg = Negative; n = No. of samples.

L. monocytogenes, typically found in areas of difficult access which shield the bacteria from cleaning and disinfection chemicals. This seems to be the case, considering the placement of drains in this zone (drain 6 is partially covered and drain 9 is hidden under a freezer).

Elimination of environmental niches requires exceptional effort and additional measures beyond increasing cleaning frequency are usually needed in these cases[38;43]. Some authors consider that changes in equipment design are key to removing *L. monocytogenes* from such sites, but these solutions are often expensive and require long breaks in production to be implemented[11;22]. Furthermore, building work in itself is a potential source of bacteria, and has been shown to aggravate contaminations with *L. monocytogenes*[43]. A different approach described by Melero et al.[37] consists of changing cleaning chemicals periodically to avoid increasing the resistance of *L. monocytogenes* to these products. Other authors have shown that while repetitive exposure of *L. monocytogenes* to sublethal concentrations of disinfectants may increase tolerance, the same chemicals remain effective when used at higher concentrations[38]. Both strategies are easy and cost-effective solutions, and thus should be the first step towards addressing the persistent contamination found in these drains. Regardless of the one employed, eradication should always be confirmed by re-sampling.

Line S samples were collected from three different zones, as shown in Figure 4: freezing (drains 13-20, 25-24), coating (drains 10, 12, 22-23 and 26-27) and packaging (drains 28-44). Before treatment, both *L. monocytogenes* and *Listeria spp.* were isolated from all three zones, and the only area free of contamination was the automatic packaging station (drains 28-32)*. After treatment, prevalence of *L. monocytogenes* was significantly reduced, but almost a third of the drains (32%) remained positive for *Listeria*. In the packaging zone, contamination was eliminated from all sites except one (drain 33). By contrast, in the coating and freezing zones, *L. monocytogenes* was eradicated but other species of *Listeria* were found, indicating poor hygiene practices *. Interestingly, the contaminated drains were located in open areas, while sites with more constrained access (drains 22, 24 and 25) tested negative. These results indicate that the presence of *Listeria* in this line is not caused by long-term persistence, but rather from repeated reintroduction of bacteria. The contamination pattern suggests that movement of personnel plays a significant role in the spread of *Listeria* between drains. Line S is one of the biggest and most complex production lines in this factory, and thus calls for a higher amount of staff during production. The majority of workers are assigned to the freezing and coating zones and tasks usually require them to circulate between the two, unlike the workers assigned to manual packing which are usually sitting down. Several authors have commented on the impact of personnel as carriers of *Listeria*[22;36;37;43]. To address contamination, Jordan et al.[43] recommend extending the application of HACCP principles to the floor of the line by defining critical control areas in the ground that should be access-restricted and clearly marked. While it may be unfeasible to physically restrain the line, warnings placed near the most susceptible drains go a long way in bringing awareness to

workers during production. Educating staff to be mindful of their routes in the food processing area is also an important step towards preventing the spread of bacteria. Lastly, cleaning and disinfection frequency should be increased in this line, namely in the freezing and coating zones where product is exposed.

Line A, Line H and Line W samples were collected from two different zones, as shown in Figure 4: freezing (drains 52-53, 56-57, 67-71 and 79-81) and packaging (drains 54-55). Akin to Line I, contamination was found only in the freezing zone, while all samples from the packaging zone tested negative*. From the metrics shown in Table 6 it is apparent that this group of production lines had the least favourable results of all. Before treatment, contamination was found in most drains sampled (71%) and *L. monocytogenes* was highly prevalent (29%). Comparing to other lines, the percentage of negative results was much lower, under-performed only by Line R. Treatment improved the overall hygiene level by decreasing the number of contaminated drains, but *Listeria* was still detected in most. In fact, this was the only case after treatment where more drains tested positive (57%) than negative (43%). It was also the only instance where the percentage of *L. monocytogenes* increased when compared to the previous samples.

Ultimately, deep cleaning had very little effect on the drains in the freezing zone. Several reasons may account for the treatment's lower efficacy compared to other product lines. On one hand, Line H and Line W have the highest production rates in this factory (Figure 1, c) and thus require more staff intervention during manufacturing. This increases both movement of personnel and job rotation in the line, which favour the re-introduction and spread of *Listeria*[22;37]. On the other hand, consistent positive results from the same area usually indicate long-term persistence[38].

While persistence of *L.monocytogenes* in food production has been extensively documented and environmental niches recognised as a key contributing factor, the mechanisms underlying this phenomenon remain a subject of debate. Some authors have reported persistent strains to have innate genetic and phenotypic traits which enhance survival in the processing environment (e.g. disinfectant resistance, biofilm formation, etc)[17;52;53]. Others maintain that any strain can become persistent in the right environmental conditions[48]. For these production lines, the results are consistent with the first hypothesis. Unlike Line I, where potential bacterial harbourage sites were identified, drains in this freezing zone are readily accessible, making environmental niches a less likely cause of persistence. A genotype of *L.monocytogenes* unique to this location would explain why the treatment failed to reduce contamination. Strains with acquired resistance could have also been carried over from one of the sites found in Line I, as described by Carpentier and Cerf[48]. In these cases, molecular subtyping of *L. monocytogenes* isolates through ribotyping or pulsed-field gel electrophoresis provides valuable insight into the strains responsible for contamination and their source. Genetic characterisation is particularly useful when (i) relationships between isolates from different areas are unclear, (ii) it is difficult to establish whether contamination is due to in-plant persistence or raw materials, and (iii) confusion remains whether samples repeatedly positive for *L. monocyogenes* or *Listeria spp.* are due to recurring sporadic contamination or persistence[45]. This practice has become increasingly more common in the industry and could help to eliminate *L. monocytogenes* from these sites. In the meantime, it is essential that personnel is made aware of these results and every precaution is taken to prevent contamination.

3 Conclusion and Future work

Conclusions This summary has reviewed food safety standards at an ice cream factory in Portugal through a practical case study approach, focused on the three essential elements of food production: equipment, personnel and processing environment.

Firstly, the performance of CIP systems for ageing tanks and continuous freezers was assessed. Monitoring results showed they are highly effective systems for eliminating product residue and microbial contaminants, but in some instances fail to adequately remove cleaning agents. For ageing tanks, it was suggested that loss of fluid pressure could result in decreased rinsing efficiency. For freezers, further studies will need to be undertaken to determine an exact cause. Otherwise, these CIP systems were considered well-suited for the removal of relevant contaminants and successfully validated.

Secondly, the role of personnel as a potential vector in the transmission of allergens and microorganisms was investigated. In contrast with previous reports, the uniforms worn by workers were not recognised as a relevant source for allergen cross-contamination. Hand sanitation protocols were found to be strictly enforced and highly effective in the eradication of enteric bacteria. Sampling of personnel's work boots showed no contamination with pathogenic *L.monocytogenes*, yet prevalence of *Listeria spp.* was significant. Though boot scrubbers are considered the industry standard for the sanitation of footwear, results from this study suggest they may be lacking as a standalone measure. Novel strategies used as a complementary measure would further improve hygiene conditions and prevent the spread of bacteria to the processing hall.

Lastly, the colonisation patterns of *Listeria spp.* and *Listeria monocytogenes* in floor drains of the production environment were studied. The adaptive and pervasive nature of these species makes total eradication unfeasible, but measures can be implemented to reduce the risk of contamination. Some lines will see instant benefits from simple changes in protocols and reinforced hygiene practices. For others, a higher level of investment is required. The annual application of a deep cleaning treatment was also found to significantly decrease the number of contaminated drains. Two niches of persistent *L.monocytogenes* were detected: one promoted by inherent inaccessibility, which reduces the efficiency of the sanitation process; the other probably by strain-specific genetic and phenotypic traits which enhance survival. For the latter, genetic characterisation of isolates is recommended for the development of a targeted eradication strategy.

Overall, results show that hygiene standards at this ice cream factory are generally satisfactory and adequate to the manufacturing of safe and high-quality products. The principles described and solutions proposed in this document may both help to further enhance conditions in this plant and be used as a benchmark for future projects pertaining to the production of safe foods.

Future work Opportunities for future studies were identified in each of the case studies presented. In Case I, CIP monitoring efforts should focus on lowering the incidence of chemical contamination. For ageing tanks, pressure losses along the piping network need to be assessed to increase rinsing efficiency. For freezers, factors such as equipment design, operation parameters, and sampling practices need to be considered. For CIP validation, the preliminary steps of review and verification need to be completed in all production lines. In Case II, the transmission of enteric bacteria through personnel's hands needs to be further assessed using an alternative method, different technique or an established con-

trol. In the transmission of *Listeria* through personnel's shoes, quantification of bacteria will clarify whether the scrubber has a removal threshold and confirm that the equipment does not increase bacterial load in shoes when contaminated. Additional sampling to assess the potential of rubber mats as a source of bacteria is also advised. In Case III, the underlying motives for persistence of *L. monocytogenes* in the freezing zone shared by the Line A, Line H and Line W should be determined, so that an effective eradication strategy could be developed.

Research needs Some of the key research gaps identified in the fields of food processing and safety were: (i) Very few researchers have investigated CIP parameters in continuous freezers, and the existent studies focus solely on microbiological contaminants. Publication of monitoring results in literature targeted at industrial audiences would help other ice cream producers to identify possible faults in design or operation parameters to be optimised. (ii) Several publications have recognised work wear as a source of cross-contamination with food allergens, but none describe their frequency, the mechanisms of transmission involved, or potential control measures. (iii) Several authors have described faults in the use of an industrial shoe scrubber as a standalone measure for the cleaning and disinfection of footwear, but effective alternatives have yet to be found. Novel strategies for cleaning and disinfection of shoe soles would help to prevent colonisation of the processing environment with *Listeria*, recognised as one of the main concerns in the ice cream industry.(iv) While persistence of *L.monocytogenes* in processing environments has been extensively documented, the mechanisms underlying this phenomenon remain a subject of debate. An improved understanding of *L. monocytogenes* persistence will contribute to prevent colonisation of processing environments and thus increase safety standards for food production.

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