TÉCNICO LISBOA

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

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Thesis to obtain the Master of Science Degree in

**Biological Engineering**

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**June 2021**

# Preface

The work presented in this thesis was performed in an ice cream factory in Portugal, during the period October 2018 - May 2019, under the supervision of Eng. Helena Maria Batista. The thesis was co-supervised at Instituto Superior Técnico by Prof. Helena Maria Rodrigues Vasconcelos Pinheiro.

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

# Acknowledgments

I would like to thank the following people, without whom I would not have been able to complete this dissertation:

To my supervisor Eng. Maria Helena Batista, for giving me the opportunity to join her team and the invaluable experience this internship has rewarded me with. Without her, none of this would have been possible. To all the quality experts, engineers, managers, and personnel who work at this ice cream factory, thank you for your kindness, hospitality and cooperation.

To my supervisor at Instituto Superior Técnico, Prof. Helena Pinheiro, for her infinite patience and encouragement, and for always enthusiastically helping in any way she can.

To the examination committee, for the time they invested in the assessment of my work. To Prof. Marília Mateus, for her amazing Food Technology class which first inspired this project.

To my parents, the most generous, supportive and caring people I have ever known. I am so incredibly grateful for everything you have helped me achieve, and so incredibly proud to be your daughter. To my sister Marta Lopes da Costa, the most amazing person in the world and my greatest inspiration.

To my grandmother, Maria Cecília Lopes da Costa, for patiently waiting for her chance to review this dissertation. To my grandmother, Ana Maria Valente Pereira for her unparalleled kindness. To my grandfathers and my uncle, who I wish could have been here today. To all of my friends and family, for the support they have shown me through the last year.

Last but certainly not least, to my boyfriend Diogo Abreu, for his genuine, unconditional and everlasting love.

# 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

# Resumo

Nos últimos 50 anos a indústria alimentar sofreu alterações consideráveis: globalização das cadeias de abastecimento, evolução dos hábitos dos consumidores e mudanças na agricultura causadas por alterações climáticas. Estas transformações exigem uma atualização contínua dos sistemas de controlo de qualidade. Nesta dissertação, os padrões de segurança numa fábrica de gelados em Portugal foram revistos através de uma abordagem prática, baseada na aplicação de casos-estudo aos três elementos fundamentais da produção alimentar: equipamento, trabalhadores e ambiente. Em primeiro lugar, os sistemas de *cleaning-in-place* de tanques de maturação e máquinas de congelação foram avaliados e validados. Posteriormente, estudou-se o papel dos trabalhadores na propagação de microrganismos e alergénios. Os uniformes utilizados não foram considerados uma fonte de contaminação relevante e verificou-se que os procedimentos para a higienização das mãos são eficazes na eliminação de bactérias entéricas. Por contraste, foi detetada uma prevalência significativa de *Listeria* spp. no calçado dos operadores. Por último, investigou-se a presença de *Listeria* nos ralos da sala de produção. No total, encontraram-se 55% de ralos contaminados e 26% positivos para a espécie patogénica *L.monocytogenes*. A aplicação de um tratamento de higienização reduziu o índice de contaminação em 25%, mas não conseguiu eliminar dois nichos de persistência identificados. Em suma, os resultados desta dissertação mostram que a unidade de produção aplica elevados padrões de higiene, adequados ao fabrico de produtos seguros e de elevada qualidade. Os princípios e soluções descritos podem simultaneamente melhorar as condições desta fábrica e servir como base a futuros projetos relacionados com segurança alimentar.

# Palavras Chave

Gelado ; Segurança alimentar ; HACCP ; *Listeria* ; Validação de CIP ; Monitorização

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# Acronyms and Abbreviations

**AFM1** Aflatoxin $M_1$.

**c.f.u** colony forming units.

**C&D** Cleaning and Disinfection.

**CAC** Codex Alimentarius Commission.

**CCP** Critical Control Point.

**CDC** Centers for Disease Control and Prevention.

**CIP** Cleaning-In-Place.

**CL** Critical Limit.

**Cond** Conductivity.

**$\text{Cond}_{\text{RW}}$** Rinse Water sample with conductivity beyond the range of acceptance.

**COP** Cleaning-Out-of-Place.

**EC** European Comission.

**EFSA** European Food Safety Authority.

**Entero** *Enterobacteriaceae*.

**$\text{Entero}_{\text{RW}}$** Rinse Water sample with *Enterobacteriaceae* beyond the range of acceptance.

**$\text{Entero}_{\text{S}}$** Surface swab sample with *Enterobacteriaceae* beyond the range of acceptance.

**EU** European Union.

**FAO** Food and Agriculture Organization.

**FSMS** Food Safety Management System.

**HACCP** Hazard Analysis and Critical Control Point.

**HTST** High Temperature Short Time).

**IgE** Immunoglobulin E.

**KO** Samples beyond the range of acceptance.

**L** *Listeria* spp..

**LM** *Listeria monocytogenes*.

**NASA** National Aeronautics and Space Administration.

**Neg** Negative.

**OK** Samples within the range of acceptance.

**OPC** Open-Plant-Cleaning.

**P&ID** Piping and Instrumentation Diagram.

**$pH+Cond_{RW}$** Rinse Water sample with pH and conductivity beyond the range of acceptance.

**$pH_{RW}$** Rinse Water sample with pH beyond the range of acceptance.

**PMO** Pasteurised Milk Ordinance.

**RTE** Ready-To-Eat.

**RW** Rinse Water sample.

**S** Surface swab sample.

**TVC** Total Viable Count.

**$TVC+Entero_{RW}$** Rinse water sample with Total Viable Count and *Enterobacteriaceae* beyond the range of acceptance.

**$TVC+Entero_{S}$** Surface swab sample with Total Viable Count and *Enterobacteriaceae* beyond the range of acceptance.

**$TVC_{RW}$** Rinse Water sample with Total Viable Count beyond the range of acceptance.

**$TVC_{S}$** Surface swab sample with Total Viable Count beyond the range of acceptance.

**UHT** Ultra High Temperature.

**USPHS** United States Public Health Service.

**WHO** World Health Organization.

# 1

# Introduction

## Contents

## 1.1 Background and motivation

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 ingredients and 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 comprehensive and 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; 4–11].

Several authors have reported an improvement in conditions, costs and overall quality from the application of this program [1; 4; 8–13]. 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; 8].

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 [14; 15]. 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, owing to the strong equity of its brands [16]. In light of these considerations, assessment of food safety standards and revision of HACCP elements in this factory has never been more relevant.

## 1.2 Objectives

This dissertation aims to assess food safety standards at an ice cream factory in Portugal. The primary objective is to determine the potential of contamination from food safety hazards (microbial, chemical and allergens) through equipment, personnel and the processing environment. The secondary objective is to propose corrective actions for non-conformities and solutions for the continuous improvement of sanitary conditions.

## 1.3 Dissertation outline

This dissertation is divided into four chapters: “Introduction”, “Literature review”, “Practical case studies”, and “Conclusions and future perspectives”.

In Chapter 1, the background and motivation for this dissertation are presented. Afterwards, the main objectives are described and the structure of this document outlined.

In Chapter 2, a literature review was performed to provide the reader with a better understanding of the ice cream manufacturing process, elementary notions of cleaning and disinfection, an introduction to the core elements and principles of HACCP and an overview of potential hazards in food production.

In Chapter 3, the concepts presented in the literature review are applied to practical case studies focused on the three essential elements of food production: equipment, personnel and processing environment. This chapter is the core of this dissertation, as the safety and quality standards for this plant are assessed and reviewed. Firstly, the performance of Cleaning-In-Place (CIP) systems for ageing tanks and continuous freezers was assessed. Secondly, the role of personnel as a potential vector in the transmission of allergens and microorganisms was investigated. Thirdly, the the colonisation patterns of *Listeria* spp. and *Listeria monocytogenes* in floor drains of the production environment were studied.

In Chapter 4, a summary table for the three case studies is provided, the conclusions of this dissertation are summarised and future work and further research needs identified.

# 2

# Literature Review

## Contents

## 2.1 Ice Cream Production

### Overview

The manufacturing process of ice cream begins with the weighing and blending of raw ingredients. This creates a liquid mix which is then homogenised, pasteurised, cooled, and stored in an ageing tank. From there, it is pumped to a freezer and whipped vigorously to create a semi-frozen slurry. The subsequent steps of processing and packaging vary according to the final product but usually involve assembly, packaging, and hardening. Toppings such as candy, cookies, fruit pieces, fudge or caramel may be also be added. Lastly, the finished product is transferred to cold storage and distribution. Leftover ingredients, known as ”rework”, may be re-pasteurised and incorporated in subsequent productions to reduce waste and costs [9–11; 15; 17–19].

The plant layout illustrated in Figure 2.1 displays the product flow and steps involved in the production of ice cream at an industrial scale. To guide the reader through this chapter, each operation was numbered after the corresponding section in this document and the corresponding block in the process diagram on Figure 2.2. The real layout for the production hall of this ice cream factory is also supplied in Appendix C.

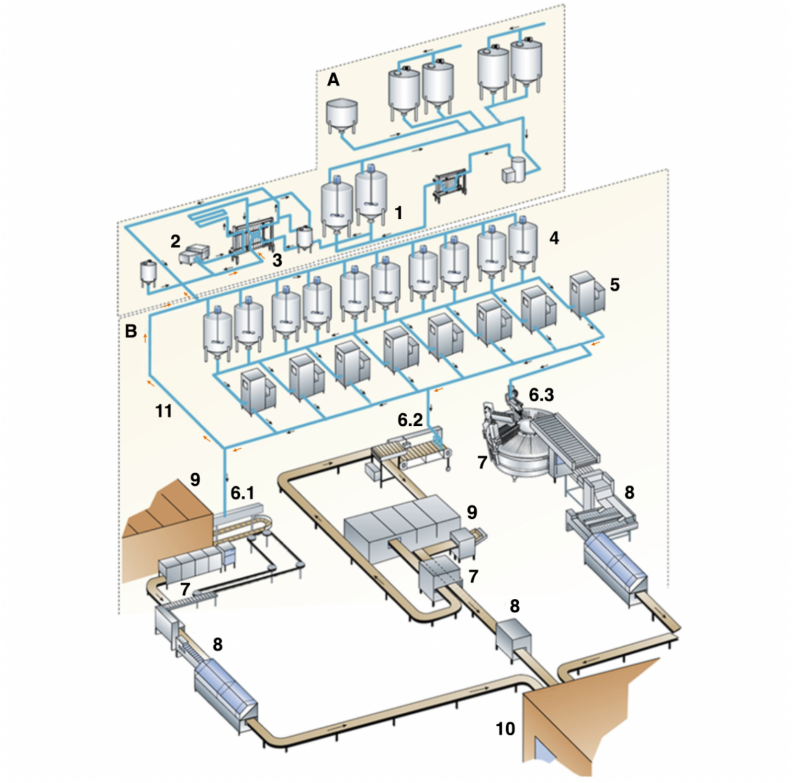


**Figure 2.1:** Illustrated layout of a large ice cream plant. A. Mix preparation room. B. Production hall. 1. Mixing unit 2. Homogeniser 3. Plate heat exchanger 4. Ageing tanks 5. Continuous freezers 6.1 Tray tunnel extruder 6.2 Cup / cone filler 6.3 Rotary bar freezer 7. Addition of flavours, coatings and decorations 8. Wrapping and packaging 9. Hardening tunnel 10. Cold storage 11. Rework [11] [adapted]

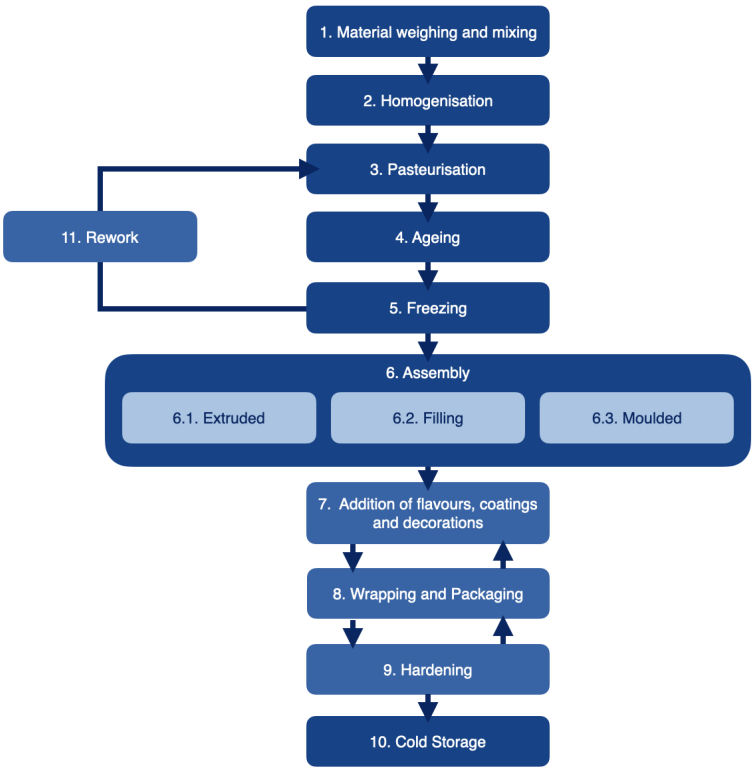


**Figure 2.2:** Process diagram for the production of ice cream

### 1. Material weighing and mixing

The manufacturing of ice cream products begins by weighing the necessary ingredients as specified in the recipe. They are then combined in a mixing vat and stirred together to create an unfrozen liquid mixture, the ice cream mix [9; 11; 20]. The order of addition follows specific guidelines, designed to achieve maximum product quality [18]. These guidelines are described in detail in the Ice Cream Category Good Manufacturing Practices [18], but the most important recommendations are outlined in the following paragraphs. Water is always added first to allow the stirrer to start effectively and enhance agitation throughout the tank. This will help to melt, dissolve and disperse the following ingredients [18].

Stabilisers and emulsifiers are added second, as they need free access to water to solvate properly. Adding these compounds with (or after) other ingredients will increase competition for the water available. Such conditions make it more difficult for the molecules to hydrate properly and thus keep them from functioning as expected. Addition at this stage will also ensure they get the highest temperature possible, further enhancing their performance [18].

Sweeteners, milk ingredients and fats may be added in parallel or in sequence, depending on the capabilities of the plant, provided certain conditions are met. Skimmed milk powder and whey protein are heat sensitive in nature, so they must not be added until the mix temperature is lower than 75 °C. Corn syrup should be added after skimmed milk powder to reduce foaming. Fats will compete with

stabilisers and emulsifiers for water, so they must not be added until these compounds have solvated completely (at the earliest, 3 minutes after stabilisers and emulsifiers) [18]. Most flavours and colourants are too heat sensitive to be added at this stage and will only be introduced after the pasteurisation step. If they need to be incorporated at this time, they should be the last ingredients added [18]. The mix needs to be agitated for 2 minutes after the last ingredient has been added, before homogenisation and pasteurisation can take place [18].

**2. Homogenisation**

Ice cream mix is an oil-in-water emulsion, in which fat globules are dispersed in a continuous aqueous phase. If left to stand, these fat globules tend to rise and collect in a cream layer at the top of the liquid, a phenomenon known as "creaming" [21]. Homogenisation is a mechanical process used to break-up fat globules into smaller, more uniform droplets. This stabilises the mix and prevents separation of the two phases [9; 19; 21; 22].

Three factors contribute to the enhanced stability of the emulsion:

1. Reduction of droplet size
   The creaming velocity of a small, spherical particle in a fluid is given by the application of Stokes' Law (equation B.4), as shown in Appendix B.
   Creaming velocity is proportional to the square of the particle's radius, and thus varies greatly with its size. Smaller fat droplets in an ice cream mix will have less tendency to rise to the surface and separate from the aqueous phase [21].

2. Reduction of droplet size distribution
   If fat droplets are more uniform in size they will rise at a similar speed and have less tendency to cluster during creaming [21].

3. Increase of droplet density
   Milk fat globules have a native membrane, created at the time of secretion, made of amphiphilic molecules (i.e., those with both hydrophilic and hydrophobic sections). Homogenisation disturbs the milk fat globules and destroys their original membrane, but the increase in surface area allows proteins from the aqueous phase to adsorb to the surface. This increases the overall density of the globules, bringing it closer to the density of the continuous phase. It can be seen from the Stokes' law equation (Appendix B) that doing so will decrease creaming velocity and the droplets will have less tendency to aggregate [19–22].

The break-up of fat globules is achieved by forcing the ice cream mix through a small opening at high pressure and velocity, as shown in Figure 2.3 [15; 19; 20; 22]. When the liquid passes through the gap between the valve and the seat, pressure and turbulence increase and a different flow profile is instated. The stability of fat globules suspended in the stream is compromised and they eventually collapse.

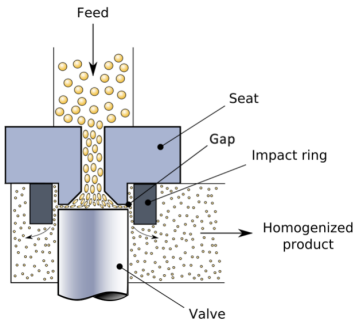


**Figure 2.3:** Homogenising valve [23] [adapted]

Several theories have been proposed to explain the mechanism responsible for the disintegration of these globules (see reference [24] for further detail), but turbulence and cavitation are believed to be the main phenomena involved [22; 24]. Turbulent eddies ("micro whirls") are a specific type of vortical flow pattern in fluid dynamics [25]. During homogenisation, high velocity liquid jets are created when the liquid passes between the valve and seat (Figure 2.3). As they exit the gap, these jets break-up and create energy-rich eddies. If an eddy collides with an oil drop of similar size, the latter becomes deformed and splits into smaller sized particles [22]. Cavitation is the formation of vapor bubbles within the liquid in regions of depressurization and high velocity, such as the gap shown in Figure 2.3. These bubbles implode when leaving the gap, creating shock waves that cause the adjacent oil drops to burst [22; 24].

Homogenisation has a great impact on the overall physical structure of the ice cream mix and sensory perception of the final product. Besides creating a more stable emulsion, smaller fat droplets of narrow size distribution improve the whipping property of the mix, reduce sensitivity to fat oxidation and generate a whiter, more appetizing colour. The adsorbed proteins increase hydration, viscosity and smoothness [9; 11; 19; 20; 22].

### 3. Pasteurisation

Pasteurisation is one of the main biological control points in the ice cream production process, designed to eliminate microorganisms that increase the risk of disease or spoilage. This is achieved by heating the mix to a specific temperature and holding it at that temperature for a certain amount of time [9; 11; 18; 19].

Pasteurisation can be performed using a batch or continuous system. Batch systems have some advantage from an investment and maintenance point of view, but they are more susceptible to error and more difficult to control than continuous systems. For this reason, automated HTST (High Temperature Short Time) is the preferred operation for pasteurisation of ice cream mixes in many factories [18]. With this system, the homogeniser usually plays the role of timing pump, restricting the HTST units to its maximum flow-rate and ensuring the correct holding time.

Using the appropriate time and temperature combination is essential, since excessive pasteurisation will demote the product's flavour and quality, while insufficient heating will be ineffective against pathogens [9; 11]. Based on the recommendation of the United States Public Health Service (USPHS), the minimum standard accepted in many industries is heating at 79.4℃ for 25 seconds, or other time and temperature combination with equal microbiological lethality [18]. It should be noted that pasteurisation is a legal requirement in most countries in addition to being a guideline in specific factories, thus the stricter standard of the two should always be applied [18]. Correctly designed and operated HTST units will provide a minimum 6-log reduction of vegetative microorganisms [18].

**4. Ageing**

Following pasteurisation the ice cream mix must be rapidly cooled until it reaches a temperature of 10℃ or less. This prevents the regrowth of microorganisms and extends longevity. This cooling step should be carried out in a separate plate heat exchanger, since it will take longer to achieve the target temperature inside the pasteurizer vessel [18]. The chilled mix is then transferred to hygienic clean tanks and stored for a minimum of 4 hours. It is during this time that the ageing process takes place [9; 11; 19; 22].

Ageing refers to the chemical and structural transformations that occur in the ice cream mix, in the storage period that anticipates production. This step is essential to develop the sensory attributes associated with high-quality ice cream [18]. During this time, milk proteins and stabilisers are allowed to fully hydrate, fat globules crystallise and their membrane is rearranged (emulsifiers displace proteins from the surface, which destabilises the fat globules and encourages partial coalescence). The result of these changes is a smoother, creamier mix with improved body, texture and viscosity, that exhibits better whipping properties and is more resistant to melting [9; 11; 19; 22].

Since this process takes place after pasteurisation, storage conditions must be carefully controlled to prevent re-growth of microorganisms and subsequent contamination of the final product [9; 11; 18]. Mix temperature should be maintained as low as possible without freezing and the product kept for the shortest time possible, without compromising the aging process (overnight is the recommendation for best results under average plant conditions) [9; 11; 18; 19]. Table 2.1 shows the combinations of temperature and storage time accepted as best practice by this factory. All mixes which exceed the maximum recommended storage time must be either discarded or re-pasteurised. In countries where the maximum storage time is specified in legislation the stricter standard should apply (for example, factories in North America must comply with the Pasteurised Milk Ordinance, which stipulates a maximum storage of 72 hours at 2℃) [18].

Water ice mixes contain no egg or milk products, so the growth of bacterial pathogens is less likely to occur (the main microbiological risk in these cases is spoilage by fungi). Ice Cream Category Good Manufacturing Practices [18] allow water ice to be stored for up to a week, provided that:

**Table 2.1:** Storage times for pasteurised ice cream mix. Reprinted from: Ice Cream Category Good Manufacturing Practices (Internal document GMP15) [18]

| Mix storage temperature (°C) | Maximum safe storage time (hours) |
|---|---|
| 1 | 210 |
| 2 | 130 |
| 3 | 108 |
| 4 | 96 |
| 5 | 84 |
| 6 | 72 |
| 7 | 65 |
| 8 | 58 |
| 9 | 50 |
| 10 | 46 |

- Storage temperature is lower than 5°C;
- Mix pH is lower than 4.0;
- No initial contamination was detected.

Under these conditions growth of bacterial pathogens will not occur and growth of spoilage organisms (including yeasts and moulds) is very unlikely. [18]

**5. Freezing**

The freezing process is responsible for turning the mix into ice cream and defining the final product's consistency, palatability, and yield [15; 18]. It is performed in a special type of refrigeration unit: the freezer. Batch models are used for smaller operations, whereas continuous models (like the one shown in Figure 2.4) are the preferred choice for industrial scale [19; 20]. Ice cream mix enters the freezer though an inlet at the bottom and is pumped upwards to the freezing cylinder, shown in Figure 2.5. The freezing cylinder consists of two concentric tubes. The external tube is protected by a stainless steel cover (jacket) and filled with refrigerant liquid. The inner tube is reserved for the circulation of ice cream mix. At the centre, a rotating unit with a beater, a dasher and knives is mounted.

Once the mix enters the inner tube, it is quickly cooled by the refrigerant and small crystals of ice start to form near the wall. As the dasher rotates, knives scrape the icy layer from the wall and the blender combines it with the warmer mix remaining in the center of the barrel. Initially the icy layer will melt when scraped from the wall, but after some time the bulk of the mix will be cool enough for crystals to survive and grow [19]. The advantage of this method is that it produces ice crystals of very small size, which will increase smoothness and creaminess [15; 19; 20]. While this process occurs, compressed air, termed the overrun, is injected into the cylinder and whipped into the mix. Overrun is necessary to produce desirable body and texture. The incorporated air forms tiny bubbles within the mix, which provide stability and lightness [9; 11; 20]. Figure 2.6 illustrates the structural changes that occur with this type of freezing. Crystallised fat globules frame the air bubbles introduced and help to maintain structure, while ice crystals are dispersed in the unfrozen phase [19; 20; 22].

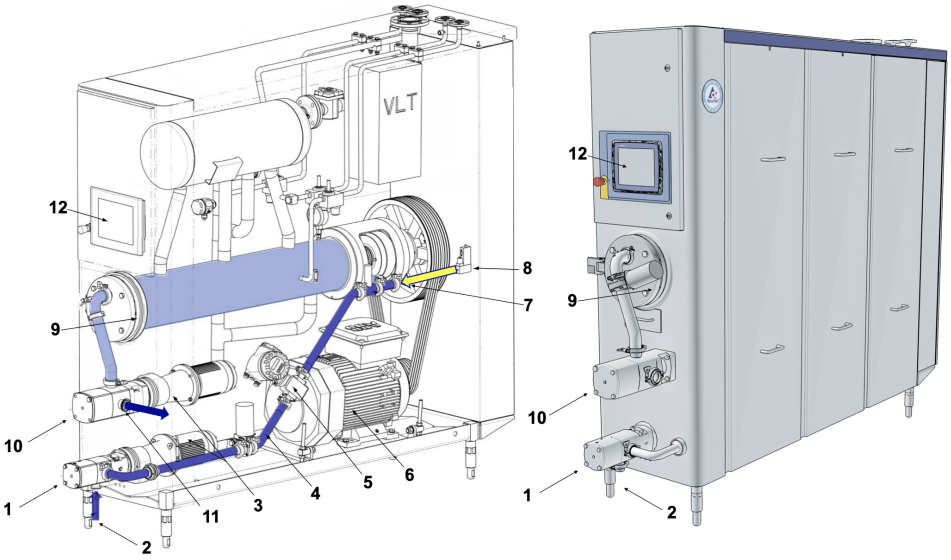


**Figure 2.4:** Continuous ice cream freezer. 1. Mix pump 2. Mix inlet 3. Gearmotors 4. Mix piping 5. Flow meter 6. Dasher motor 7. Air inlet 8. Air controller 9. Freezing cylinder 10. Ice cream pump 11. Ice cream outlet 12. Control panel. Blue: Mix and ice cream circuit. Yellow: Air circuit. [22] [adapted]

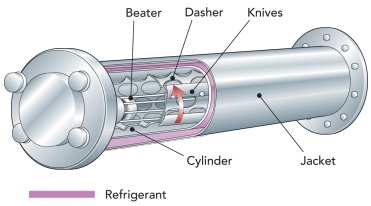


**Figure 2.5:** Continuous ice cream freezer cylinder. [22]

Ice cream that results from such processing is flowable, light, creamy, and soft. The consistency is similar to frozen yogurt or soft-serve desserts (not liquid but also not completely solid). This half-frozen mixture is ideal for assembling and decorating the product before it is packaged [15].

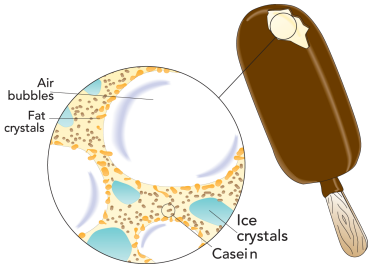


**Figure 2.6:** Ice cream structure after freezing. [22]

### 6. Assembly

Configuration of the production line will differ from this point on, depending on the desired outcome. In terms of assembly method, products will fall into one of the following categories:

### 6.1. Extruded

Ice cream is continuously extruded onto a cold tray and cut as desired, usually with a wire. A variety of products can be produced using this method. Horizontal extruding with vertical cutting is used to produce logs or sandwiches, while vertical extruding with horizontal cutting is used to produce stick novelties with more detailed forms. Additional coatings, flavours and decorations may be added if desired [19; 22].

### 6.2. Filled

Ice cream or water ice is filled directly from the freezer into cups, cones or containers/tubs. Additional flavours and decorations may be added if desired [19; 22].

### 6.3. Moulded

Ice cream or water ice supplied from the freezer is filled into moulds to produce stick novelties. Moulds, usually made from stainless steel, are refrigerated from the outside with a brine solution. After partial freezing, sticks are inserted into the product. An extractor unit grabs the sticks and pulls the ice cream from the mould. Extracted products may be dipped in chocolate or other coatings if desired [19; 22].

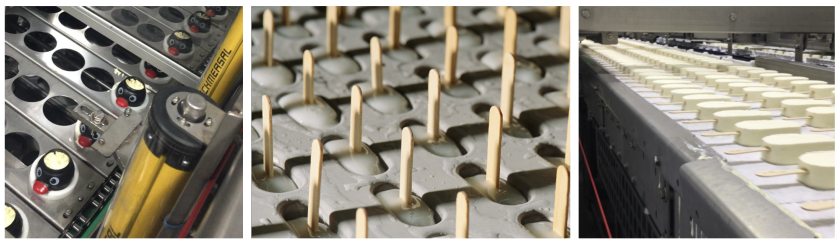

**Figure 2.7:** Ice cream categories in terms of assembly method: Filled (left, original photo), Moulded (centre, [26]), Extruded (right, original photo).

Filling, moulding and extrusion expose pasteurised ice cream to the factory environment, as seen in Figure 2.7. As such, every possible precaution should be taken to minimise the risk of contamination [18; 27].

## 7. Addition of flavours, coatings and decorations

The safest way to introduce materials into the mix is prior to pasteurisation. However, some ingredients are heat degradable or contain seeds which cannot pass through the homogeniser [11; 18]. This type of materials may be added [22]:

- Directly to the ageing tank;
- By in-line dosing;
- By use of flavour tanks in the production hall.

Caution is required to prevent contamination when adding ingredients post-pasteurisation. The use of ingredient dosers is generally recommended as the most hygienic choice (Figure 2.8) [18].

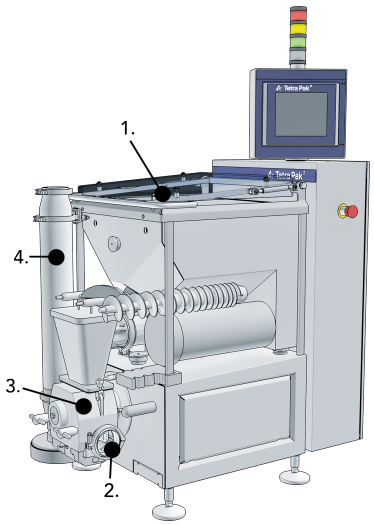


**Figure 2.8:** In-line ingredient doser. 1. Funnel for ingredient 2. Ice cream feed 3. Pump 4. In-line mixer. [22]

In-line dosers allow for the continuous and controlled addition of ingredients to the ice cream. Different types can be accommodated by the feeder [22]:

- Dry ingredients (e.g. nuts, cookies, chocolate chips);
- Soft ingredients (e.g. pieces of fruit, cookie dough, marzipan);
- Liquid ingredients (e.g. sauces, marmalade, jam, caramel).

**8. Wrapping and Packaging**

Wrapping and packaging choices are based on product type and capacity and varying degrees of manual and automatic operations can be employed. The process must be carefully controlled to avoid damages, especially those from temperature abuse. Irreversible quality degradation will occur at this stage if the energy absorbed is sufficient to change the product's organoleptic properties [9; 18; 27].

**9. Hardening**

The manufacturing process is not complete until ice cream has been thoroughly hardened to a core temperature of at least -18℃. This allows most of the remaining water to be frozen and the final ice cream shape to be fixed [9; 11; 15; 18–20; 27].

Hardening is achieved by carrying products through a freezing tunnel at -40℃, where they are flash-frozen with rapid air circulation [15; 22]. Systems can be divided into two groups: those that harden wrapped or packaged product and those that harden naked product. Products that undergo this step before packaging are more susceptible to contamination from the circulating air [18; 27]. Rapid freezing is essential to obtain small-sized ice crystals. When hardening is slow, water still remaining

in the ice cream will migrate to already existing crystals and increase their size [9; 11; 15; 19]. Ideally, crystal size should not exceed the threshold of sensory detection at the time of consumption (40-50 µm) [19].

**10. Cold Storage**

After hardening, products are transferred to cold storage and placed in shelves or pallet racks. Subsequent distribution must maintain these temperatures to protect the shelf life, structure and textural quality of products [15; 19].

**11. Rework**

Rework is defined as finished or unfinished food products returned from any step of the manufacturing process for later reincorporation (e.g. mix leftovers from the freezer can be re-pasteurised and used in the next production) [18; 28; 29]. Rework is considered a valuable foodstuff and high quality ingredient. As such, disposal should be kept to a minimum (both for economical and ethical reasons), while also not compromising the standards and safety of the final products [18; 30].

Factories must have a clear policy for handling and incorporation which contemplates both quality aspects and legal requirements [27; 29]. From a legal perspective, rework is considered an ingredient of the final product, therefore all of its components must be included in the list of ingredients. Warnings such as ”may contain traces of” cannot be used to account for items omitted from this list, since they only concern accidental contamination and the addition of rework is intentional [18]. Special claims like ”organic” or ”made with Madagascan vanilla beams” have an impact on usage too. Rework which is not compliant with the relevant claims must not be used in such products [18]. Traceability must be ensured thorough all stages of the process [18]. From a quality standpoint, precautions must be taken to ensure microbiological safety. Rework must be collected in dedicated containers and kept in a refrigerated room that is not used for storing already pasteurised materials. Incorporation should be performed before the pasteurisation stage to further reduce the risk of contamination. Allergens are also a concern and adequate control of this material is necessary to verify that cross-contamination does not occur [27; 29–31]. Rework containing allergenic ingredients should only be used in products containing the same allergens and segregated from rework free of allergens [15; 28; 29; 31]. All sensory traits of the final product must be safeguarded, including taste, texture, colour and aroma [27].

## 2.2 Cleaning and Disinfection

Cleaning and disinfection (C&D) are a vital part of any manufacturing operation. These procedures maintain the process equipment and factory environment clean and free of contaminants, thus ensuring the quality and safety of the final products [2; 32; 33]. This section outlines the parameters which most influence cleaning and disinfection, the main steps involved, and different types of processes.

### 2.2.1 Parameters of cleaning and disinfection

The efficiency of cleaning and disinfection processes relies mainly on four parameters [22; 34–36]:

- Temperature (higher temperatures tend to favour solubility and thus facilitate dirt removal and microbe elimination);
- Mechanical strength (removal of soil is more effective if the action of the chemical agent is accompanied by a mechanical action, carried out by instruments or turbulence);
- Chemical concentration (concentrated solutions will make the process more efficient);
- Contact time (increasing the contact time between the chemical agent and the soil will lead to a more effective removal).

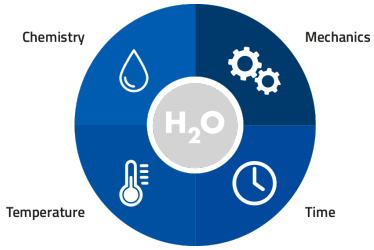


**Figure 2.9:** The four variables of Sinner's Circle [37]

They are often grouped in a circular diagram known as the Sinner's circle, as shown in Figure 2.9. These four variables are interconnected, and thus changing one of them would entail adjusting the other three to achieve the same result. For instance, a cleaning process carried out at a low temperature with little mechanical activity would require a more powerful detergent than one carried out at a high temperature with vigorous agitation. This concept is widely used in the industry and has a significant role in the design, optimisation, and overall assessment of cleaning and disinfection operations.

For further information on the mechanics and kinetics of these interactions readers are referred to Holah et al. [38]; Tamime [39] and Sansebastiano et al. [36].

### 2.2.2 Cleaning and Disinfection Processes

#### 2.2.2.A Fundamental steps

There is no universal program for cleaning and disinfection. Each facility must be considered unique and processes must be adapted to suit the features of the plant, type of food being produced and operations carried out [36]. Generally, a normal cleaning an disinfection procedure will consist of the following steps.

1. Gross soil removal / Purge

   Before cleaning and disinfection operations begin, all gross soil should be removed and placed in suitable containers. Dry methods such as scraping, brushing and shovelling should be used

whenever possible. Hosing waste down the drain should be avoided, since this practice not only increases the risk of clogging but may also spread bacteria to food contact surfaces and other areas of production [32; 40].

Product that is still in the line and is eligible for rework should also be removed at this point. Purging should be performed with clean warm water, at a temperature high enough to liquefy fatty material for easy removal and low enough to keep proteins from coagulating (40-50℃ is recommended) [22; 32].

2. Pre-rinse

   After gross soil is eliminated, surfaces should be rinsed with warm water to release debris. This step should be carried out as soon as possible to prevent increased adhesion from dehydration and prolonged contact [22; 36]. Rinsing removes a considerable amount of dirt and makes subsequent cleaning steps more efficient [36]. Recovered water may be used if not heavily soiled. For greasy residues, a temperature of 40-50℃ is recommended to prevent coagulation of proteins (see previous paragraph) [22; 32; 39; 40]. Pre-rinsing must continue until the water leaving the system is clear, as any loose dirt left will increase detergent consumption during cleaning [22; 32]

3. Cleaning

   During this step the remaining food soil is removed with a solution of water and detergent [35; 36]. Water used at this stage should be potable, clear and colourless. A low mineral content is preferred to avoid interactions that compromise the efficacy of cleaning compounds [36]. Adding a detergent will lower the surface tension of the water, which helps to detach residues, encapsulate suspended particles and prevent flocculation [22; 36; 39]. A suitable product should be selected based on the type of soil and equipment, as improper chemicals can fix soils and make them more difficult to remove (e.g., acid cleaners can precipitate protein). A good rule of thumb is to use acid cleaners to dissolve alkaline soils (minerals) and alkaline cleaners to dissolve acid soils and food waste [35]. In automated systems the first 50 liters of solution should be drained, since they contain 90% of the detached food residues. The remaining liquid can be recuperated for re-use and losses made-up by adding concentrated detergent to the solution tank, maintaining the target concentration [32]. Besides cleaning the surfaces they come into contact with, detergents will also reduce the total microbial load which favours the subsequent disinfection step (Figure 2.10) [36; 40].

4. Post-rinse

   After cleansing with detergent solution, surfaces must be flushed with potable water at room temperature to remove all traces of cleaning product [32; 36; 39]. Softened water is preferred to prevent deposition of lime scale on the cleaned surfaces. Hard water with a high content of calcium salts must therefore be softened in ion exchange filters [22]. In automated systems the liquid may be recovered for use at the pre-rinsing stage in the next cleaning cycle [32; 39]. Effective rinsing is essential to remove any organic residues and optimise the disinfecting procedure.

5. Disinfection

The purpose of disinfection is to reduce microbial load and eliminate pathogens from food contact surfaces [34; 36; 38]. Processes can be classified into two categories [22; 34–36]:

- Thermal disinfection (involves the use of boiling water, hot water, or steam);
- Chemical disinfection (involves the use of an approved chemical agent, such as chlorine, acids, iodophors, hydrogen peroxide).

Novel disinfection technologies, such as electrolysed water and ozonated water, have also emerged in recent years as more environmentally conscious alternatives to traditional chemical agents [5; 34; 41].

Several factors need to be considered when selecting a disinfection method. For use in food processing equipment, the disinfectant must be non-toxic to humans and convey no offensive odour to the food. It is also important that it does not corrode or damage the equipment. If chemical agents are used, they must be stable enough to remain active during the process, but not so long that they become an environmental hazard when discarded. Lastly, the method selected must be compatible with the level of decontamination required [34; 36]. Production of certain dairy products (e.g. UHT milk, infant formula) requires sterilisation of the processing equipment (i.e. elimination of all living microorganisms, viable spores, viruses, and viroids). For ice cream, a disinfection process capable of eliminating vegetative bacteria and reducing microorganisms to a level that does not compromise food safety is considered sufficient (Figure 2.10) [32].

6. Final rinse

   Lastly, a final rinse with cold potable water should be performed to eliminate any chemical residue [22; 36; 38]. The efficiency of this process can be confirmed by measuring the conductivity and pH of rinse water. Since cleaning and disinfection solutions are more conductive and alkaline/acidic, the equipment is considered free of chemicals when rinse water values are the same of incoming water [32; 34].


**Figure 2.10:** Reduction of microbial load through cleaning and disinfection [34]

#### 2.2.2.B Types of processes

Based on the level of automation and disassembly required, cleaning and disinfection processes can be grouped into three categories:

**Cleaning-In-Place (CIP)**

CIP is an automatic cleaning method where water and chemical agents are circulated through equipment and process lines without dismantling or opening. The passage of the high-velocity liquids generates a mechanical scouring effect that promotes the removal of dirt deposits. It is a system engineered to provide fast and consistent cleaning and disinfection of product contact surfaces to a predetermined level [5; 15; 22; 32; 34; 36; 39; 42].

CIP units vary in complexity and degree of automation, hence their efficiency and cost-effectiveness are also variable. In single-use CIP units, water and cleaning chemicals are flushed to drains immediately after use. This type of process considerably reduces the potential for contamination but tends to be expensive to operate. Such units are generally implemented in plants with higher levels of soil [22; 34; 36; 39; 42]. In recovery CIP systems, water and cleaning chemicals are partially retrieved and stored in tanks to be reused. This step makes the process more economical, but close monitoring is required to prevent soil build-up. Recovery systems are used in processing plants where equipment is not heavily soiled and pre-rinsing successfully removes a high percentage of dirt [22; 34; 36; 39; 42]. CIP units have several benefits over manual cleaning methods [36]:

1. Reduced maintenance costs;
2. Optimised C&D process;
3. Reduced variability of C&D and increased standard of hygiene for the system;
4. Reduced duration of stopping times in production and the possibility of increasing the frequency of the cleaning procedures;
5. Elimination of the risk of damage due to disassembly of the mechanical parts to be cleaned;
6. Elimination of the risk of re-contamination of the equipment in the assembly stage;
7. Reduced risk for the personnel involved, namely in the handling of dangerous substances or access to the parts of the system for cleaning (e.g. tanks).

As such, this is currently the preferred method for cleaning and disinfection operations and should be implemented whenever possible [18; 36; 42]. In the ice cream industry, most large pieces of equipment are now CIPable. However, some components such as ingredient feeders still have to be disassembled for adequate cleaning [36].

**Cleaning-Out-of-Place (COP)**

COP is used for smaller equipment which cannot be cleaned through CIP. Parts are manually disassembled and cleaned in COP baths, special pressurised containers where hot solutions are used, together with turbulence and pressurised chemical agents [15; 32; 36]. The type of COP equipment varies from manual to fully automated. In manual types, cleaning results rely solely on the performance of the operator. In a fully automated COP tank, reproducible cleaning results can be achieved if the process is well optimised [32]. Since thermal disinfection is not practical in this case, a separate chemical

disinfection step should be carried out [32; 36].

**Open-Plant-Cleaning (OPC)**

For open equipment that cannot be disassembled and surroundings (e.g., floors, walls, ceilings), cleaning is performed through OPC. Procedures and products used are tailored to the plant's equipment and conditions (i.e. dry or wet environment). In the ice cream industry, it usually consists of applying specially formulated gels/foams and rinsing with water at low pressure[32]. In OPC it is critical to prevent the formation of aerosols, since these particles promote the spread of bacteria and allergens, increasing the risk of contamination [18; 32; 43–45].

## 2.3 Food Safety Management and HACCP

### 2.3.1 Hazard Analysis and Critical Control Point (HACCP) System

#### 2.3.1.A Introduction to HACCP

Food Safety Management Systems (FSMSs) are networks of interrelated elements designed to ensure that food does not endanger the health of consumers [29]. Hazard Analysis and Critical Control Point (HACCP) is a food safety management system (FSMS), used to identify, prevent and control all safety hazards associated with production of a particular food [9; 12; 13; 29; 46]. Described by Arvanitoyannis and Kassaveti [12] as the original FSMS, it was created by NASA and the Pillsbury Company in 1959 to produce astronaut-safe meals for the Mercury, Gemini and Apollo spaceflight programs [4; 12; 13; 33; 47]. The HACCP system was made public in 1971 at the Conference of Food Protection and recommended for general use, but it was not fully implemented until the 1990s, after an outbreak of *Escherichia coli* from raw ground beef infected hundreds [12]. Over the past decades, HACCP has become widely accepted by food manufacturers and relevant authorities, with many countries adopting its principles as legal requirements [4]. Nowadays, it is considered an instrumental tool in food quality and safety in nations all over the world (United States of America, United Kingdom, European Union, Japan), as well as international organizations such as the World Health Organization (WHO), Food and Agriculture Organization (FAO), and Codex Alimentarius Commission (CAC) [9; 11].

Wright and Teplitski [47] credit the success of HACCP to its proactive nature. Classical quality control methods focus solely on the hygienic quality of the final product and thus fail to control contamination at earlier stages of the manufacturing process [10; 13]. In contrast, HACCP takes the whole chain of food production into consideration and tackles safety hazards before they compromise the quality of final products [10; 47].

The implementation of the HACCP system has been reported to be an efficient and cost-effective approach to food safety regulations [4; 9; 46]. Various studies on the application of HACCP programs in

ice cream factories have proven to have positive effects on the safety and the quality of the final product [1; 8–11; 48; 49].

#### 2.3.1.B Principles and Elements of HACCP

As the name suggests, HACCP programs consist of two major components: Hazard Analysis and Critical Control [1; 4; 9; 11; 33; 46; 50].

**Hazard Analysis:** Process of assessing the entire food production process and identifying potential factors that may render products unsafe.

**Critical Control:** Process of devising control measures to eliminate or reduce the identified hazards to an acceptable level. The application of these control measures is performed at key points where loss of control compromises the safety of food products, defined as critical control points (CCP). The acceptable levels to which hazards must be controlled at these points are defined as critical limits (CL).

Implementation of HACCP in a food production process is achieved through the application of the following seven principles, described in the *Codex Alimentarius* [1; 2; 33; 50]:

Principle 1. Conduct a hazard analysis

Principle 2. Identify the critical control points in the process (CCPs)

Principle 3. Establish critical limits (CL) for the preventative measures associated with each CCP.

Principle 4. Establish a system to monitor and maintain control of each CCP.

Principle 5. Establish corrective actions to be taken when monitoring indicates that a particular CCP is not under control.

Principle 6. Establish verification procedures to confirm that the HACCP system is working efficiently.

Principle 7. Establish documentation and record-keeping procedures concerning all operations and the application of these seven HACCP principles.

#### 2.3.1.C Monitoring, Verification and Validation

Monitoring, verification and validation are core concepts of the HACCP system and key activities to guarantee the safety of food products. The National Advisory Committee on Microbiological Criteria for Foods [1] and the Codex Alimentarius Commission [2] define them as follows:

- Monitoring: Consists in routinely collecting data from critical points to ensure that the system is under control and to produce an accurate record for future use (see HACCP principles 4 and 5).
- Verification: Consists in confirming through documentation that the HACCP plan is followed as outlined (see HACCP principle 6).
- Validation: Consists in collecting and evaluating scientific and technical information to determine if the HACCP plan is capable of effectively controlling hazards.

## 2.4 Hazards in food production

Hazard analysis is considered the foundation of a HACCP plan and the first step towards its implementation. However, before one can assess the risks, a working knowledge of potential hazards must be obtained [50; 51]. Hazards can be categorized into four general areas: physical (external particles), chemical (toxic substances), microbiological (pathogens), and allergens.

### 2.4.1 Physical

Physical hazards include any foreign object with the potential to cause harm or injury. These items may be introduced in food by accidental contamination (e.g. plastic pieces) or be naturally present and removed during processing (e.g. bone fragments) [1; 33; 50; 52]. This category encompasses a wide variety of materials of different origins. A summary of the main physical hazards in food processing and their sources is provided in Table 2.2. Ice cream manufacturers must also take into account promotional items intentionally included in the product (e.g. toys), especially when it is intended to be consumed by children.[18]

**Table 2.2:** Main physical hazards of food processing and common sources [50] [adapted]

| Source | Examples of physical hazards |
|---|---|
| Raw Materials | Seeds, Pits, Leaves, Bark, Bone fragments, Feathers, Insects, Shells |
| Packaging | Wood splinters, Plastic pieces, Metal clips, Glass fragments, Cardboard, String |
| Personnel | Buttons, Coins, Jewellery, Hair, Fingernails, Band-aids, Pens |
| Equipment | Screws, Metal bolts, Paint chips, Rust, Duct tape, Belt frays, Rubber pieces |
| Environment | Dirt, Sand, Stones, Insulating material, Pests, Rodent droppings |

The strategies employed for the control of foreign material are as diverse as their sources: metal detectors and magnets can be used to find metal objects; X-ray inspection systems are available for stone, bone and glass; recent developments in phase contrast imaging provide enough level of discrimination to detect insects, hair and vegetable matter [1; 4; 10; 52]. Manufactures usually use an integrated approach, combining two or more of these strategies [52]. Sourcing units should also have an effective pest control program in place [1; 10; 18]

Risk assessment and control of physical hazards in ice cream production is outside of the scope of this dissertation. For further information on this topic readers are referred to Mortimore and Wallace [1]; Papademas and Bintsis [4]; Drosinos and Siana [33]; Varzakas [46]; Arvanitoyannis et al. [48]; Pierson and Corlett Jr. [50]; Keener [52].

### 2.4.2 Chemical

Chemical hazards include any compounds that may cause illness or injury, either from immediate or long-term exposure. They can be separated into 2 categories [50]:

1. Naturally occuring chemicals

   This category includes poisons and toxins that are naturally present in food and not the result of environmental, agricultural or industrial contamination [12; 51]. Examples can be found in plants, animals and microorganisms. Many species of wild mushrooms produce toxins which make them poisonous to humans. Shellfish may accumulate toxins produced by the microscopic algae on which they feed. Fish that have been temperature abused contain high levels of histamine, which cause an intoxication reaction [1; 50; 51]. Certain moulds produce secondary metabolites (mycotoxins) which cause acute toxic reactions and have long-term carginogenic effects [1; 50; 51; 53]. In the ice cream industry, presence of cow milk contaminated with the mycotoxin AFM1 is of particular concern, since this compound has been shown to resist standard pasteurisation treatments [53].

2. Added chemicals

   This category refers to substances that are added to foods at some point between growing, harvesting, processing, storage, and distribution. These chemicals are generally not considered hazardous if proper conditions of use are followed. However, when misapplied or when their permitted levels are exceeded they become potential hazards [50; 51]. Examples include compounds used in agriculture and farming (e.g. pesticides, herbicides, fungicides, antibiotics), heavy metals (e.g. lead, zinc, copper, cadmium, mercury, arsenic, antimony), food additives (e.g. preservatives, nutritional additives, colourants, flavours) and factory chemicals (e.g. refrigerants, solvents, acids, lubricants, cleaning and disinfection chemicals) [1; 18; 50; 51].

### 2.4.3 Microbiological

Microbiological contaminants include a wide variety of microbes (protozoa, algae, bacteria, fungi, and viruses), as well as any toxins or metabolic products produced by them [3; 12; 33; 51]. For some types of food, a permanent and stable microflora is desired as part of the production process (e.g. some in-house strains of bacteria are believed to contribute to fermentation). However, production processes usually require low contamination to ensure safe, high-quality products [51; 54]. Most pathogens, virus and protozoa can be eliminated through the application of an adequate heat-treatment, such as pasteurisation. As such, precautions must be taken to prevent post-pasteurisation recontamination, notably through dosed ingredients and the processing environment [15; 33; 43; 55]. Microbial spores (e.g. from *Bacillus cereus*) and heat resistant toxins (e.g. *Staphylococcus aureus* enterotoxin) which are able to survive pasteurisation must also be considered. The most relevant microorganisms to be

controlled vary strongly with product category and geographical region [7]. Pathogenic organisms most associated with ice cream and dosed ingredients include [15; 18]:

- *Salmonella*;
- *Escherichia coli*;
- *Listeria monocytogenes*;
- *Bacillus cereus*
- *C*oagulase positive *Staphylococci*.

#### 2.4.3.A Concept of indicator microorganisms

Routine examination of environmental samples for multiple pathogens is often expensive, complex, and time-consuming. One way to overcome the problems inherent to specific pathogen detection is through the use of indicator microorganisms [3; 43; 56].

The indicator concept relies on the fact that certain non-pathogenic bacteria are common to the excrements of all warm-blooded animals. These microorganisms can often be isolated and quantified by simple methods, more easily than pathogenic microbes. Presence of these bacteria suggests that fecal contamination has occurred, and thus that enteric pathogens can be present [43; 56]. Indicators may also provide additional insight on changes in the micro-flora or conditions of the environment [43]. Depending on the goal of the monitoring project, various groups of microorganisms have been suggested and used as indicators of contamination. The microbiological counts most frequently used as indicators of good hygiene in ice cream production are the Total Viable Count (TVC) and the *Enterobacteriaceae* or coliform count [15; 18]. Recommended values are is usually included in specifications and legislation covering bacterial standards (e.g. the EU regulations for microbiological criteria of foodstuff (EC) No 2073/2005 [57]).

#### 2.4.3.B Total Viable Count (TVC)

The Total Viable Count (also called Aerobic Colony Count or Standard Plate Count) is one of the most widely used and simple tests in food microbiology [38; 58]. It refers to the enumeration of viable microorganisms, capable of growing aerobically in a non-selective medium at mesophilic temperatures [54; 58]. This test is often used to monitor levels of bacteria after a cleaning process as a general indicator of the overall standards of hygiene. However, it does not allow the distinction between pre and post-pasteurisation contaminants because bacterial spore formers included in this count survive the pasteurisation process [18; 54].

#### 2.4.3.C *Enterobacteriaceae* and Coliform Counts

Coliform bacteria occur naturally in the intestines of all warm-blooded animals and are excreted in great numbers in feces. This group has been used as the standard for assessing fecal contamination for almost a century [56; 59]. It is comprised of nonspore-forming Gram-negative rod-shaped bacteria that produce gas upon lactose fermentation in prescribed culture media (within 48 h at 35 ℃). Examples include species from the *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* genera [56].

*Enterobacteriaceae* is a larger family of nonspore-forming Gram-negative rods, of which coliform are a sub-group (Figure 2.11) [15; 56]. Members can be trivially referred to as enteric bacteria, since several species live in the intestines of animals.

The choice between the two indicators depends on the level of detection desired. In plants that experience very low counts of coliform bacteria, a more rigorous test for contamination may be required. Adding of 1% glucose to coliform culture media allows any member of the *Enterobacteriaceae* family to grow, which increases the test's sensitivity [15].

All members of *Enterobacteriaceae* are heat sensitive, thus the presence of these bacteria in final product is a valuable indicator of ineffective pasteurisation, possible fecal contamination and overall poor hygiene standards [15; 18; 54].

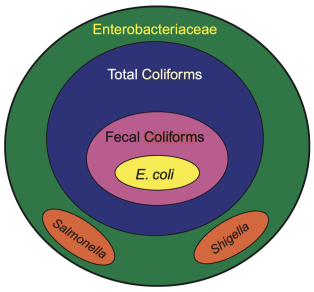


**Figure 2.11:** Relationship between coliforms and *Enterobacteriaceae* [56]

#### 2.4.3.D *Listeria* spp. and *Listeria monocytogenes*

The concept of indicator microorganisms can also be applied to a specific pathogen, rather than several genera. Attributes for the scientific support of an indicator in this context are described in the *Codex Alimentarius* as follows:

- Similar survival and growth characteristics;
- Shared common source for both organisms;
- Direct relationship between the conditions that promote the presence of the pathogen and the indicator organism;
- Practical isolation, detection and enumeration methods for the potential indicator organism [2].

In ice cream production, *Listeria* spp. is commonly used as an indicator of *Listeria monocytogenes*. *Listeria* in general and *L. monocytogenes* in particular are also used as hygiene indicators in all stages of the food processing chain [18; 60; 61]. As of 2020, the genus *Listeria* contains 21 species, but only *L. monocytogenes* causes disease in humans [62].

*Listeria monocytogenes* is a gram-positive, non-sporeforming bacteria, and one of the most concerning pathogens in the food industry [2; 14; 44; 45; 63–68]. It is the microorganism responsible for the bacterial infection listeriosis. In healthy adults, the disease is relatively uncommon and usually presents with gastroenteritis and fever. However, for patients in high-risk groups (pregnant women, newborns, immunocompromised people and the elderly) symptoms are far more severe and include sepsis, infection of the central nervous system, and death. In pregnant women, it can cause stillbirth or spontaneous abortion and preterm birth is common [59; 63; 64; 66; 69; 70].

While prevalence of listeriosis is fairly low, it ranks third among foodborne diseases in overall number of deaths, with a fatality rate of 15-20% [67; 69]. Its severity makes *L. monocytogenes* one of the most dangerous bacteria in the food industry, more lethal than other common pathogens such as *Salmonella spp.* and *Campylobacter spp.* [64; 71]. In the United States, the CDC (Centers for Disease Control and Prevention) estimates there are 1600 cases of listeriosis every year, 260 of which fatal [72]. Regulatory initiatives implemented between 1998 and 2008 have reduced outbreaks from meats and poultry, but outbreaks from dairy products have shown no decrease in frequency [67]. In the European Union, the EFSA (European Food Safety Authority) has also reported an upward trend of listeriosis in recent years, with 2536 cases confirmed across 28 member states in 2016, causing by far the highest number of deaths related to food-borne illness [71].

Because of its adaptive and ubiquitous nature, the control of *L. monocytogenes* has some challenges [66]. While most bacteria do not grow in temperatures below 4 ℃, *L. monocytogenes* cultures have excellent tolerance for cold environments and are able to multiply in refrigeration temperatures (-1.5 ℃ to 10 ℃ range) [59; 69; 70; 73]. Cultures are also able to grow in a wide range of pH values (4.4 to 9.4) [66; 73] and have been shown to resist the antimicrobial effect of sanitising products commonly used in the food industry [14; 73; 74]. Conversely, *L. monocytogenes* is sensitive to heat and will rarely survive temperatures higher than 60 ℃. In most cases, the cooking processes will destroy any bacteria that may be present, but since ready-to-eat foods do not require preparation before consumption they pose an increased risk of infection. In fact, most listeriosis outbreaks have been associated with such items [59; 61; 64; 65; 73]. This makes ice cream a high risk product for food poisoning with *L. monocytogenes* and further emphasises the importance of avoiding contamination during production.

### 2.4.4 Allergens

Allergens are a specific type of antigen (usually a protein) capable of triggering an intense immunological reaction in some people [29; 75]. Over 170 foods are known to be allergenic, but only a handful

of these are responsible for the vast majority of food intolerances [28; 30; 75]. The following list outlines the 14 allergens known as the most common sources of food intolerance (also shown in Figure 2.12):

1. Cereals containing gluten (wheat, rye, barley, oats, spelt, kamut) or their hybridised strains and products thereof;
2. Crustaceans and products thereof;
3. Eggs and products thereof;
4. Fish and products thereof;
5. Peanuts and products thereof;
6. Soybeans and products thereof;
7. Milk and products thereof (including lactose);
8. Nuts and products thereof (almonds, hazelnuts, walnuts, cashews, pecan nuts, Brazil nuts pistachio nuts, macadamia or Queensland nuts);
9. Sulphur dioxide and sulphites (over 10 mg/kg or 10 mg/litre in total $SO_2$);
10. Lupin and products thereof;
11. Molluscs and products thereof [29; 75].


**Figure 2.12:** The 14 most common food allergens. [76]

The expression ”food allergy” is often used as an overarching term to describe any adverse reaction to food [28; 30]. Although symptoms can be similar, risks from a true food allergy are much more serious than those of food intolerance, so it is important to differentiate the two [77] (Figure 2.13).

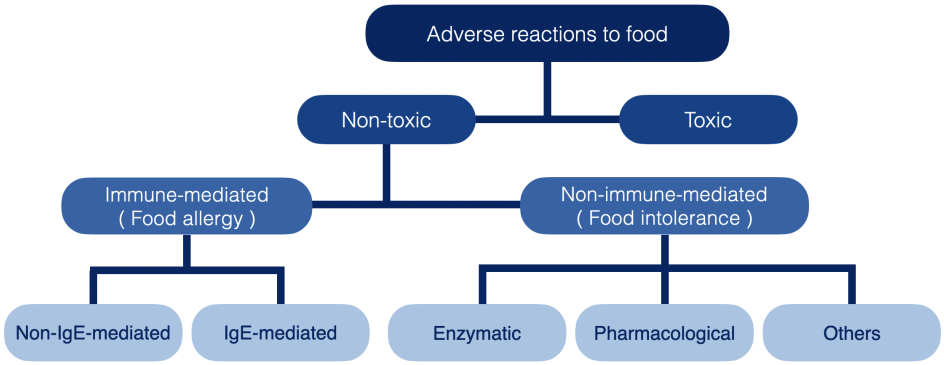


**Figure 2.13:** Classification of adverse reactions to food

Adverse reactions to foods may be toxic or nontoxic. Toxic reactions are not related to individual sensitivity but occur in anyone who ingests a sufficient quantity of tainted food (e.g. puffer fish poisoning by tetrodotoxin or accidental ingestion of bacterial toxins in food) [1; 78]. Non-toxic reactions depend on individual susceptibility and are either non–immune-mediated (food intolerance) or immune-mediated (food allergy) [28; 78; 79]. Food intolerances are adverse reactions which typically involve the digestive system. They can be caused by the pharmacologial action of certain ingredients (e.g caffeine in coffee, phenylethylamine in chocolate), enzymatic factors specific to the individual (e.g. lactose intolerance) or other elements (e.g. clinical symptoms of eating disorders). Such reactions can make the consumer feel extremely unwell, but symptoms are not severe. [28; 29; 79]. Food allergies involve a reaction from the immune system and symptoms can vary from mild to life-threatening. IgE-mediated reactions are the most common and usually have a rapid onset, while non-IgE-mediated have a more delayed response, hours or days after exposure (e.g. celiac disease) [28–30; 75; 79; 80].

Symptoms of food allergies include one or more of the following [28–30; 75; 80]:

- Skin problems (itching, rash, hives, dermatitis, eczema, swelling of lips, conjunctivitis);
- Respiratory tract (difficulty breathing, asthma, rhinitis, swelling of the throat);
- Gastrointestinal problems (nausea, stomach ache, vomiting, diarrhoea).

A systemic response can occur in severe allergies, leading to anaphylaxis (sudden drop in blood pressure, severe constriction of the airways, and multiple organ failure) and possibly death. While only a small number of people with food allergies have such extreme reactions, multiple cases of death resulting from unintentional ingestion of an offending food have been documented [29; 30; 81].

Food allergies and intolerances have increased significantly over the past 30 years, but accurate incidence rates are elusive [28; 77; 79]. Different numbers have been reported in literature, influenced by study populations, geographic region, methodology and type of pathology. The European Food Safety Authority [82] performed a comprehensive literature review on the prevalence of food allergies and most publications report prevalence rates of 4-5% in adults and 7-8% in children (for further detail readers are referred to that report). Statistics also indicate that children tend to outgrow most of their food allergies by the age of 5-7 (namely milk, eggs, and soy) but some tend to persist (peanut, shellfish, fish) [29; 75].

For all practical purposes, there is no cure for these reactions and consumers must avoid foods containing the ingredients to which they are allergic or intolerant [30; 75; 79]. To that end, they rely on food manufacturers to produce safe food products, with labels that accurately communicate the presence of allergens [81].

# 3

# Practical case studies

## Contents

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

### 3.1.1 Background and motivation

Cleaning and disinfection is a vital part of any manufacturing operation to ensure the quality and safety of the final products. Effective processes 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; 5–7]. As such, it is indispensable to periodically review cleaning programs and ensure they remain effective and compliant with the established HACCP plan [2; 6; 36]. 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 [38; 39]. Assessment of CIP processes has hence become an essential part of cleaning and disinfection operations [22; 36].

Monitoring refers to the routine measurements performed after cleaning and disinfection that serve as indicators that these processes are in a state of control [6; 36; 38; 39]. 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 [6; 36].

Validation studies are key activities to assess cleaning and disinfection operations and thus an instrumental part of any plant's HACCP program. A thorough study provides evidence that protocols in place are effective at controlling relevant hazards (microbiological, chemical, physical and allergens) and should be repeated on a regular basis to maintain a seamless and reliable quality assurance regime [1; 6; 29; 39; 83]. Ice Cream Category Good Manufacturing Practices recommend that a formal evaluation be performed once a year, or in case of any major alteration to the process. When completed, a formal report must be prepared, stating that the cleaning and disinfection process was successfully validated [6; 18].

While cleaning and disinfection validation is not necessarily difficult, it is nonetheless an intricate and lengthy project which requires the cooperation of several different departments (Production, Quality Assurance, Maintenance, etc.) [1; 6; 39; 83]. To perform a successful C&D validation, it is helpful to structure all of the tasks involved into steps and create a detailed master plan that prevents insufficient or overlapping work. A structure similar to that shown in Figure 3.1 is adopted in many cases [83].

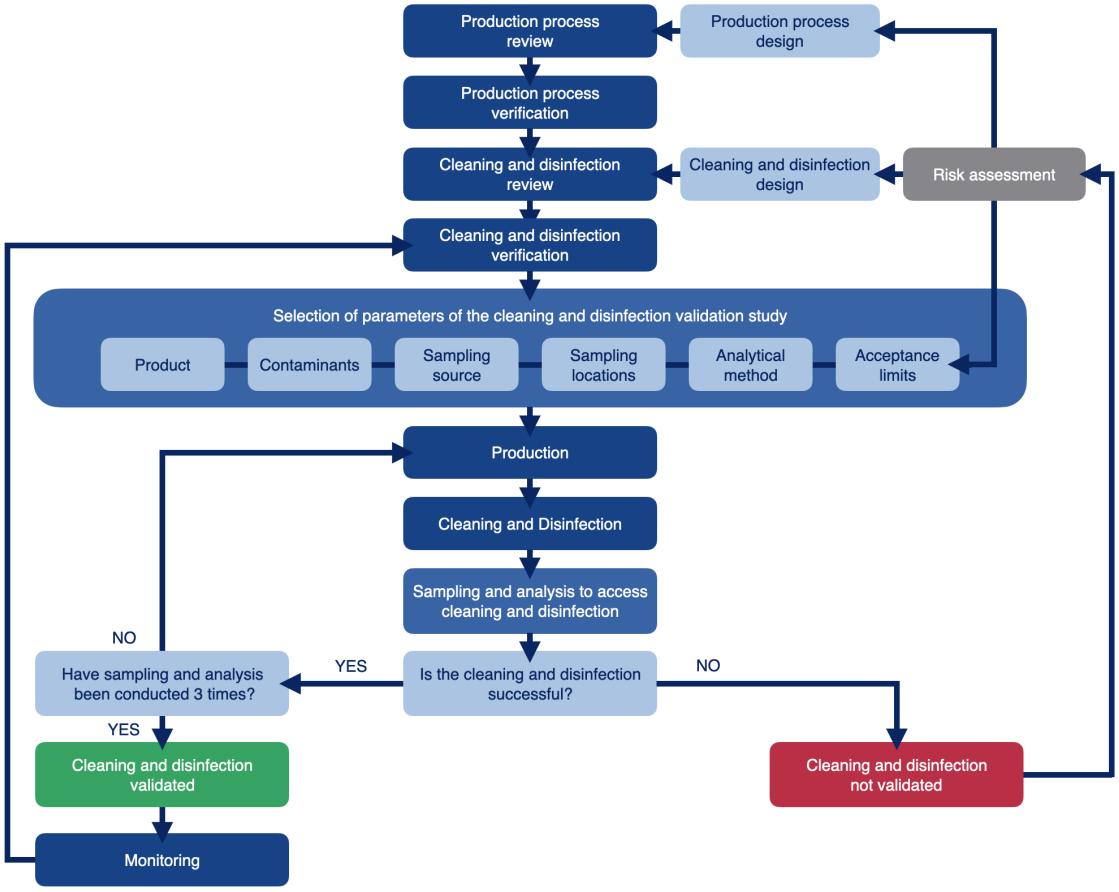


**Figure 3.1:** Cleaning and disinfection validation master plan adopted by the company and used in this study [6] [adapted]

Generally, validation studies can be divided into two parts. The first one consists of a preliminary review and verification of cleaning and production operations, to ensure that both are compliant with documentation. The second one consists of the the validation exercise itself.

The validation master plan should contain a detailed description for each of the steps shown in Figure 3.1. The guidelines adopted by the company studied are documented in the Cleaning and Disinfection Validation Master Plan [6], but the most important recommendations are outlined in the following paragraphs.

Part I - Preliminary review and verification of production and cleaning and disinfection

Step 1. Production process review

Cleaning and disinfection validation begins with a review of the production process. During this step, documents detailing the operation are collected and inspected to ensure that its contents accurately reflect factory settings. It is also important to identify the most difficult units to clean (e.g. due to the aggregation of fouling or deposits) and worst-case parameters (e.g. lowest flow, maximum run length, maximum idle time).

Step 2. Production process verification

After reviewing the production process, one must confirm that the equipment and monitoring instruments are working as described. Maintenance and calibration records must be analysed to ensure parameters are compatible with the documentation. Evidence must be obtained that moving parts (e.g. valves and pumps) are functioning correctly.

Step 3. Cleaning and disinfection review

Next, the cleaning and disinfection program must be reviewed. Documentation on these procedures should be as comprehensive as possible, especially where manual cleaning operations are concerned. Relevant parameters such as contact time, chemicals type and concentration, temperature and flow rate must be specified for every step.

Step 4. Cleaning and disinfection verification

As with production, after reviewing the cleaning and disinfection program one must confirm that the equipment and monitoring instruments are working as described. Maintenance and calibration records must be analysed to ensure that the cleaning and disinfection parameters are respected. Evidence must be obtained that moving parts are able to disperse the cleaning and disinfection solutions to all product contact areas. Manual cleaning operations must be audited.

Part 2 - Cleaning and disinfection validation

Steps 5-10. Selection of parameters of the cleaning and disinfection validation study

Step 5. Selection of product

Production lines are usually not dedicated to a single product, but rather a range of them. Supposedly, the validation exercise would be repeated for each, but this would make the process extremely difficult and complex. To reduce workload, validation is performed for the most difficult to remove item, assuming is that if the cleaning and disinfection protocols are effective for the worst-case they will also be effective against less persistent product residue.

Step 6. Identification of relevant contaminants

After selecting the worst-case product, the most relevant contaminants of the process must be defined.

The following categories usually need to be considered:

- Microbiological
- Chemical
- Physical
- Allergens
- Product residue

Step 7. Selection of sampling sources

Samples must be collected to confirm that cleaning and disinfection effectively eliminate the contaminants identified in the previous step. Different strategies can be used depending on the production line, equipment, and type of contaminant. The main features and applications of each source are summarised in Table 3.1.

**Table 3.1:** Sampling sources for cleaning and disinfection protocols. Adapted from: Cleaning and Disinfection Validation Master Plan (Internal document UQG2001) [6]

| Sampling source | Sampling method | Nature of contaminant | Applications | Limitations |
|---|---|---|---|---|
| Rinse medium | During the final stage of cleaning and disinfection the rinsing medium is collected and tested. | Detaches from contact surfaces. Uniformly dispersed in rinse medium. | Inaccessible systems (e.g. pipework). Units that can not be routinely disassembled (e.g. heat exchangers). Large sampling areas. | Indirect method (indicates when removal of contaminant has stopped, not if surface is clean). |
| Surface | Swabs or contact plates are pressed directly against the surface and tested. | Attached to contact surfaces. Insoluble in rinse medium. Trapped in areas of difficult access. | Open areas (e.g. open plant). Difficult to clean areas (e.g. dead ends). Non-product contact surfaces (e.g. floors). | Only a small area can be sampled at a time. May not be representative of the whole. |
| Product | Product flowing through the pipework and equipment acts as a rinse fluid, collecting residue along the line. Products can be directly used for testing. | Detaches from contact surfaces. | New lines and product releases. | Retrospective (samples can only be collected after the lot is produced). |
| Growth medium | Growth medium is inserted in the line to promote rapid growth of microorganisms from low counts. After incubation, it is retrieved and tested. | Low counts of microbial contaminants. | Aseptic lines | Requires expert microbiological analytical skills. |

Step 8. Selection of sampling locations

In addition to the source, the specific location from where each sample is taken must also be identified. Rinse medium is normally gathered from drains or collection points (e.g. tank outlets, purges). Surface samples must include representative locations and worst-case areas. To choose representative locations, similar types of equipment can be grouped and an example selected for each (e.g. if a line has multiple filling nozzles, one must be selected). To choose worst-case areas, the following aspects should be considered:

- Nature of fouling: The most difficult units to clean, identified in Step 1;
- Hygienic design: Areas of difficult access where cleaning may be less efficient;
- Cleaning conditions: Zones where the optimal cleaning parameters, identified in Step 3, are more difficult to achieve (e.g. temperature, flow rate).

Step 9. Selection of analytical method

An appropriate method must be selected to analyse all the samples collected. The choice will be determined by the nature of the contaminant, type of sample and desired precision of result.

Step 10. Determination of acceptance limits

The acceptance limits define a range within which results of analytical methods are considered satisfactory, and the process line adequately clean. A range must be defined for every combination of contaminant, analytical method and location (e.g. microbiological criteria needs to be more stringent after the pasteurisation step to prevent re-contamination). Local legislation, factory requirements and GMPs must be taken into consideration when defining these values.

Step 11. Sampling and analysis

Once the parameters of the validation study have been defined, sample collection and analysis can begin. These activities take place after the cleaning and disinfection process and must be repeated at least three times, to verify that consistent results are obtained.

Step 12. Evaluation of the results

Cleaning and disinfection is considered successful if all the samples collected show contamination levels within the acceptance range. If three repetitions are successful, the process is validated. In line with the principles of HACCP, monitoring and verification activities must still be carried out to maintain a state of control. Cleaning and disinfection is considered unsuccessful if some of the samples show contamination levels above the acceptance limits. In this case, it must be verified that validation was performed according to protocol. If the study was correctly implemented, an investigation must be performed to determine the cause of failure and the necessary parameters adjusted. A risk assessment must also be performed to determine the course of action for any production batch affected (e.g. quarantine and re-sampling, destruction, production shutdown). If the validation study shows frequent failures modifications to the production process or cleaning and disinfection protocols need to be implemented and the validation study restarted.

### 3.1.2 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.

### 3.1.3 Materials and methods

#### 3.1.3.A Validation and monitoring of CIP in ageing tanks

In ageing tanks, the entire cleaning and disinfection process is performed using a CIP system, so this is the only component which requires validation and monitoring. The studied plant has 44 tanks in total, arranged into 6 lines as shown in Figure 3.2.

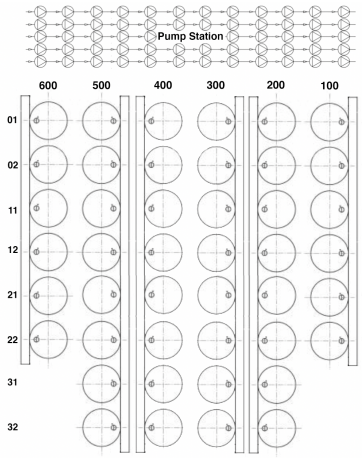


**Figure 3.2:** Layout of ageing tanks in the mix ageing room

Cleaning and disinfection is performed using two separate CIP circuits:

- CIP A is used for the tanks in lines 600, 500 and 400;
- CIP B is used for the tanks in lines 300, 200 and 100.

The validation plan described in subsection 3.1.1 was applied to each of these CIP circuits separately. Sampling protocols and validation criteria are summarised in Tables 3.2 and 3.3. The monitoring protocol was identical to the one used for validation, with the exclusion of allergen testing.

#### Selection of product

Generally, ice cream mixes produced in this plant have the same allergens in their composition (milk, gluten and sulphites). Exceptions include water ices, which do not contain milk, and a few mixes which contain egg or nuts. To minimise allergen cross-contamination, the same tanks are used when producing these recipes and tested for the appropriate allergen before change-over (i.e. milk before water ices and egg or nuts before changing to a different recipe). For each tank, the worst-case scenario was chosen and tested accordingly.

#### Identification of relevant contaminants

The mix has already been pasteurised at this stage, so avoiding microbiological contamination is paramount. Microorganisms must be kept at a level which is not harmful to the consumer and food pathogens must be eliminated to guarantee the safety of the product. Product residue is also an important contaminant, both as a source of microorganisms (e.g. bacteria, mould), and as a threat to organoleptic properties of the product (e.g. colour, scent, flavour). Chemical contaminants must also be monitored, namely detergent residue from CIP. Allergens transferred from the mix were also considered and measured accordig to the recipes produced in each tank. Since the ageing tanks are part of a closed circuit, physical contaminants are highly unlikely, and thus were not considered during the validation process.

### Selection of sampling source

A combination of rinse medium and swabs (COPAN SRK R4160 sterile polyester transport swabs) were used, depending on the nature of the contaminant. All samples were collected by the author.

### Selection of sampling locations

The specific locations for each sampling source are shown in Figure 3.3. Rinse medium was collected during the final rinsing stage of CIP, from an outlet under the tank. Swabs were collected from the inner surface of the tank vent, identified as the most difficult to access area within the tank. For visual inspection, the whole inner surface of the tank was considered.

**Figure 3.3:** Sampling locations for validation and monitoring of CIP in ageing tanks: Outlet for rinse medium (left, original photos), Tank vent from the outside and inside of the tank (centre, original photos), Inner surface of tank (right, original photo)

### Selection of analytical method

In line with standard industry practices, product residue was detected through sensorial inspection, generally more stringent than other qualitative methods [6]. For microbiological analysis, total viable count (TVC) and *Enterobacteriaceae* were selected as hygiene indicators and samples were tested in the internal laboratory by microbiology analysts, using a plate count method. For chemical analysis, pH and conductivity were measured using a Mettler Toledo SevenCompact Duo S213. Allergen testing was performed using Reveal 3-D Food Allergen Kits from Neogen. Steps followed are shown in detail in another case study (3.2, Figure 3.14).

### Determination of acceptance limits

Tanks were considered free of product residue if none was visible and no smell was detected. For microbiological contaminants, factory guidelines based on EU regulations for microbiological criteria of foodstuff were followed ((EC) No 2073/2005 [57]). For chemical analysis, rinse water was considered free of contaminants if the pH and conductivity values were similar to those of incoming water (maximum difference of +0.5 and +0.05 mS/cm, respectively). Lastly, tanks were considered free of allergens when fast detection kits had negative results.

**Table 3.2:** Sampling protocol for monitoring and validation of CIP A

| Product | Contaminant | Sampling location | Sampling source | Analytical method | Acceptance limit |
|---|---|---|---|---|---|
| Mix containing allergens (worst-case) | Product residue | CIP A - 400<br>CIP A - 500<br>CIP A - 600 | Surface<br>Surface<br>Surface | Sensorial inspection | Visually clean<br>Absence of scent |
| | Microorganisms | CIP A - 400<br>CIP A - 500<br>CIP A - 600<br>CIP A - 400<br>CIP A - 500<br>CIP A - 600 | Surface<br>Surface<br>Surface<br>Rinse water<br>Rinse water<br>Rinse water | Microbiological analysis (TVC, *Enterobacteriaceae*) | TVC:<br>$<$100 c.f.u / 100 cm$^2$<br>$<$100 c.f.u / 100 mL<br>Entero:<br>$<$5 c.f.u / 100 cm$^2$<br>$<$1 c.f.u / 100 mL |
| | Chemicals | CIP A - 400<br>CIP A - 500<br>CIP A - 600 | Rinse water<br>Rinse water<br>Rinse water | Chemical analysis (pH, Conductivity) | pH: $<$8.764<br>Conductivity: $<$1.006 mS/cm |
| | Allergens | CIP A - 400<br>CIP A - 500<br>CIP A - 600<br>CIP A - 400<br>CIP A - 500<br>CIP A - 600 | Surface<br>Surface<br>Surface<br>Rinse water<br>Rinse water<br>Rinse water | Fast detection allergen kits | Negative |

**Table 3.3:** Sampling protocol for monitoring and validation of CIP B

| Product | Contaminant | Sampling location | Sampling source | Analytical method | Acceptance limit |
|---|---|---|---|---|---|
| Mix containing allergens (worst-case) | Product residue | CIP B - 100<br>CIP B - 200<br>CIP B - 300 | Surface<br>Surface<br>Surface | Sensorial inspection | Visually clean<br>Absence of scent |
| | Microorganisms | CIP B - 100<br>CIP B - 200<br>CIP B - 300<br>CIP B - 100<br>CIP B - 200<br>CIP B - 300 | Surface<br>Surface<br>Surface<br>Rinse water<br>Rinse water<br>Rinse water | Microbiological analysis (TVC, *Enterobacteriaceae*) | TVC:<br>$<$100 c.f.u / 100 cm$^2$<br>$<$100 c.f.u / 100 mL<br>Entero:<br>$<$5 c.f.u / 100 cm$^2$<br>$<$1 c.f.u / 100 mL |
| | Chemicals | CIP B - 100<br>CIP B - 200<br>CIP B - 300 | Rinse water<br>Rinse water<br>Rinse water | Chemical analysis (pH, Conductivity) | pH: $<$8.764<br>Conductivity: $<$1.006 mS/cm |
| | Allergens | CIP B - 100<br>CIP B - 200<br>CIP B - 300<br>CIP B - 100<br>CIP B - 200<br>CIP B - 300 | Surface<br>Surface<br>Surface<br>Rinse water<br>Rinse water<br>Rinse water | Fast detection allergen kits | Negative |

#### 3.1.3.B Validation and monitoring of CIP in continuous freezers

In production lines, cleaning and disinfection is highly complex and involves several different processes. Each step must be carefully assessed and validated, to ensure the line is hygienic and does not compromise the safety of food products. This study focuses on the validation of cleaning and disinfection in continuous freezers, which is performed using a CIP system.

Three CIP circuits may be used for freezers (C, D or E), and each machine is usually connected to more than one. For this reason, validation is performed for each processing line individually, rather than for each CIP circuit. To this end, the plan described in subsection 3.1.1 was applied to the three CIP circuits combined. Sampling protocols and validation criteria are summarised in Table 3.4. The monitoring protocol was identical to the one used for validation.

### Selection of product

If allergens are not taken into consideration (see "Identification of relevant contaminants"), ice cream mixes have very similar properties and thus similar behaviour in terms of removal. As such, no worst-case product was selected and validation was performed according to the production schedule of each line.

### Identification of relevant contaminants

As discussed for ageing tanks, avoiding post-pasteurisation contamination is paramount, so microbiological criteria must be established. Chemical contaminants must also be monitored, namely detergent residue from CIP. Allergens were excluded from C&D validation since (i) allergen validation in processing lines is complex and requires sampling of specific equipment and packaging downstream (ii) a successful C&D validation of ageing tanks makes the risk of allergen cross-contamination minimal, and thus inconsequential to the validation process. Like ageing tanks, freezers are in a closed circuit, which makes physical contaminants highly unlikely. For this reason they were also not considered.

### Selection of sampling source

Since swabbing would require a complete disassemble of the freezer, rinse medium was the only source used. Samples were collected by personnel during production, as established in the monitoring program of this plant.

### Selection of sampling locations

Rinse medium was collected during the final rinsing stage of CIP, from the same outlet used for ice cream (shown in Figure 2.4).

### Selection of analytical method

The analytical methods used were identical to the ones used for the validation of ageing tanks. For microbiological analysis, total viable count (TVC) and *Enterobacteriaceae* were selected as hygiene indicators and samples were tested in the internal laboratory by microbiology analysts, using a plate count method. For chemical analysis, pH and conductivity were measured using a Mettler Toledo SevenCompact Duo S213.

### Determination of acceptance limits

The acceptance limits defined were identical to the ones used for the validation of ageing tanks. For microbiological contaminants, factory guidelines based on EU regulations for microbiological criteria of foodstuff were followed ((EC) No 2073/2005 [57]). For chemical analysis, rinse water was considered free of contaminants if the pH and conductivity values were similar to those of incoming water (maximum difference of +0.5 and +0.05 mS/cm, respectively).

**Table 3.4:** Sampling protocol for monitoring and validation of CIP C/D/E

| Product | Contaminant | Sampling location | Sampling source | Analytical method | Acceptance limit |
|---|---|---|---|---|---|
| Aged Mix | Microorganisms | CIP C/D/E - Line A<br>CIP C/D/E - Line B<br>CIP C/D/E - Line C<br>CIP C/D/E - Line I<br>CIP C/D/E - Line G<br>CIP C/D/E - Line S<br>CIP C/D/E - Line H<br>CIP C/D/E - Line R<br>CIP C/D/E - Line W | Rinse water | Microbiological analysis<br>(TVC, *Enterobacteriaceae*) | TVC: <100 c.f.u / 100 mL<br>Entero: <1 c.f.u / 100 mL |
| | Chemicals | CIP C/D/E - Line A<br>CIP C/D/E - Line B<br>CIP C/D/E - Line C<br>CIP C/D/E - Line I<br>CIP C/D/E - Line G<br>CIP C/D/E - Line S<br>CIP C/D/E - Line H<br>CIP C/D/E - Line R<br>CIP C/D/E - Line W | Rinse water | Chemical analysis<br>(pH, Conductivity) | pH: <8.764<br>Conductivity: <1.006 mS/cm |

## 3.1.4 Results and discussion

### 3.1.4.A 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 3.5, Table 3.5) had generally good results, with only two samples outside of the acceptable range. Further detail on samples which failed to meet the appropriate requirements can be found in Table 3.8. 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 (shown in Figure 3.4). CIP B (Figure 3.6, Table 3.6) 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.

**Figure 3.4:** Placement of vents in ageing tanks: Old model (left, original photo), New model (right, original photo)

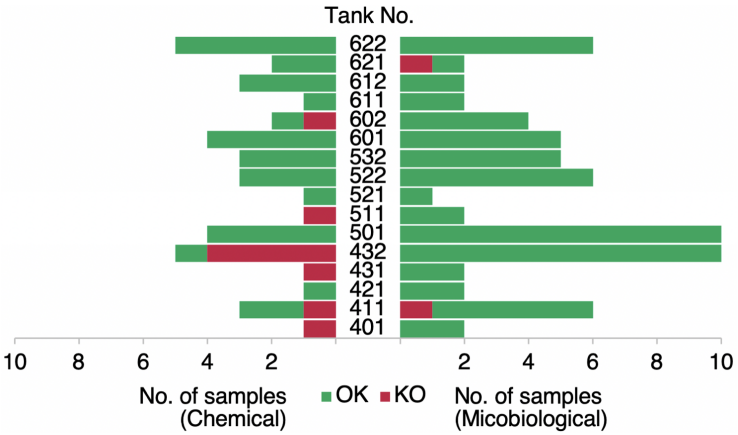


**Figure 3.5:** Chemical and microbiological analysis of samples from CIP A.
OK (■) = samples within the range of acceptance; KO ((■) = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH <8.764, conductivity <1.006 mS/cm
Range of acceptance (Microbiological): TVC <100 c.f.u / 100 cm$^2$ or 100 mL, Entero <5 c.f.u / 100 cm$^2$ or <1 c.f.u / 100 mL

**Table 3.5:** Chemical and microbiological analysis of samples from CIP A (absolute frequency and percentage)

| | Chemical | | | Chemical (%) | | Microbiological | | | Microbiological (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OK | KO | Total | OK | KO | OK | KO | Total | OK | KO |
| CIP A - 400 | 4 | 7 | 11 | 36 | 64 | 21 | 1 | 22 | 95 | 5 |
| Tank 401 | 0 | 1 | 1 | 0 | 100 | 2 | 0 | 2 | 100 | 0 |
| Tank 402 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 411 | 2 | 1 | 3 | 67 | 33 | 5 | 1 | 6 | 83 | 1 |
| Tank 412 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 421 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 422 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 431 | 0 | 1 | 1 | 0 | 100 | 2 | 0 | 2 | 100 | 0 |
| Tank 432 | 1 | 4 | 5 | 20 | 80 | 10 | 0 | 10 | 100 | 0 |
| CIP A - 500 | 11 | 1 | 12 | 92 | 8 | 24 | 0 | 24 | 100 | 0 |
| Tank 501 | 4 | 0 | 4 | 100 | 0 | 10 | 0 | 10 | 100 | 0 |
| Tank 502 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 511 | 0 | 1 | 1 | 0 | 100 | 2 | 0 | 2 | 100 | 0 |
| Tank 512 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 521 | 1 | 0 | 1 | 100 | 0 | 1 | 0 | 1 | 100 | 0 |
| Tank 522 | 3 | 0 | 3 | 100 | 0 | 6 | 0 | 6 | 100 | 0 |
| Tank 531 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 532 | 3 | 0 | 3 | 100 | 0 | 5 | 0 | 5 | 100 | 0 |
| CIP A - 600 | 16 | 1 | 17 | 94 | 6 | 20 | 1 | 21 | 95 | 5 |
| Tank 601 | 4 | 0 | 4 | 100 | 0 | 5 | 0 | 5 | 100 | 0 |
| Tank 602 | 1 | 1 | 2 | 50 | 50 | 4 | 0 | 4 | 100 | 0 |
| Tank 611 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 612 | 3 | 0 | 3 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 621 | 2 | 0 | 2 | 100 | 0 | 1 | 1 | 2 | 50 | 2 |
| Tank 622 | 5 | 0 | 5 | 100 | 0 | 6 | 0 | 6 | 100 | 0 |
| CIP A | 31 | 9 | 40 | 78 | 23 | 65 | 2 | 67 | 97 | 2 |

OK = samples within the range of acceptance; KO = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH <8.764, conductivity <1.006 mS/cm
Range of acceptance (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

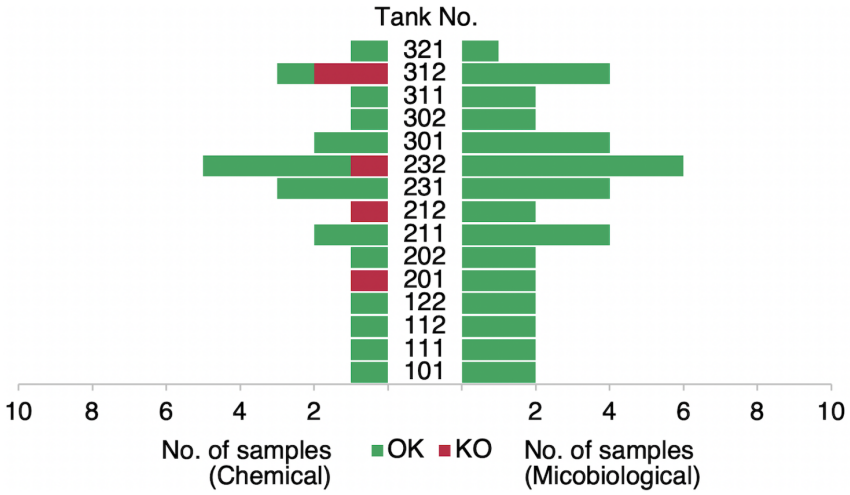


**Figure 3.6:** Chemical and microbiological analysis of samples from CIP B.
OK (■) = samples within the range of acceptance; KO (■) = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH <8.764, conductivity <1.006 mS/cm
Range of acceptance (Microbiological): TVC <100 c.f.u / 100 cm$^2$ or 100 mL, Entero <5 c.f.u / 100 cm$^2$ or <1 c.f.u / 100 mL

**Table 3.6:** Chemical and microbiological analysis of samples from CIP B (absolute frequency and percentage)

| | Chemical | | | Chemical (%) | | Microbiological | | | Microbiological (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OK | KO | Total | OK | KO | OK | KO | Total | OK | KO |
| CIP B - 100 | 4 | 0 | 4 | 100 | 0 | 8 | 0 | 8 | 100 | 0 |
| Tank 101 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 111 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 112 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 121 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 122 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| CIP B - 200 | 10 | 3 | 13 | 77 | 23 | 20 | 0 | 20 | 100 | 0 |
| Tank 201 | 0 | 1 | 1 | 0 | 100 | 2 | 0 | 2 | 100 | 0 |
| Tank 202 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 211 | 2 | 0 | 2 | 100 | 0 | 4 | 0 | 4 | 100 | 0 |
| Tank 212 | 0 | 1 | 1 | 0 | 100 | 2 | 0 | 2 | 100 | 0 |
| Tank 221 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 222 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 231 | 3 | 0 | 3 | 100 | 0 | 4 | 0 | 4 | 100 | 0 |
| Tank 232 | 4 | 1 | 5 | 80 | 20 | 6 | 0 | 6 | 100 | 0 |
| CIP B - 300 | 6 | 2 | 8 | 75 | 25 | 13 | 0 | 13 | 100 | 0 |
| Tank 301 | 2 | 0 | 2 | 100 | 0 | 4 | 0 | 4 | 100 | 0 |
| Tank 302 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 311 | 1 | 0 | 1 | 100 | 0 | 2 | 0 | 2 | 100 | 0 |
| Tank 312 | 1 | 2 | 3 | 33 | 67 | 4 | 0 | 4 | 100 | 0 |
| Tank 321 | 1 | 0 | 1 | 100 | 0 | 1 | 0 | 1 | 100 | 0 |
| Tank 322 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CIP B | 20 | 5 | 25 | 80 | 20 | 41 | 0 | 41 | 100 | 0 |

OK = samples within the range of acceptance; KO = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH <8.764, conductivity <1.006 mS/cm
Range of acceptance (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

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. As shown in Figure 3.5, 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, time-consuming 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. Requirements for equipment selection according to factory guidelines can be found in the validation master plan [6]. 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. Figure 3.7 shows the layout of the ageing tanks and chemical analysis results. Notably, tanks with samples outside of the acceptable range (marked with an ”X”) are concentrated towards central lines (200, 400) and rows (12), which are more difficult to access and thus fluids lose more pressure.

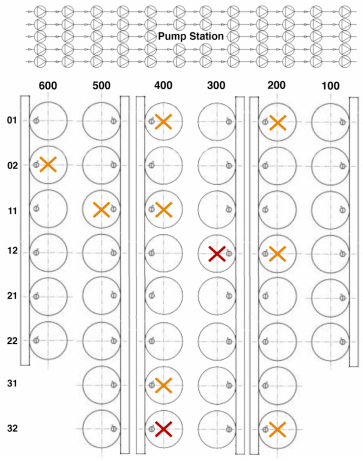


**Figure 3.7:** Layout of ageing tanks in the mix ageing room and results of chemical analysis. Tanks marked with ”X” had results outside of the acceptable range of pH and conductivity. Orange: single unsatisfactory sample Red: multiple unsatisfactory samples

The piping network for these machines is extremely complex, (Figure 3.8), so information from this diagram 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

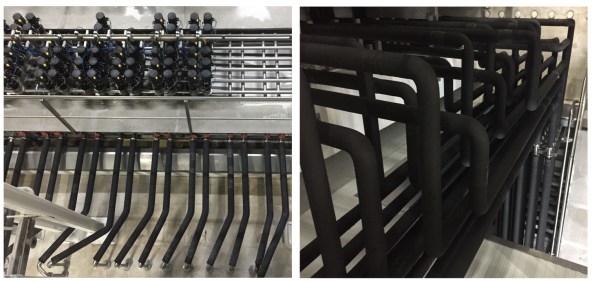

**Figure 3.8:** Pumps and piping network for ageing tanks: Pump station (left, original photo), Piping behind the tanks (right, original photo)

studied to confirm which tanks are more vulnerable to pressure loss and define strategic positions in the piping network to measure flow rate. Using a ultrasonic flow metre would be the best approach, since this device is inexpensive and provides a real-time, accurate representation of system conditions, unlike using in-line measures to calculate an approximate value. These measurements can also be compared to CIP records (temperature, concentration, velocity, etc) to ensure that all variables are within the defined range and all sensors properly calibrated.

For freezers (CIP C/D/E) microbiological analysis results were identically encouraging, as 98% of samples were within the acceptance range (Figure 3.9, Table 3.7). 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, as shown in Table 3.8. 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 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% (Table 3.7).

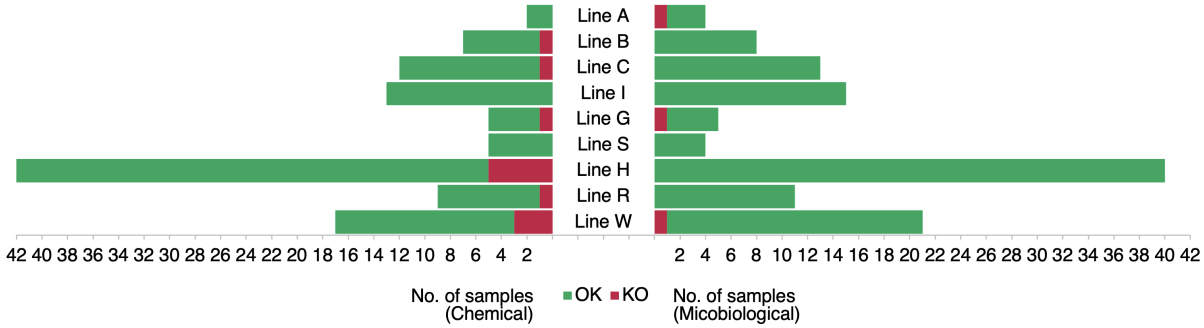


**Figure 3.9:** Chemical and microbiological analysis of samples from CIP C/D/E.
OK (■) = samples within the range of acceptance; KO ((■) = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH $<$8.764, conductivity $<$1.006 mS/cm
Range of acceptance (Microbiological): TVC $<$100 c.f.u / 100 mL, Entero $<$1 c.f.u / 100 mL

**Table 3.7:** Chemical and microbiological analysis of samples from CIP C/D/E (absolute frequency and percentage)

| | Chemical | | | Chemical (%) | | Microbiological | | | Microbiological (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OK | KO | Total | OK | KO | OK | KO | Total | OK | KO |
| Line A | 2 | 0 | 2 | 100 | 0 | 3 | 1 | 4 | 75 | 25 |
| Line B | 6 | 1 | 7 | 86 | 14 | 8 | 0 | 8 | 100 | 0 |
| Line C | 11 | 1 | 12 | 92 | 8 | 13 | 0 | 13 | 100 | 0 |
| Line I | 13 | 0 | 13 | 100 | 0 | 15 | 0 | 15 | 100 | 0 |
| Line G | 4 | 1 | 5 | 80 | 20 | 4 | 1 | 5 | 80 | 20 |
| Line S | 5 | 0 | 5 | 100 | 0 | 4 | 0 | 4 | 100 | 0 |
| Line H | 37 | 5 | 42 | 88 | 12 | 40 | 0 | 40 | 100 | 0 |
| Line R | 8 | 1 | 9 | 89 | 11 | 11 | 0 | 11 | 100 | 0 |
| Line W | 14 | 3 | 17 | 82 | 18 | 20 | 1 | 21 | 95 | 5 |
| CIP C/D/E | 100 | 12 | 112 | 89 | 11 | 118 | 3 | 121 | 98 | 2 |

OK = samples within the range of acceptance; KO = samples beyond the range of acceptance.
Range of acceptance (Chemical): pH $<$8.764, conductivity $<$1.006 mS/cm
Range of acceptance (Microbiological): TVC $<$100 c.f.u / 100 mL, Entero $<$1 c.f.u / 100 mL

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 [14]. In other types of heat exchanger, fouling and corrosion have been shown to increase the conductivity of rinse water during CIP [84]. The use of drinking water is generally adequate to prevent either condition, but the manufacturer has advised that sensitivity may vary with operating parameters [34]. 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 (for example, in the middle of the rinsing process instead of the last 30 seconds), 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 minimal as possible.

**Table 3.8:** Distribution of samples beyond the range of acceptance (KO) for chemical and microbiological analysis from all CIP circuits (A, B, C/D/E)

| | | Chemical | | | Microbiological | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | $pH_{RW}$ | $Cond_{RW}$ | pH + $Cond_{RW}$ | $TVC_{RW}$ | $TVC_S$ | $Entero_{RW}$ | $Entero_S$ | TVC+$Entero_{RW}$ | TVC+$Entero_S$ |
| CIP A | 11 | 1 | 6 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| Tank 401 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 411 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Tank 431 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 432 | 4 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 511 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 602 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 621 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| CIP B | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 201 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 212 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 232 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tank 312 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CIP C/D/E | 16 | 4 | 8 | 0 | 2 | - | 0 | - | 1 | - |
| Line A | 1 | 0 | 0 | 0 | 1 | - | 0 | - | 0 | - |
| Line B | 1 | 0 | 1 | 0 | 0 | - | 0 | - | 0 | - |
| Line C | 1 | 0 | 1 | 0 | 0 | - | 0 | - | 0 | - |
| Line G | 2 | 0 | 1 | 0 | 1 | - | 0 | - | 0 | - |
| Line H | 5 | 3 | 2 | 0 | 0 | - | 0 | - | 0 | - |
| Line R | 1 | 0 | 1 | 0 | 0 | - | 0 | - | 0 | - |
| Line W | 4 | 1 | 2 | 0 | 0 | - | 0 | - | 1 | - |
| Grand Total | 31 | 5 | 19 | 2 | 2 | 2 | 0 | 0 | 1 | 0 |

RW = Rinse water sample ; S = Surface swab sample; + = Both parameters simultaneously
Range of acceptance (Chemical): pH $<$8.764, conductivity $<$1.006 mS/cm
Range of acceptance (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

#### 3.1.4.B CIP Validation

As noted by Tamime [39], there are always opportunities for improving the quality, safety and productivity of CIP operations, and it is important that these are reviewed and captured on a regular basis. In this case, monitoring results of aging tanks and freezers showed some possible advancements, namely in the prevention of chemical contamination. Nevertheless, the CIP system proved to be generally effective against the most relevant contaminants of each type of equipment. Expecting any process to have a constantly flawless performance is highly unrealistic, and thus should never be set as the standard for cleaning and disinfection. Instead, food manufactures must endorse a system which consistently minimises the risk of contamination, to a point where food is considered safe. This is precisely the purpose of validation, to confirm that the C&D system in place is capable of reducing contaminants according to a predefined set of criteria.

For ageing tanks, a preliminary review and verification of the production process and cleaning and disinfection confirmed that both operations were compliant with the documentation and that all parts were working as expected. As mentioned in section (3.1.1), three successful rounds of sampling and analysis are required for validation. The timeline of results obtained for ageing tanks is shown in Figure 3.10. Since each line (100-600) had three consecutive samples within the ranges of acceptance established, validation of CIP A and B was successfully completed within the first trimester of production.

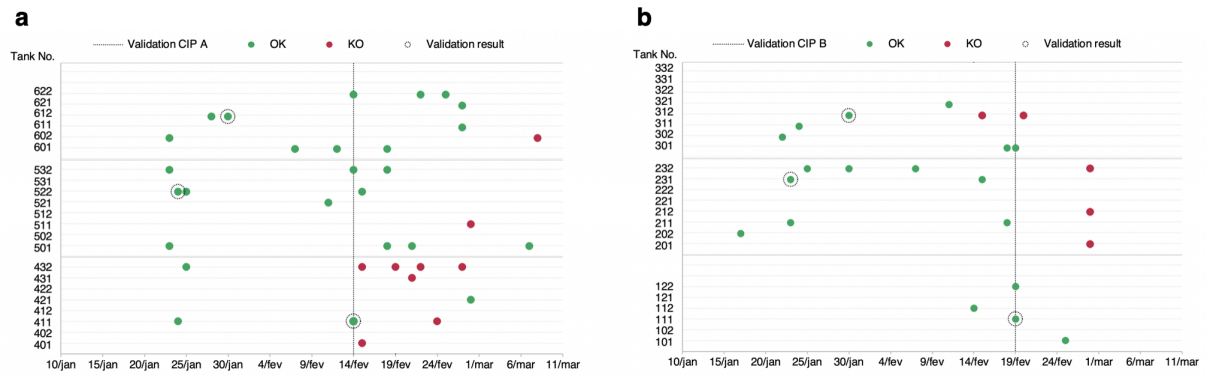


**Figure 3.10:** Validation timeline for CIP circuits in the mix ageing room: CIP A (**a**) and CIP B (**b**).
OK (■) = samples within the range of acceptance; KO ((■) = samples beyond the range of acceptance;
Validation result = Third consecutive green sample for the same line.
Range of acceptance (Product residue): Visually clean, Absence of scent
Range of acceptance (Allergens): Negative
Range of acceptance (Chemical): pH $<$8.764, conductivity $<$1.006 mS/cm;
Range of acceptance (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

For freezers, the preliminary steps of review and verification are performed in conjunction 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. The timeline of results obtained is shown in Figure 3.11.

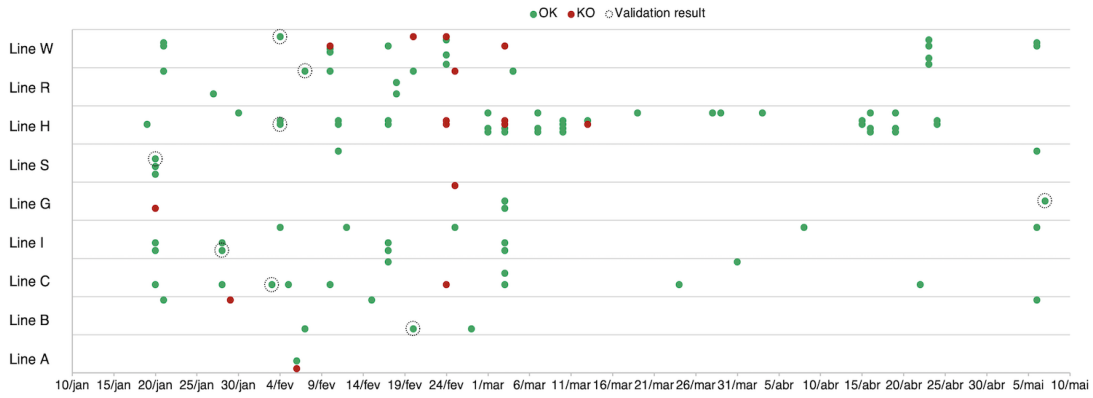


**Figure 3.11:** Validation timeline for CIP circuits in the production hall: CIP C/D/E
OK (■) = samples within the range of acceptance; KO ((■) = samples beyond the range of acceptance;
Validation result = Third consecutive green sample for the same production line.
Range of acceptance (Chemical): pH $<$8.764, conductivity $<$1.006 mS/cm;
Range of acceptance (Microbiological): TVC $<$100 c.f.u / 100 mL, Entero $<$5 c.f.u /$<$1 c.f.u / 100 mL

For Line A, only two samples of rinse water were collected and only one was within the criteria established. As such, CIP operations could not be validated for this line. For all others, three consecutive samples within the range of acceptance were obtained. Validation was thus successfully completed, within the second trimester of production for Line G and within the first trimester for all others. However, it should be noted that validation of the freezer CIP system does not necessarily translate into a successful

cleaning and disinfection validation for the whole line, since results from other sampling locations must also be considered.

In any case, 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.

### 3.1.5 Conclusions

Cleaning and disinfection are vital in any manufacturing process to guarantee the quality and safety of final products. Regular assessment and review of these programs ensure that they remain effective and compliant with the established HACCP plan.

This study focused on the CIP systems used for cleaning and disinfection of ageing tanks and continuous freezers. Samples from each type of equipment were collected and tested to monitor cleaning performance. These samples were also used to validate the CIP system, as part of an annual cleaning and disinfection validation study required for all the factories of the studied company.

Monitoring results showed that CIP systems were highly effective at eliminating microbial contaminants from both types of equipment, which is critical for preventing post-pasteurisation recontamination of ice cream mixes. Conversely, several instances of chemical contamination were discovered and attributed to the presence of detergent residue. For ageing tanks, it was reasoned that this likely stemmed from the equipment itself (e.g. defective spray ball, loss of pressure in the piping network). For freezers this was also listed as a potential cause, but other factors such as operating conditions and sampling practices are equally possible. Further research is proposed to devise an effective solution for each case and thus improve the overall CIP system's efficiency.

Validation was performed using a pre-established plan, which serves as the basis for all cleaning and disinfection studies performed in the studied company's plants. This workflow requires three successful rounds of sampling and analysis for the process to be validated. For ageing tanks, CIP systems were able to meet the required criteria, and thus cleaning and disinfection were successfully validated. For freezers, three successful repetitions were achieved in every line except Line A. However, validation of the freezer CIP system does not necessarily translate into a successful cleaning and disinfection validation for the whole line, since results from other sampling locations also need to be considered.

Altogether these results show that the CIP systems used in this plant for the cleaning and disinfection of ageing tanks and freezers effectively remove contaminants and thus contribute to the production of safe and high-quality ice cream. This case study also further establishes monitoring and validation as pillars for the continuous improvement of cleaning and disinfection activities.

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

### 3.2.1 Background and motivation

Ready-to-eat (RTE) food products that have been submitted to an adequate heat-treatment during processing are free of vegetative pathogens. Nevertheless, RTE items have been implicated in various food-borne illnesses [43; 55], which are often caused by recontamination during subsequent production steps, when the product is unintentionally exposed to bacteria, allergens, chemicals, and foreign bodies [8; 43; 55].

Food handlers are a well recognised route of post-pasteurisation recontamination in ice cream production, namely for microbiological and allergen contaminants [1; 8–11; 29; 33; 43]. As such, it is essential to regularly review the procedures in place for the hygiene of personnel and to assess the need to introduce additional policies.

Upon consideration of the most likely pathways for contamination via personnel, three risk assessments were designed for this case study:

1. Transfer of allergens from personnel's clothing

   Allergen management has emerged as a recent concept in the food industry, but it has quickly become an important safety and quality concern of manufacturers [28; 29; 81; 85]. Over the past decades, significant efforts have been made to establish control measures in processing facilities which decrease the risk of contamination [28; 81]. Most of them, however, are based on pre-existing GMPs and mainly focused on the segregation of allergenic ingredients. While certainly an improvement on past regulations, the industry recognises there is still much to be done ([75; 85]). Cross-contamination (i.e. the accidental introduction of allergens in food products which do not list them as ingredients) is a major concern in the control of allergens across all stages of food production [15; 30; 81]. To reduce the risk of cross-contamination, quality experts must design risk assessment studies to determine potential sources of contaminating allergens and whether the existing systems can manage the associated risk under normal operating conditions [29; 30]. Company guidelines and best practices for allergen management are comprehensive in terms of equipment and raw materials, but less detailed in what regards personnel [31; 86; 87]. For instance, quality standards for personnel hygiene and employee facilities [87] state that food must not be consumed outside of designated areas to avoid contamination of products with allergens, but fail to consider employees themselves as carriers and thus possible sources of cross-contamination.

   In this study the role of personnel as a vector of allergens and promoter of cross-contamination was evaluated by testing uniforms for both specific allergens and total protein.

2. Transfer of enteric bacteria from personnel's hands

   Transfer of pathogens from food handlers, in particular from hands, has been identified in many publications as one of the main pathways for recontamination after pasteurization. Deficient hand washing practices allow bacteria to survive and spread to food products, which may lead to severe cases of contamination [8; 43; 44; 66; 88–93]. This is especially relevant for enteric pathogens with low infectious doses such as *Shigella* and *Escherichia coli* [43; 44]. Aside from contaminating food products directly, these species of bacteria may also be transferred from hands to equipment and, under the right conditions, form biofilms which increase resistance to regular cleaning and disinfection. For instance, Gunduz and Tuncel [44] documented a case of persistent post-pasteurisation contamination in an ice cream plant, from *Shigella* biofilms which had originated from a worker's hands. To prevent this type of contamination, food manufactures must ensure that the equipment and procedures in place are appropriate for maintaining good levels of personnel hygiene.

   In this study the efficiency of hand sanitation protocols and equipment were assessed by testing the hands of personnel and jet hand dryers for contamination with enteric bacteria.

3. Transfer of *Listeria* from personnel's shoes

   *Listeria monocytogenes* is an opportunistic pathogen and one of the main causes of foodborne illness [2; 14; 44; 45; 63–68; 73]. Ubiquitous in nature, it is able to adapt and survive in a wide range of environmental conditions (see 2.4.3.D). Although this species is inactivated by the thermal treatments used in the production of ready-to-eat foods, recontamination from the equipment, environment and personnel represents a major concern. As such, control of *L. monocytogenes* in food production facilities requires constant focus by risk managers [2; 65–68; 73]. Presence of *L. monocytogenes* in the processing environment is thought to be the primary source of recontamination after pasteurisation. Once this pathogen enters the production hall, it finds environmental niches which provide protection from lethal stress and allow the bacteria to grow [2; 43; 65–68]. Personnel's shoes are a well recognised entry route of *L. monocytogenes* in the production hall. In a study performed by Rückerl et al. [68], 48.4 % of shoes from workers in a cheese factory were contaminated with *L. monocytogenes*. Melero et al. [73] found personnel movements to be one of the main transmission vehicles for *L. monocytogenes* in a newly established dairy processing facility. Rørvik et al. [94] also reported footwear to be a significant factor for contamination in ready-to-eat smoked salmon.

   The presence and persistence of *L. monocytogenes* in this factory's processing environment is analysed in another case study (3.3). The present study focuses on the role of personnel's shoes as an entry route of *L. monocytogenes* in the production hall and the efficiency of a boot scrubber as a countermeasure for contamination.

### 3.2.2 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.

### 3.2.3 Materials and methods

#### 3.2.3.A Sampling and detection of allergens in clothing

To investigate the transmission of allergens through personnel's clothing, a total of 60 uniforms were swabbed and tested over the course of a month (2 almond, 4 peanut, 9 hazelnut, 10 gluten and 35 total protein). All samples were collected in the afternoon, after employee's lunch break. The testing population was selected at random. Samples were collected by swabbing the front of the uniforms in the chest area. A surface of 600 $cm^2$ (30 x 20 cm) was swabbed, using horizontal and vertical zigzag strokes across the area (Figure 3.12).

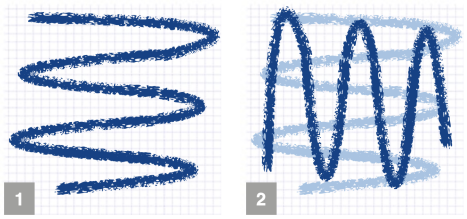


**Figure 3.12:** Swabbing technique used for allergen testing

Total protein was detected using Clean-Trace$^{TM}$ Surface Protein Plus Test Swabs from 3M (catalogue no. PRO100, 50 µg/100 cm $^2$) [95]. Steps followed are shown in Figure 3.13.

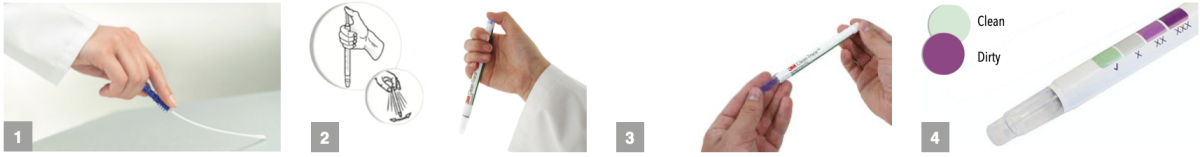


**Figure 3.13:** Directions for Clean-Trace$^{TM}$ Surface Protein Plus Test Swabs. Clean - green, Dirty - purple, Inconclusive - grey. [95] [adapted]

Specific allergens were detected using Reveal 3-D Food Allergen Kits from Neogen [96]: Almond (item no. 902086G, 1 µg/100 cm $^2$), Peanut (item no. 901041L, 3-4 µg/100 cm $^2$ ), Hazelnut (item no. 90208E, 10 µg/100 cm $^2$), and Gluten (item no. 8505, 80 µg/100 cm $^2$). Steps followed are shown in Figure 3.14.

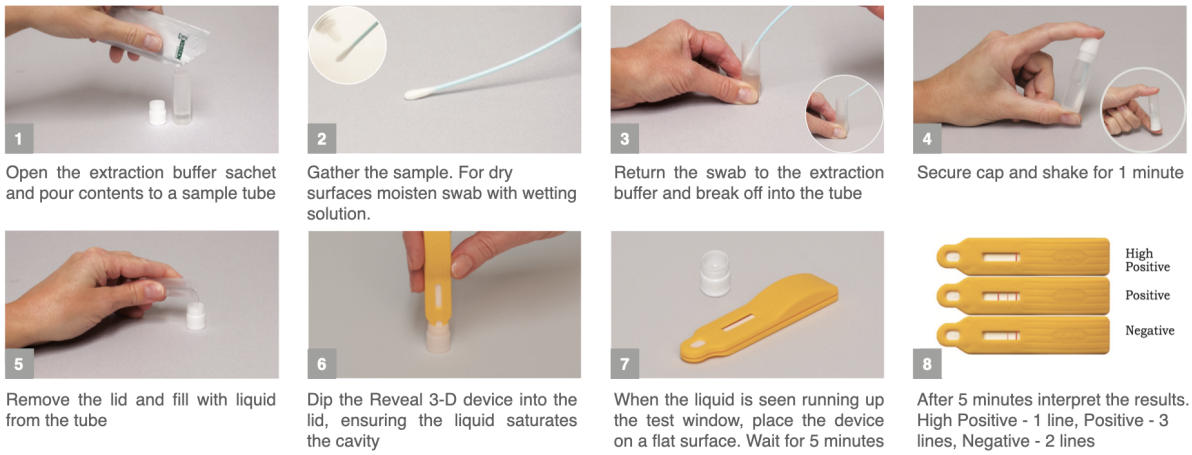


**Figure 3.14:** Directions for Reveal 3-D Food Allergen Kits with swab sampling. High Positive - 1 line, Positive - 3 lines, Negative - 2 lines. [96] [adapted]

### 3.2.3.B Sampling and detection of *Enterobacteriaceae* in hands and hand dryers

To investigate the transmission of *Enterobacteriaceae* through food handlers, a total of 86 hands were swabbed over the course of a month (74 bare, 12 wearing gloves). The testing population was selected at random. Samples were collected by swabbing personnel's hands with sterile cotton wool dry transport swabs (COPAN 150C), according to the manufacturer's instructions. Both the palm and back of the hand were swabbed, using horizontal and vertical zigzag strokes across the area (Figure 3.12). Special attention was given to areas frequently neglected during hand washing, shown in Figure 3.15. Jet hand dryers used by personnel before entering the production hall (Dyson Airblade dB) were also sampled by swabbing the interior surface.

After collection samples were transported to the internal laboratory and tested for *Enterobacteriaceae* by microbiology analysts, using a plate count method. Results were obtained after 5 days.

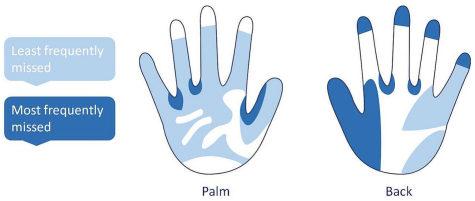


**Figure 3.15:** Areas most frequently neglected during hand washing [97]

#### 3.2.3.C Sampling and detection of *Listeria* in shoes and boot scrubber

To investigate the transmission of *Listeria monocytogenes* and *Listeria* spp. through personnel's shoes, a total of 28 soles were swabbed over the course of 15 days, half in the morning shift and half in the evening shift. The testing population was selected at random. Samples were collected by swabbing personnel's shoe soles with sterile cotton wool dry transport swabs (COPAN 150C), according to the manufacturer's instructions. All soles were sampled twice, before and after being cleaned in an industrial boot scrubber, using horizontal and vertical zigzag strokes across the area (Figure 3.12). Special attention was given to sole crevices and details. The boot scrubber was sampled twice a day over the same 15 days (morning and evening shift) by swabbing a representative area of the brush roll.

After collection swabs were kept in a chilled environment at 4℃ before being transported to an external laboratory. Detection of *Listeria monocytogenes* and *Listeria* spp. was performed by Silliker Portugal, S.A., using the ALOA® One Day qualitative method (internal protocol numbers PAM.16.4 and PAM.09.0, respectively). This method uses a patented chromogenic medium (ALOA® One Day) to detect the activity of the enzyme β-glucosidase, common to all Listeria species. Presence of *Listeria monocytogenes* is highlighted by the activity of a phospholipase, involved in the infection process of this pathogen. After incubation at 37℃, *Listeria monocytogenes* and *Listeria* spp. grow round blue-green colonies which are confirmed with the appropriate protocol. Results are obtained within 3-5 days [98].

### 3.2.4 Results and discussion

#### 3.2.4.A 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 achieve this, a sampling strategy was designed, based on the factory's features and regular production routines. Two different sources were considered: (i) ingredients used in the ice cream products made in this factory and (ii) ingredients found in food consumed by employees during shift breaks, which can accidentally be transferred to clothing. With these routes in mind, specific allergens were selected for testing among the 14 most commonly known to cause adverse reactions (see 2.4.4). Tests for total protein were also performed to account for any allergen not included in this group. While hands have more contact with allergens, they are also frequently washed and thus sampling of this surface would not accurately translate the potential for carryover. Instead, samples were collected from the chest area of personnel's uniforms, where food particles are likely to attach when close to topping dispensers or after lunch. Results for specific allergens and total protein samples are shown in Table 3.9.

**Table 3.9:** 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 in total, 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 [95].

Several publications have recognised work wear as a source of cross-contamination with food allergens [28; 29; 99], but little is known about the exact conditions in which it occurs. In this factory, results from this study confirm that the uniforms worn by personnel are not a relevant vector in the transmission of allergens, and thus cross-contamination through garments is very unlikely to occur. They also demonstrate that the practices established for their use and laundering are effective at eliminating traces of food. Risk assessment projects such as the present one provide valuable insight into cross-contamination sources and help to develop a tailored and comprehensive allergen management program. Obtaining negative results should not be seen as a fruitless effort, but rather as an opportunity to investigate more relevant contamination routes and focus prevention efforts on areas that will have a more significant impact.

#### 3.2.4.B 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 the hands of personnel are a relevant source of contamination with *Enterobacteriaceae* in the production hall. Swabs were collected from bare and gloved hands, as well as jet dryers used by employees after hand washing. Samples were tested for *Enterobacteriaceae* and the results obtained are shown in Table 3.10.

**Table 3.10:** 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. 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. Park et al. [89] audited the manufacturing process of ten soft-ice cream brands and found higher concentrations of heterotrophic bacteria in the hands of operators who has worn the same pair of gloves for an extended period of time, when compared to operators who had not worn gloves. Lynch et al. [100] analysed the levels of bacteria on foods handled by gloved and bare hands at fast food restaurants and found a higher concentration of coliform and heterotrophic bacteria in samples handled by gloved hands. The authors also observed that workers who wore gloves tended to wear the same pair for extended periods of time and wash hands less frequently, making this measure counterproductive in preventing bacterial contamination.

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 [101; 102]. Additionally, Scott and Bloomfield [102] 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. [103] reported a consistent ten-fold increase in the amount of bacteria recovered from hands by going over the same area five times. Defining a positive control would also help to validate this sampling method. For example, a small amount of swabs could be collected from volunteer employees after using the restroom and before proceeding to hand washing.

Jet hand dryers are used by personnel before entering the production hall, an essential step to decrease the survival and transmission efficiency of microorganisms from hands. The importance of thorough hand drying to reduce contamination is well documented [2; 88; 90; 91; 102–104]. However, authors disagree on the best method to achieve this goal. Several studies have shown that air dryers increase aerosolisation and dispersion of microorganisms, making paper towels a more suitable alternative in places where hygiene considerations are paramount, such as healthcare settings and the food industry [88; 104; 105]. Others recognise hand dryers as the most efficient and ecological method to eliminate bacteria from hands [90–92]. Results from the swabs collected were consistent with these findings, since no enteric bacteria was found in any of the jet dryers. Having said that, the primary purpose of these samples was not to compare different drying strategies, but rather to assert that these units are not a source of re-contamination for personnel's hands before entering the production. To

certify them as a suitable method to reduce microbial contamination, further studies are required.

#### 3.2.4.C 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 (3.3).

Although no contamination with *L. monocytogenes* was discovered, several swabs tested positive for *Listeria* spp., as shown in Figure 3.16.

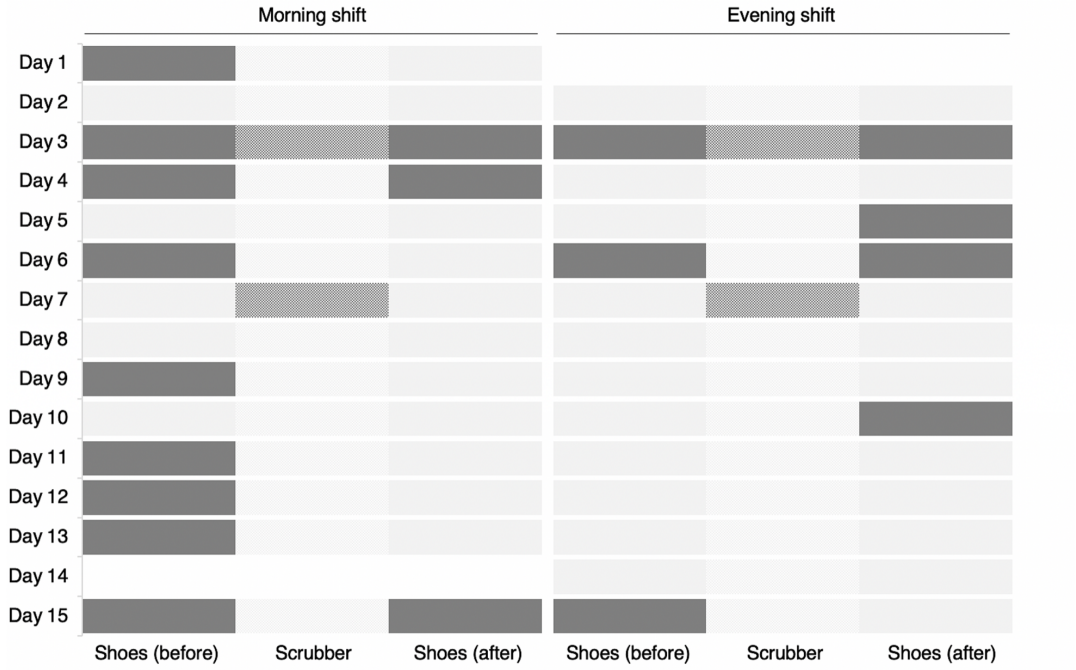


**Figure 3.16:** Detection of *Listeria* spp. in personnel's shoes and boot scrubber (84 samples), in the morning shift (left) and evening shift (right). For each shift, the first column represents shoes sampled before scrubbing, the second samples collected from the scrubber and the third shoes sampled 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 [68; 73; 94; 106]. 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 (Appendix A, Table A.1), 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 3.11.

**Table 3.11:** 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 same 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. Microbiological tests performed on swabs were only meant to determine the presence of *Listeria* species, 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 the cleaning and

disinfection process, as explained in 2.2.1. Scrubbing duration is defined by equipment programming to ensure optimal cleaning and disinfection, but instances of incorrect use have been observed. To avoid this situation 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* [66]. A study by Lappi et al. [107] 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 [44; 66; 108]. 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 4 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 [18], 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. [106] 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. [65] 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.

### 3.2.5 Conclusions

Food handlers are well recognised as a route of post-pasteurisation contamination in ice cream production, namely for microbiological and allergen contaminants. As such, it is vital to regularly review the procedures in place for the hygiene of personnel and assess the need to introduce additional policies.

This case study aimed to investigate the role of personnel as a potential vector of transmission of microorganisms and allergens in the production hall of this plant. To that end, three different contamination pathways were investigated.

Firstly, the transmission of allergens through clothing was considered. Samples were collected from personnel's workwear and tested for selected allergens and total protein. The results obtained were markedly negative, suggesting that the uniforms worn by personnel are not a relevant vector in the transmission of allergens, and thus cross-contamination through garments is very unlikely to occur in this plant.

Secondly, the transmission of enteric bacteria through hands was assessed. Samples were collected from bare and gloved hands, as well as jet dryers used by employees after hand washing, and tested for *Enterobacteriaceae*. Results registered no occurrences of enteric bacteria, indicating that the hand sanitation protocol implemented in this factory is highly effective and strictly enforced by food handlers. Nevertheless, further work needs to be carried out to validate the sampling method used.

Lastly, the transmission of *Listeria* through footwear was examined. Swabs were collected from shoe soles, before and after cleaning, and from the boot scrubber used. The results showed no contamination with *L. monocytogenes*, but a significant prevalence of *Listeria* spp. was recorded, notably in samples from the morning shift. The use of the boot scrubber as a cleaning method was assessed by analysing sampling results as a sequence of events. The scrubber appears to quite adequately remove *Listeria* from personnel's shoes without accumulating significant amounts of bacteria throughout the day, albeit not entirely effectively.

Ultimately, this study has shown that personnel is a key element in the production of safe food products. It is essential that employees understand the importance of good hygiene practices and their significance in preventing contamination. Quantifying the different risks associated with the impact of workers in the food preparation process provides a scientific basis for risk management efforts and allows quality experts to effectively implement the HACCP plan.

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

### 3.3.1 Background and motivation

The processing environment has been well established as a source of contamination in the production of ready-to-eat foods [54; 61; 66; 67]. Surfaces like floors and walls act as indirect sources of bacteria, which are then carried by air, staff and cleaning systems [44]. Some pathogens become established and find niches where they can survive for long periods of time [43; 54].

*Listeria monocytogenes* is a perfect example of such a pathogen. A wide range of growth conditions (see 2.4.3.D) and potential to adapt under stress allow the bacteria to survive and persist in processing plants for years or decades, with specific strains isolated repeatedly over time [44; 61; 66; 67; 69; 73; 109]. A variety of studies have described this trend, notably in refrigerated premises. Contamination of an ice cream plant recorded by Miettinen et al. [110] showed that the dominant strain had survived for at least seven years. Fox et al. [111] discovered several persistent strains of *L. monocytogenes* in a cheese factory, one of which suggested to have survived for 10 years. A comprehensive review by Ferreira et al. [66] describes 31 other cases of persistent *L. monocytogenes* in various processing environments (dairy, meat, fish and ready-to-eat foods).

Upon colonization of the facilities, bacteria are easily spread to food contact surfaces and, ultimately, to the final product. Several authors have linked the presence of *L. monocytogenes* in food processing environments to the contamination of food products [43–45; 66–68; 73; 93; 94; 108; 112].

While elimination of *Listeria monocytogenes* is a priority in the ice cream industry, resilience of this species to environmental factors makes this a difficult challenge. Most authors agree that it is virtually impossible to permanently eradicate *L. monocytogenes* from manufacturing settings, so the elimination of bacteria must be actively managed and should remain a constant concern of quality experts [61; 65; 67; 69].

Monitoring of areas prone to contamination and application of corrective measures are important tools in the efforts to control *L. monocytogenes* [61; 65; 67–69]. Contamination during production occurs mostly in the production hall, since pasteurisation is generally effective against *L. monocytogenes* [45; 68; 69].

Environmental niches are the main areas that enable the survival and persistence of this microorganism. They provide growth permissive conditions and protection from lethal stress, allowing bacteria to replicate. These sites are usually areas of difficult access that cannot be adequately reached during the application of cleaning and sanitation protocols (e.g. cracks in the equipment, crevices of surfaces, seals and gaskets) [61; 64–67; 70; 73].

*L. monocytogenes* is also commonly found in wet locations [18; 44; 66]. Special attention should be paid to [18; 27; 45]:

- Stagnant water in equipment;
- Condensation around freezers, hardening tunnels and poorly isolates pipes;
- Leaking connections between equipment;
- Cleaning utensils, such as mops, brushes and buckets;
- Cleaning activities adjacent to a running production line;
- Floor drains.

Routine environmental sampling is indispensable to reduce the spread of *L. monocytogenes*. A thorough analysis of the processing environment will provide information on the efficacy of cleaning and disinfection procedures and other preventive measures (e.g. zoning, limitations of movements of personnel and goods, knowledge of staff, etc.). This type of surveillance 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 [18; 43; 61; 65–67; 69].

### 3.3.2 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.

### 3.3.3 Materials and methods

#### 3.3.3.A Sampling strategy

The sampling plan used to investigate the presence and persistence of *L. monocytogenes* in floor drains is presented in Appendix A. A total of 69 drains were sampled across the nine processing lines of the production hall (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). Sites were strategically selected from a pre-existing plan, based on accessibility and regular production routines. Samples were collected on two separate occasions, before and after a deep cleaning treatment was performed during the factory's annual December production break (samples collected in November 2018 and January 2019). A summary of the specific location of each site and results obtained for both occasions is provided in Table 3.12 and Table 3.13.

#### 3.3.3.B Sample collection

Samples of water (100 mL) were collected from floor drains with a sterile pipette and stored in sterile cups. Containers were kept in a chilled environment at 4 ℃ before being transported to an external laboratory.

#### 3.3.3.C Detection of *Listeria monocytogenes*

Detection of *Listeria monocytogenes* and *Listeria* spp. was performed by Silliker Portugal, S.A., as described in section 3.2.3.C.

### 3.3.4 Results and discussion

It is common industry practice for processing plants to have an annual shutdown period, when no production occurs. This allows for essential repair and maintenance work to be carried out, new equipment to be installed, and several improvement measures to be implemented. It is also a good opportunity to perform deep cleaning operations which would otherwise not be possible.

In this factory, routine environmental sampling of the processing environment had identified several drains contaminated with *Listeria monocytogenes* and *Listeria* spp.. To address this, a deep cleaning treatment was performed during this annual break period. It should be noted that the products used could not be applied during regular production, since they release aerosol particles which may contaminate the product, even if diluted. After manufacturing was resumed, new samples were collected from the same drains. Results for each drain before and after the application of this product are presented in Table 3.12 and Table 3.13. The results obtained for production lines with common areas or equipment (e.g. Line A, Line H and Line W) could not be allocated to a single line, so they were grouped together and evaluated as a whole.

To ascertain the efficacy of the cleaning treatment, three performance indicators were calculated from these results for each production line or group:

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

Values are summarised in Table 3.14.

**Table 3.12:** Occurrence of *Listeria monocytogenes* and *Listeria* spp. in drains of the production hall, before and after the application of a deep cleaning treatment

| Production Line / Zone | Drain No. | Before treatment | After treatment |
|---|---|---|---|
| Line R | | | |
| Next to Line R platform | 3 | ● | - |
| Next to Metal detector | 5 | ● | - |
| Line I | | | |
| Freezing | | | |
| Next to Freezers 23/24 | 6 | ● | ● |
| Next to Freezer 25 | 9 | ● | ● |
| Coating | | | |
| Next to Section II, Line I side | 86 | - | - |
| Next to Section II, Line S side | 83 | - | - |
| Line G & Line C | | | |
| Next to Freezers 17,18,19 | 51 | - | - |
| Next to Line S packaging | 46 | - | - |
| Next to Line G | 45 | - | - |
| Next to Freezer 16 | 50 | ◐ | - |
| Next to Line C | 49 | - | - |
| Next to Freezers 14/15 | 48 | - | - |
| Between Freezers 14/15 and Line C | 47 | - | - |
| Line A, Line H & Line W | | | |
| Packaging | | | |
| Next to APV, Chocolate tank side | 54 | - | - |
| Next to APV, Case erector side | 55 | - | - |
| Freezing | | | |
| Next to Line W | 67 | ● | - |
| Next to Line W, APV side | 68 | ● | - |
| Behind Freezer 10 | 69 | - | ◐ |
| Next to Line A | 52 | ◐ | ◐ |
| Next to Line A, APV side | 53 | ◐ | ● |
| Next to Line H | 56 | ◐ | ◐ |
| Next to Line H, APV side | 57 | ◐ | - |
| Next to Freezer 13 | 79 | ◐ | - |
| Next to Freezers 8/9 | 80 | ◐ | ● |
| Next to Freezers 6/7 | 81 | ● | ● |
| Behind Freezers 8/9 | 70 | - | ● |
| Behind Freezers 6/7 | 71 | ● | ● |
| Line B | | | |
| Behind Cone feeder | 72 | ● | - |
| Next to Cone feeder | 73 | - | - |
| Next to Freezers 1,2,3,4,5 | 78 | - | - |
| Next to Freezer 1 | 82 | - | - |
| Next to Control station, Freezer 4 side | 59 | - | - |
| Next to Control station, Frigo II side | 76 | - | - |
| Next to Freezer 4 | 60 | - | - |
| Next to Conveyor belt | 77 | - | - |

● = *Listeria monocytogenes*; ◐ = *Listeria* spp.; - = Negative

**Table 3.13:** Occurrence of *Listeria monocytogenes* and *Listeria* spp. in drains of the production hall, before and after the application of a deep cleaning treatment (continued)

| Production Line / Zone | Drain No. | Before treatment | After treatment |
|---|---|---|---|
| Line S | | | |
| Packaging | | | |
| Next to Frigo I, Line G side | 44 | ● | - |
| Next to Frigo I, centre | 43 | ◐ | - |
| Next to Frigo I, Case erector side | 42 | - | - |
| Next to Lift conveyor, right | 38 | ● | - |
| Next to Lift conveyor, left | 37 | ● | - |
| Next to Lift conveyor, back | 39 | - | - |
| Next to Manual packaging, back right | 40 | ◐ | - |
| Next to Manual packaging, back centre | 41 | ◐ | - |
| Next to Manual packaging, right | 36 | ◐ | - |
| Next to Manual packaging, centre right | 35 | - | - |
| Next to Manual packaging, centre left | 34 | - | - |
| Next to Manual packaging, left | 33 | ◐ | ● |
| Next to Automatic packaging, front right | 32 | - | - |
| Next to Automatic packaging, front centre | 31 | - | - |
| Next to Automatic packaging, front left | 30 | - | - |
| Next to Automatic packaging, right | 29 | - | - |
| Next to Automatic packaging, left | 28 | - | - |
| Coating | | | |
| Next to Coating tank, wall side | 27 | - | - |
| Next to Stamping station | 26 | ● | ◐ |
| Next to Conveyor curve | 23 | ● | ◐ |
| Behind Pick and place | 22 | ◐ | - |
| Next to Pick and Place | 10 | ● | ◐ |
| Next to Coating tank, freezers side | 12 | ● | ◐ |
| Freezing | | | |
| Next to Freezing tunnel conveyor | 25 | ◐ | - |
| Next to Freezing tunnel product exit | 24 | - | - |
| Next to Freezing tunnel product entrance | 13 | ◐ | ◐ |
| Next to Freezing tunnel entrance right | 15 | ◐ | - |
| Next to Freezing tunnel entrance left | 16 | ● | ◐ |
| Next to Platform, front left | 17 | - | - |
| Next to Platform, front center | 18 | ◐ | ◐ |
| Next to Platform, right | 19 | ● | - |
| Next to Platform, center | 20 | ◐ | ◐ |
| Next to Freezer 16 | 21 | ◐ | ◐ |
| Next to Freezer 20 | 14 | ◐ | ◐ |

● = *Listeria monocytogenes*; ◐ = *Listeria* spp.; - = Negative

**Table 3.14:** 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.

Overall, results show that the application of this treatment considerably reduced contamination with *L. monocytogenes* and *Listeria* spp.. 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. From the floor plan in Appendix A, it is apparent that 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 C and Line G 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 C and Line G, with only one contamination in total (*L. monocytogenes*). The implicated site is located behind the cone feeder at the beginning of the line, drain 72 in the sampling plan (Appendix A). During production ice cream cones are manually loaded in the feeder and the surplus kept in cardboard boxes stacked against the wall. One could argue that *L. monocytogenes* is carried from the exterior through these boxes, and from there spreads to the floor and drains. Although accumulating raw materials near the line is generally ill-advised, it does not seem to be the source of contamination in this case. Since boxes are stacked to the full length of the wall, drains 73 and 82 would also test positive for *L. monocytogenes* if bacteria came from packaging materials, but both drains had a negative result. A better explanation would be that bacteria was 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. The implication is that this site could be re-contaminated, which is particularly concerning given its proximity to raw materials. Several measures can be implemented to reduce this risk. Firstly, cone boxes can be placed in a different location during production (for instance, against the wall behind the freezers) or stored away (if possible). 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* [43–45; 66]. 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 the sampling plan (Appendix A), 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 *L. monocytogenes*, typically found in areas of difficult access which shield the bacteria from cleaning and disinfection chemicals (see 3.3.1). This seems to be the case, considering the placement of drains in this zone (drain 6 is partially covered by one of freezer 24's support legs and drain 9 is hidden under freezer 25, only accessible through a narrow space). Elimination of environmental niches requires exceptional effort, as noted by Jordan et al. [65] and Ferreira et al. [66]. Additional measures beyond increasing cleaning and sanitation frequency are usually needed in these cases. Some authors consider that modifications in equipment design are key to removing *L. monocytogenes* from such sites [14; 43]. These solutions, however, are often expensive and tend to require long breaks in production to be implemented. Furthermore, building work in itself is a potential source of bacteria, and has been shown to aggravate contaminations with *L. monocytogenes* [65]. A different approach described by Melero et al. [73] 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 sub-lethal concentrations of disinfectants may increase tolerance, the same chemicals remain effective when used at higher concentrations [66]. 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 the sampling plan (Appendix A) and Table 3.13: 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* spp.. 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* [43; 65; 73; 94]. A study by Rørvik et al. [94] on factors for contamination during processing indicated job rotation as the strongest associated risk. Melero et al. [73] investigated the process of colonisation in a newly opened cheese factory and found bacteria to spread easily via personnel movements. This issue, further explored in another case study (3.2), is especially relevant in places where product is exposed. As noted by Jordan et al. [65], it makes a difference whether a drain tests positive in a general processing area or where food is handled prior to packing. To address contamination, the author recommends 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 sampling plan (Appendix A) and Table 3.12: 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 3.14 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 (see monitoring data from 3.1) 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*, as discussed for Line S. On the other hand, consistent positive results from the same area usually indicate long-term persistence [66]. 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) [38; 113–116]. Others maintain that any strain can become persistent in the right environmental conditions [109; 117]. 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 [109]. 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 [61]. This practice has become increasingly more common in the industry and is gaining recognition as an important tool to improve environmental hygiene [61; 65–67]. In Ireland, a project funded by the Department of Agriculture to monitor *Listeria* in food processing sites was implemented in 2013. Factories send environmental samples to a laboratory for testing, which further characterises positive isolates. The results are shared with the participants and an effective combat strategy is created in cooperation with quality assurance specialists from each plant [65]. A similar approach could be employed in this case, to further investigate the reasons for persistence and create a targeted plan 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 transferring bacteria to finished products. Special attention must be paid to Line B, where drains are downstream and will likely be recontaminated until *L. monocytogenes* is permanently eradicated from this area, and to the Line A, Line H and Line W freezing zone, where persistent *L. monocytogenes* was identified. Of the latter, products from Line A have the highest risk of exposure, since assembly in this line is performed by extrusion (exposes the whole product to the processing environment for a considerable time) whilst assembly in Line H and Line W is performed by filling (only exposes the top surface of the container until the lid is placed).

### 3.3.5 Conclusions

Control of *L. monocytogenes* is a fundamental challenge of the ice cream industry due to this pathogen's ability to survive refrigeration temperatures. Routine sampling of the processing hall allows quality experts to evaluate the cleaning and disinfection practices in place and perform the necessary adjustments to prevent cross-contamination of food from the environment.

This study aimed to profile the contamination of drains in the processing hall with *L. monocytogenes* and assess the efficacy of a deep cleaning, treatment applied during the factory's annual break. Overall,

results showed a substantial decrease in the amount of drains contaminated with *Listeria* spp. and *L. monocytogenes*. Even though the treatment was successful, *L. monocytogenes* was still found in several areas, which is to be expected give the pathogen's ubiquitous nature. Complete eradication of this species from the processing environment is generally not possible, but measures can be implemented to reduce the risk of contamination.

Each production line is different and thus has a unique set of obstacles which must be addressed. Some generate products with simple formulas and ingredients, which make them less susceptible to contamination. Others face more complicated challenges, such as unhygienic design and persistence of pathogenic *L. monocytogenes*. Quality experts are responsible for analysing microbiological data, trying to decipher contamination patterns and provide targeted solutions for the problems encountered. In some cases, simple measures go a long way towards improving hygiene standards. For Line B, contamination risks can be lowered by relocating raw materials, containing cleaning procedures in adjacent lines and reinforcing hygiene practices. In Line S, *Listeria* may be contained by reducing foot-traffic and supplementing personnel training. Other cases require a higher level of investment to fully eliminate bacteria. Two areas affected by persistent *L. monocytogenes* were identified during this study. In Line I, drains of difficult access make the sanitation process less efficient and supply bacteria with a growth niche. While contamination may be managed through the application of a concentrated product, sanitary redesign constitutes a more long-term and proactive solution. In the freezing zone shared by Line A, Line H and Line W, *L. monocytogenes* was repeatedly isolated but the source and potential for persistence remain unclear. In recent years, genetic characterization has gained popularity in the food industry as a targeted strategy to address contamination with persistent strains. Analysis of isolates from these sites would help to clarify these questions and allow for the implementation of a successful decontamination strategy.

Studies such as the present one may be an effective method to reduce the risk of contamination while challenging food producers to increasing standards of safety and quality.

# 4

# Conclusions and future perspectives

## Contents

## 4.1 Summary of case studies

The three case studies presented are summarised in Table 4.1.

## 4.2 Final conclusions

This dissertation 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 *Listeria 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, the results of this dissertation show that hygiene standards at this ice cream factory are generally satisfactory and adequate to the manufacturing of safe and high-quality products. Neverthe-

less, food safety is a continuous endeavour and even the most efficient companies may find opportunities for improvement.

These case studies uncovered unique challenges for equipment, personnel and processing environment, but surprisingly some common trends have become apparent (e.g. sanitary redesign, personnel training). 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.

## 4.3 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, observing the process through an endoscope and assessing pressure losses along the piping network are key to increase rinsing efficiency. For freezers, several 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, findings for the transmission of enteric bacteria through personnel's hands need to be further assessed using an alternative method, different technique or an established control. In the transmission of *Listeria* through personnel's shoes, quantification of bacteria will provide further insight into the efficiency of scrubbing by clarifying whether the equipment has a threshold for removing bacteria and confirming that the scrubber does not increase bacterial load in shoes, even 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 *Listeria 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.

## 4.4 Research needs

Throughout this dissertation several research gaps in the fields of food processing and safety were identified. Some of the key issues to be addressed are as follows: (i) Drastically different numbers for the incidence of food allergies and intolerances have been reported, and studies are heavily influenced by populations, geographic region, methodology and type of pathology. More accurate prevalence rates would help manufacturers to devise control strategies through HACCP based on the appropriate level of risk. (ii) 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 for food safety and quality. (iii) Several publications have recognised work wear as a potential source of cross-contamination with food allergens, but to the author's knowledge no studies have been published on the frequency of these contaminations, the mechanisms of transmission involved, or the integration of adequate control measures in existing or newly implemented food safety management systems. These are important considerations in the face of rising food allergies worldwide and increasing demands for labels that accurately communicate the presence of allergens (iv) 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. (v) 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.

**Table 4.1:** Summary of case studies

| Case | Application | Objectives | Hazard type | Methods | Conclusions | Future work |
|---|---|---|---|---|---|---|
| I | Equipment (ageing tanks, freezers) | Monitoring and validation of CIP systems | Product residue<br>Microbiological (TVC, Entero)<br>Chemical<br>Allergens (milk, egg, nuts) | Sensorial inspection<br>Plate count<br>pH , Conductivity<br>Fast detection kits | CIP systems are highly effective at eliminating product residue and microbial contaminants, but may fail to adequately remove cleaning agents. CIP successfully validated for ageing tanks and freezers (exc. Line A) | Investigating causes for chemical contamination. Concluding review and verification steps for product line validation. |
| II | Personnel (clothing, hands, shoes) | Assessing the role of personnel as vector for cross-contamination | Allergens (almond, peanut, hazelnut, gluten, total protein)<br>Microbiological (Entero)<br>Microbiological (*Listeria*) | Fast detection kits<br>Plate count<br>ALOA®One Day | Uniforms were not considered a relevant source of allergens. Hand sanitation protocols were effective at eliminating enteric bacteria. High prevalence of *Listeria* was found in shoes. | Further sampling of personnel's hands and shoes.<br>Assessing the potential for contamination with *Listeria* from rubber mats. |
| III | Environment (floor drains) | Studying the presence of *Listeria*.<br>Finding persistence niches.<br>Evaluating the efficacy of an annual cleaning treatment. | Microbiological (*Listeria*) | ALOA®One Day | Drains contaminated with *Listeria* spp. (55%) and *L.monocytogenes* (26%).<br>Treatment reduced contamination by 25%, but two persistent niches remained. | Investigating persistence in the Line A, Line H and Line W freezing zone. |

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# A

# Production of ice cream at placement ice cream plant

This Appendix contains supplementary information on the production of ice cream specific to this plant.

## A.1 *Listeria* sampling plan for floor drains

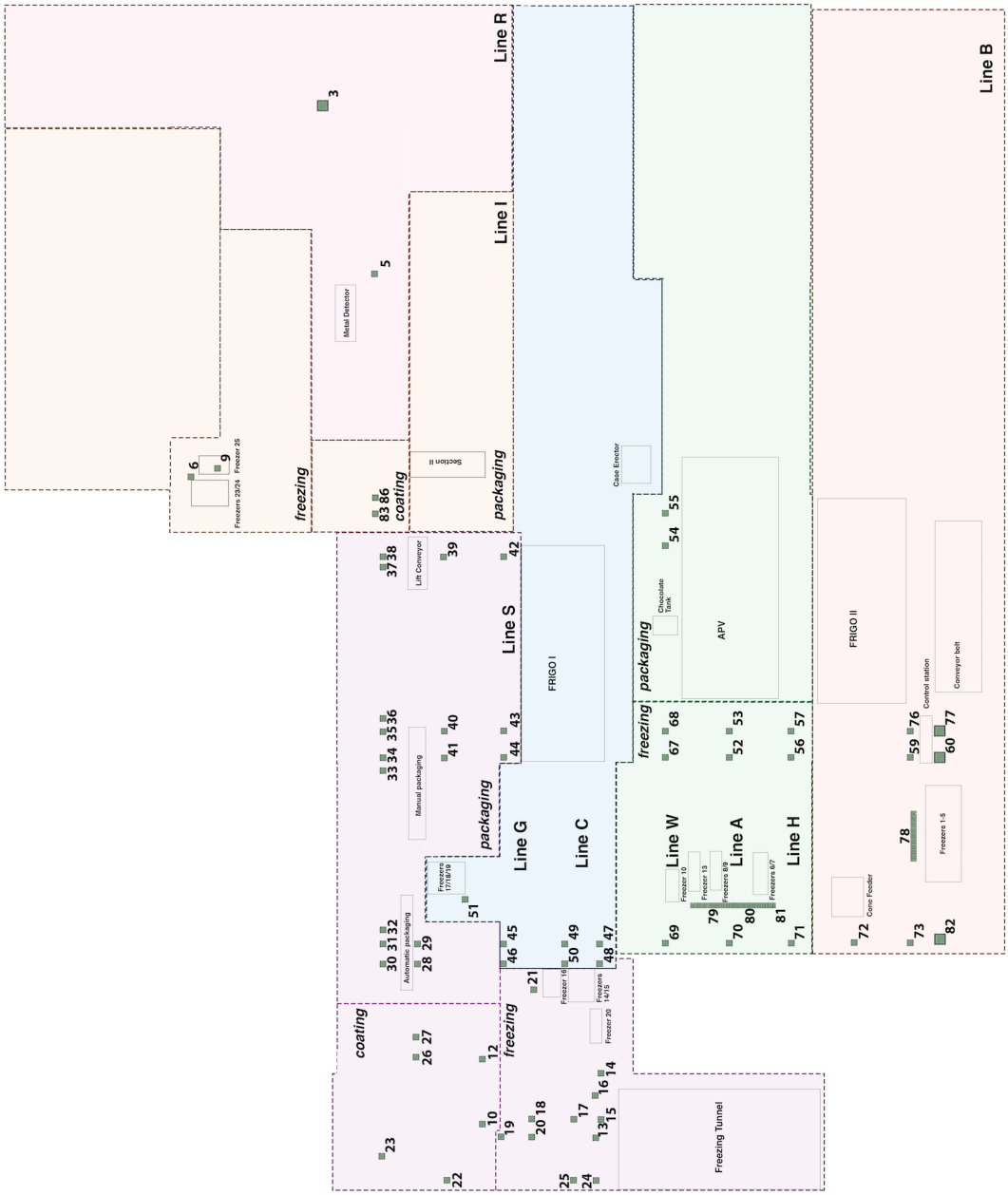


**Figure A.1:** Sampling plan used to investigate the presence and persistence of *Listeria* in floor drains

## A.2 Production shifts

**Table A.1:** Schedule of production shifts and office hours at the ice cream factory

| Production Shifts | |
|---|---|
| Morning | 07:00 a.m. - 03:00 p.m. |
| Evening | 03:00 p.m. - 11:00 p.m. |
| Night | 11:00 p.m. - 07:00 a.m. |
| Office hours | |
| Workday | 09:00 a.m. - 06:00 p.m. |

# B

# Scientific background

This Appendix contains supplementary scientific background for the phenomena involved in the production of ice cream.

## B.1 Stokes' Law and terminal velocity

When a particle falls through a fluid, two forces of opposite direction act upon it. On one hand, the particle accelerates due to its own gravity. On the other hand, the fluid exerts resistance on the particle and counteracts the effect of gravity (Figure B.1). The latter increases as the particle moves through the fluid until the two forces balance each other. At this point, acceleration ceases and the particle falls with a constant velocity, which is referred to as "terminal velocity".

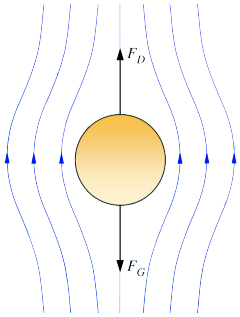


**Figure B.1:** Schematic of terminal fall velocity of a particle. $F_G$ is the submerged weight of the particle and $F_D$ is the fluid drag. Flow streamlines are also shown. [118] [adapted]

The mathematical expression for the terminal velocity of a small spherical particle can be derived by equating the two forces (equation B.1).

$$F_g = F_d \tag{B.1}$$

The force of gravity ($F_g$) is given by the difference between the weight and buoyancy of the sphere. In equation B.2, $g$ refers to the gravitational acceleration, $R$ to the radius of the particle and $\rho_p$ and $\rho_f$ denote the mass densities of the sphere and fluid, respectively.

$$F_g = \frac{4}{3}\pi(\rho_p - \rho_f)gR^3 \tag{B.2}$$

The force of the resistance is given by Stokes' law (equation B.3), which describes the frictional force exerted on a small, spherical particle as it falls through a fluid.

$$F_d = 6\pi\mu v R \tag{B.3}$$

The friction force ($F_d$), also known as Stokes' drag, is proportional to the particle's radius ($R$), the viscosity of the fluid ($\mu$) and the velocity of the particle ($v$). Stokes' law is derived by solving the Stokes flow limit of the Navier-Stokes equations for small Reynolds numbers. As such, the following assumptions must be considered: (1) the fluid moves in laminar flow; (2) the particle is spherical; (3) the particle is uniform in composition and has a smooth surface; and (4) the interaction between particles is

negligible.

By combination equations B.3 and B.3, we obtain the expression for the terminal velocity (B.4).

$$v = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\mu} g R^2 \tag{B.4}$$

# C

# Confidential Information

This Appendix contains confidential information on the production of ice cream specific to this plant. The contents of this chapter are privileged and available only to readers upon authorisation from Instituto Superior Técnico and the placement ice cream factory.