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REVIEW

Microbiological aspects and challenges of whey powders – I thermoduric, thermophilic and spore-forming bacteria

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For dairy processors, spoilage and pathogenic spore-forming bacteria are key sources of concern, not only due to their ability to remain dormant in a desiccated state in powders and to survive heat treatments, but also their ability to form biofilms in the vegetative state that lead to contamination of foods. These include members of the genera Bacillus, Geobacillus, Anoxybacillus, Brevibacillus, Paenibacillus *and* Clostridium, *many of which are associated with food poisoning and spoilage. Here, we review the common bacterial species that form spores in whey powders and their sources and provide insights into their risks and strategies to control them.*

Keywords Whey powder, Dried dairy products, *Clostridium botulinum*, *Bacillus*, Spore-forming bacteria.

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INTRODUCTION

Whey is typically described as a greenish-yellow liquid formed following the separation of curd during the manufacturing of cheese. This dairy by-product is a highly valuable commodity. Whey is categorised into two different types; (i) sweet whey (min pH 6.3) obtained through the separation of casein coagulated using rennet enzyme; and (ii) acid whey (pH 4.6) obtained by the separation of casein coagulated using acid (direct or indirect acidification). Whey composition and properties vary according to the quality of cheese-milk used and the type of cheese produced (Magalhães *et al*. 2011; Carvalho *et al*. 2013; Barukcic 2018).

Traditionally, only liquid whey was processed in small artisanal production facilities. Value-added whey components are being isolated using cutting-edge technologies including chromatography and membrane filtration (ultrafiltration, nanofiltration, microfiltration, reverse osmosis, ion exchange, electrodialysis), evaporation or drying. Whey protein (WP) powder ingredients include whey protein concentrate/isolate (WPC/WPI), whey powder with low lactose content, demineralised whey powder, hydrolysed whey powder, whey protein fractions, lactose powder and milk minerals (Patel *et al*. 1991; Marshall 2004). WP powders are rich in vitamins, calcium and high-quality protein, all of which are crucial for both human and animal nutrition. In order to produce various whey beverages and products such as protein bars, WP components are widely utilised (Güler *et al*. 2006; Sousa *et al*. 2012; Pires *et al*. 2021). Whey products and their average composition (% by weight), production and applications are summarised in Table 1.

Whey powder is created by processing approximately two thirds of the world's whey production (Božanić *et al*. 2014). Whey powder has a very low water activity (a_w), making it an unsuitable environment for the growth and multiplication of microorganisms. However, when the powder ingredients absorb moisture during

Table 1 Products from whey and their average composition (% by weight), production and applications

Product	Protein	Lactose	Minerals	Fat	Production	Applications
WP	11–14.5	63–75	8.5	1–1.5	Produced from SW which is clarified, pasteurised and dried.	Used in breads, bakery and snack items and dairy foods.
Demineralised (70%/90%) WP	13.7/15.0	75.7/83.0	3.5/1.0	~	WP is demineralised through ion-exchange, electrodialysis or by nanofiltration 90% demineralised WP have a mineral content of <1%. Nanofiltration may be used to remove salt and water and retain lactose. However, divalent salt removal is lower than electrodialysis.	Used in same way as WP but avoids imparting strong salty flavour into sweet products. 90% demineralised whey may be used in infant formula.
WPC	25–89	4.0–52.0	3.0–5.0	1–9	WPCs are produced by ultrafiltration. Different ratios of protein to total solids may be obtained using diafiltration.	Most common and affordable form. Used in protein beverages and bars, bakery and confectionary products, dairy and nutritional foods.
WPI	88.0–95.0	<1	2.0–3.5	0.5–1	Produced by defatting whey streams using microfiltration, or isolating native whey proteins by IEC, followed by concentration utilising ultrafiltration, evaporation and drying.	Have a minimum protein content of 90%. Used in protein supplements, beverages, bars and other nutritional food products.
Hydrolysed WPC	>80	<8	~	<10	Produced using enzymatic hydrolysis; typically using proteases. The degree of hydrolysis affects functional properties and intended usage.	Used in sports nutrition products.
Hydrolysed WPI	>90	0.5–1	~	0.5	Produced using enzymatic hydrolysis; typically using proteases. The degree of hydrolysis affects functional properties and intended usage.	Highly digestible form containing highly digestible peptides that reduce risk of allergenic reactions among susceptible individuals. Commonly used in infant formula and sports nutrition.

WP, whey powder; WPC, whey protein concentrate; WPI, whey protein isolate.

rehydration, the a_w increases, and optimum conditions occur for the germination of bacterial spores such as *Clostridium botulinum* and for toxin production (Lindström *et al*. 2010; Pal *et al*. 2016). Consequently, spore-forming bacilli numbers are undesirable (Hill and Smythe 2012). *Bacillus cereus* spores can withstand pasteurisation and the production of powdered milk. Upon reconstitution, spores can germinate and thrive in rehydrated products that are improperly handled or stored, as well as in dry foods like rice cereal in conditions where dry foods are processed for extended periods of time (Pal *et al*. 2014). This situation is also dangerous for dry baby food products (infant formula) where dairy powders are used, especially through the germination of spores of thermophilic bacteria and has been linked to a case of newborn botulism. The production of certain enzymes, such as lipases and proteases, might result in flaws in the milk product (Sadiq *et al*. 2016). For these reasons, good manufacturing practices (GMPs) and Hazard Analysis and Critical Control Point (HACCP) systems should aim to prevent cross-contamination of high-moisture foods with pathogens or spoilage microorganisms from low-moisture foods that are microbiologically stable. After rehydrating powders, these should be consumed quickly or kept for a brief period of time in the refrigerator or freezer before being consumed. If not, there is a significantly increased risk that certain meals will result in an illness or poisoning (Beuchat *et al*. 2011). While mesophilic spore formers are less problematic, they remain of note because of their potential to adapt (McHugh *et al*. 2018).

Spore-forming bacteria are common and are found in soil, the gastrointestinal tracts of insects, and the reproductive organs of warm-blooded animals. They are Gram-positive organisms with over 200 species that can produce endospores, making them resistant to harsh environmental factors like pressure, extremely high or low temperatures, drought, starvation, biocides and UV radiation (Moeller *et al*. 2007, 2008; Postollec *et al*. 2012). The Bacillota (previously known as Firmicutes), which includes the spore-forming bacteria, can be further broken down into five different classes: Bacilli, Clostridia, Erysipelotrichia, Negativicutes and

Thermolithobacteria. Bacilli and Clostridia, which consist of 16 and 21 families, respectively, and are perhaps the most significant classes with regard to the dairy industry, continue to be the most prevalent classes among the Bacillota despite ongoing development and reclassifications (Galperin 2013).

Due to the strong neurotoxins they produce, anaerobic spore-forming bacteria of the genus *Clostridium*, particularly *C. botulinum* and *C. perfringens*, are considered to be the deadliest foodborne bacteria. However, aerobic spore-forming bacteria (*Bacillus* and related genera), have a significant impact on quality, food safety, and economy due to their ability to induce food spoiling and, to a lesser extent, disease. According to Gopal *et al*. (2015) and Carroll *et al*. (2019), these genera include *Bacillus*, *Geobacillus*, *Paenibacillus*, *Brevibacillus*, *Sporocarcina* and *Paenisporosarcina*.

On the stainless steel surfaces of heat exchangers and evaporators, vegetative cells can also continue to build biofilms, and the spores of these bacteria may survive pasteurisation (Flint 1998). When cells or biofilms enter the processing stream, the quality of the final product is reduced the processing plant's operational time shortens (Hinton *et al*. 2002). Dormant spores have the potential to germinate under the right circumstances, generating enzymes that modify the composition and organoleptic characteristics of powders (Flint *et al*. 2007). The resulting powder is typically of low value and is always considered out-of-specification, failing to meet production standards in the case of significant spore contamination in milk (over 10^5 cfu/g; Seale *et al*. 2008; Gopal *et al*. 2015).

In this review, we highlight the current status in the field of bacterial spore contamination of whey powders, the sources of contamination as well as legislation and regulations governing microbial contamination in dairy and whey powders. The factors affecting heat resistance of bacterial spores, their rate of inactivation and mitigation strategies to increase the deactivation rate of spores are also discussed.

QUALITY OF WHEY AS A RAW MATERIAL

Whey powder's composition and quality are influenced by a number of variables, such as the milk used to make cheese, the technology used to process it, the type of coagulation employed (rennet or acid), the temperature and duration of the coagulation, and the method used to cut the curd. Whey can degrade very quickly due to its high moisture content and organic components and, as a result, preservation techniques such as refrigeration and/or the addition of preservatives must be applied correctly (Almeida *et al*. 2001). A maximum cooling temperature of 10°C is advised for carrying chilled whey in isothermal tanks, and the maximum duration for collecting and industrial processing of whey is 72 h (Brasil 2013).

Since whey is released during the processing of milk into cheese, all steps involved in cheese production, starting from the quality of raw milk, affect the quality of whey and its products. After whey is collected, the processes of storing the whey and processing it into powder as well as the storage conditions of the final product also affect the quality of the final powder product. Milk processed into cheese must be: (i) stored under hygienic conditions; (ii) cooled if not processed immediately after milking; and (iii) pasteurised to eliminate pathogens. To prevent microbial contamination during cheesemaking, hygienic conditions should be applied. In this sense, clarification and/or bactofugation are the first pretreatments to be applied during the processing of whey. In this process, by applying centrifugal force, curd pieces and bacterial spores are removed from the whey. This process is among the most important processes that affect the sensory and solubility properties of the final powder. Another important process applied to whey is pasteurisation to stabilise microbial quality. If pasteurisation is not applied immediately after whey removal, whey should be cooled below 5°C.

PROCESSING STEPS AND THEIR CONTRIBUTION TO BACTERIAL SPORE CONTAMINATION

Contamination of whey powders during processing may originate from milk or liquid whey if an efficient heat treatment such as pasteurisation, evaporation and spray drying (Pearce *et al*. 2012) and a clean and sanitised cheese processing are not properly followed (Figure 1).

If the area around the milking cows and milking equipment is not cleaned and sanitised, raw milk may include a variety of microbial contaminants (McHugh *et al*. 2018). A small number of *C. botulinum* spores in raw milk may originate from silage, bedding, or diseased cattle's faeces, according to Doyle and Glass (2013). The number of spores in milk can be considerably decreased by thoroughly cleaning the udders before milking. Additionally, contamination from negligent handling and infected tools can occur both inside the processing plant itself (Figure 1) and during transport from the farm to the processing facility (Pantoja *et al*. 2011). High spore content in supplementary feeds have been linked to a large increase in the contamination of raw milk (Wedel *et al*. 2022). The variety of thermotolerant or mesophilic spore formers may also be influenced by the type of farming practice (organic vs. conventional). The number of thermotolerant organisms was somewhat higher in typical dairy farms (Coorevits *et al*. 2008). It is important to take precautions to reduce contamination because raw whey can be a favourable environment for microbial growth (Varnam and Sutherland 2001; Lazzi *et al*. 2004). The spray dryer needs to be cleaned and sanitised regularly to keep the processing area safe (Keener 2019).

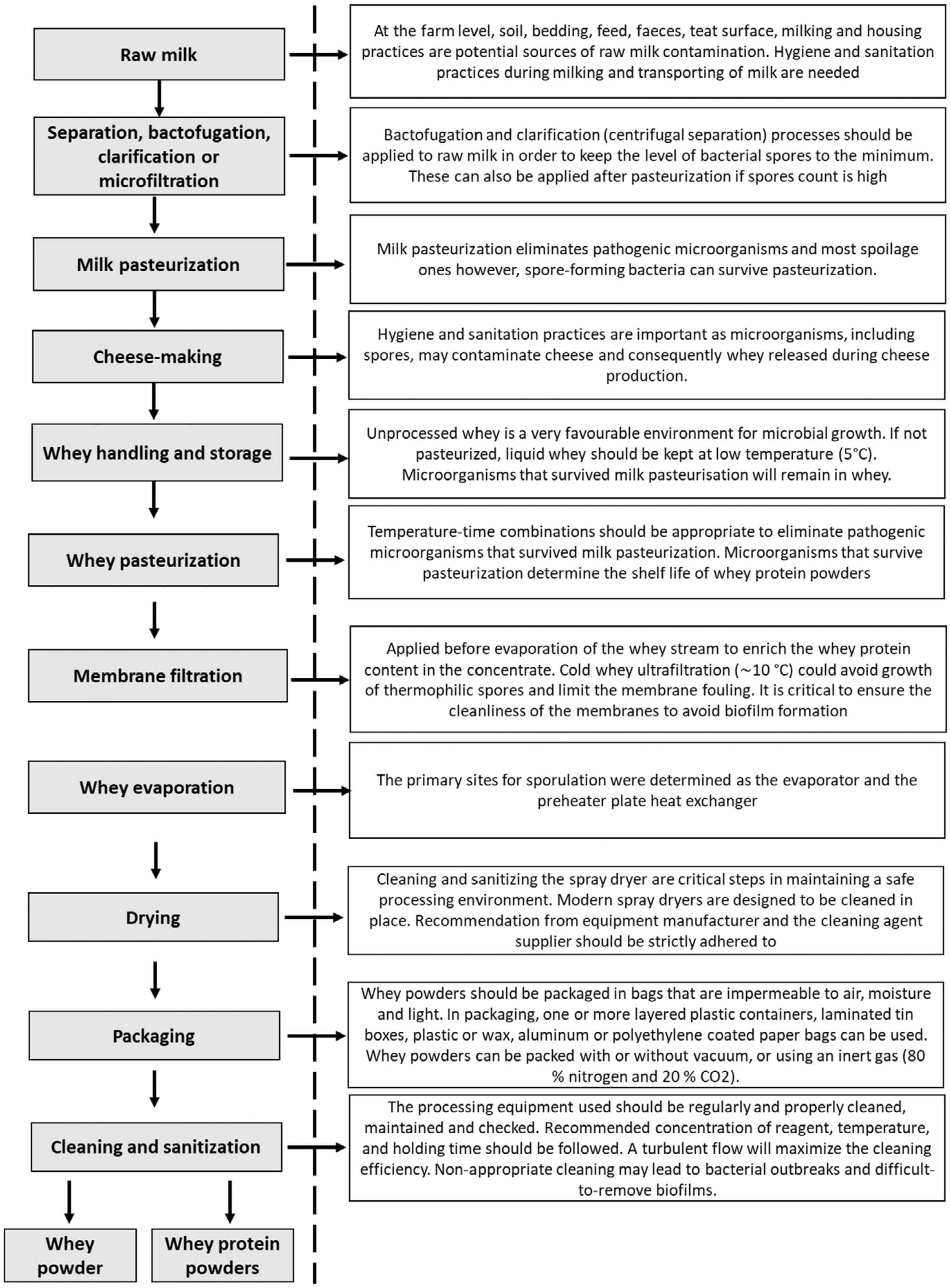

Figure 1 Sources of contamination of whey powders during processing (left) and approaches to reduce the risk of contamination (right).

Although dried dairy products have been linked to a number of outbreaks of foodborne illness, these have frequently been caused by pathogen contamination after pasteurisation. Although they cannot multiply, foodborne pathogenic bacteria can endure for a very long time in dried dairy products. Outbreak cases associated with dairy powders or other products such as liquid milk produced from them are summarised in Table 2.

To render any pathogenic bacteria present in milk and whey inactive, pasteurisation should be performed at the proper temperature and duration. The pathogens' vegetative cells in milk may typically be destroyed by high-temperature short-time (HTST) treatment (72–75°C for at least 15 s), but not their spores (Kessler 2002). Thermophilic organisms, both in the vegetative and spore form, can survive pasteurisation (73°C, 20 s) (Reich *et al*. 2017).

Table 2 Outbreak cases commonly seen in various dairy products

Microorganism	Outbreak	Sources	Reasons	References
Salmonella	Salmonellosis In 2005, powdered infant formula contaminated with *S. agona* in France involving 104 infants.	Raw milk	Lack of hygiene, sanitation, and process control. Small cracks in the spray dryer wall can carry contaminated powder with *Salmonella*. Poorly designed equipment, difficult to clean effectively.	Pal *et al*. (2016); Brouard *et al*. (2007)
Staphylococcal enterotoxins	Staphylococcal food poisoning In 2000, a massive outbreak of staphylococcal food poisoning was reported in Japan, affecting more than 13 420 people	Semiskimmed liquid milk produced using dried skimmed milk powder	Some temperature abuse during the production of dried milk Grow and produce thermostable toxins in milk powder	Smulders and Collins (2004); Ikeda *et al*. (2005); Bogdanovicova *et al*. (2017)
L. monocytogenes	Listeriosis Some research has also been done on the viability of *L. monocytogenes* during spray drying and product storage. No cases of listeriosis associated with dried dairy products have been reported.	Dried products	It was found that spray drying caused a small reduction in numbers and the viable count continued to decrease during storage, but viable *L. monocytogenes* was isolated from some samples after 12 weeks	Vardar-Unlu *et al*. (1998); Sanlıbaba *et al*. (2018); Ballom *et al*. (2020)
Cronobacter sakazakii	Powdered infant formulas contaminated with *Cronobacter sakazakii* were responsible for outbreaks among infants.	Powdered infant formulas	Studies have shown that *C. sakazakii* can survive spray drying when added to skimmed milk powder	Fernandes (2008); Akineden *et al*. (2015)
Aflatoxin M1	Mycotoxins are produced by many moulds, especially *Aspergillus*, *Penicillium* and *Fusarium*.	Dried products	Drying has been found to reduce the concentration but a significant amount of mycotoxins can survive the processing and storage of the finished product for extended periods.	Ráduly *et al*. (2020)
Bacillus cereus	In the USA, 62.5% of the milk powder samples were positive for *Bacillus cereus*, and in Brazil, the organism was isolated from 80% of the samples examined.	Dried dairy products	*B. cereus* food poisoning directly related to the consumption of dried milk	Sumon *et al*. (2021)
B. licheniformis and *B. subtilis*	In 2005 milk powder contaminated with *B. licheniformis and B. subtilis* was the cause of an outbreak in Croatia involving 12 children. The reason was found to be.	Reconstituted milk	Reconstituted milk, which was kept for 2 h without boiling before consumption	Fernández-No *et al*. (2011); Vidic *et al*. (2020)
C. botulinum	In the United Kingdom, 39 commercial infant formula samples (5 brands) were tested for the presence of anaerobic spores in the United States.	Infant formula, Cheddar cheese	Twelve samples containing *Clostridium* spores but botulinum or other neurotoxigenic *Clostridium* were not defined. While mesophilic spores were found in cheddar cheese whey commercially produced in the United States, *C. botulinum* spores were not found	Sithole *et al*. (2006); Barash *et al*. (2010)

Vegetative cells of mesophilic and thermophilic spores can also adhere to heat-exchangers through biofilm formation and contaminate the product flow (Jindal *et al*. 2016).

Before the whey stream is evaporated, membrane processing techniques like reverse osmosis, nanofiltration, ultrafiltration and microfiltration may be used to enrich the content of WP in the concentrate. According to Steinhauer *et al*. (2015), thermophilic spore germination can be prevented and membrane fouling can be minimised by using cold whey ultrafiltration (10°C). The dairy processor must make sure the membranes are clean in order to minimise the food safety hazards and maximise the membrane life. This can be carried out by closely following the instructions provided by the suppliers of the cleaning chemical and the membrane itself (Pouliot 2008; Akpinar-Bayizit *et al*. 2009; Dhineshkumar and Ramasamy 2017). Prior to spray drying, evaporation is used to increase the dry matter and lower the cost of spray drying. The most popular evaporators in the dairy sector are falling-film evaporators. The spray drying process is the final step, which aims to lower the product's moisture content to 4–5% in order to increase its stability at room temperature. The finished powder's packaging has a key role in determining how safe dairy powdered ingredients are from microbiological contaminants (Ramos *et al*. 2016; Zandona *et al*. 2021). In the spray dryer, the air is heated from 150 to 270°C at the process entrance (Schmitz-Schug *et al*. 2013). The majority of microorganisms are normally rendered inactive once the sprayed product particle comes into contact with hot air for 0.2–55 s (Schmitz-Schug *et al*. 2013). However, a number of mass transfer events (such as moisture reduction) can reduce the negative impact that high temperatures of spray drying have on microorganisms (Bhandari 2013). During spray drying, the thermophilic spores may not be reduced but the mesophilic associated spores may. Hence, inlet air temperature and product feed composition and viscosity should be considered. As an example, when spray drying a product feed inoculated with *Bacillus thuringiensis* spores (thermophilic spores), an inactivation effect could be shown for inlet air temperatures between 170 and 250°C. Nevertheless, the log-reduction was only under 1-log (Zhou *et al*. 2008). On the other hand, at inlet temperatures between 150 and 190°C, substantially larger log-reduction values were achieved for the mesophilic *B. cereus* (Alvarenga *et al*. 2018). It is also worth mentioning that the actual particle temperatures are significantly lower than the entrance or output temperatures of the spray dryer, which is particularly crucial to note.

During production, microbial biofilms formed on the equipment surfaces in processing facility may act as a protective structure for spore-forming bacteria. The spores, regardless of the sources of contamination, can withstand the process and then remain in a dormant form in powdered dairy products (McHugh *et al*. 2018). On the surfaces of storage and processing equipment, *B. cereus* produces biofilms that can be challenging to remove. These spores then multiply during the production of whey powder, with the evaporator and the preheater plate heat exchanger identified as the main locations for sporulation (Scott *et al*. 2007; Glass and Marshall 2013; Stoeckel *et al*. 2013). The processing equipment should be cleaned, maintained and tested on a regular basis. A large bacterial load can be caused by improperly cleaned equipment and poor whey storage conditions (Figure 1) (Monfredini *et al*. 2011).

Spore populations in dairy powders

Dairy powders have a wide range of applications including the formulation of soups, frozen desserts, bakery products, confectionery, baby foods and sports drinks. Among these powders, whole milk powder (WMP), skimmed milk powder (SMP), WPC/WPI, whey protein hydrolysates (WPH), microparticulated whey proteins (MWPs), milk protein concentrate/isolate (MPC/MPI), casein and caseinates are available in the market (Pal *et al*. 2016; McHugh *et al*. 2017). Milk powders have high economic value and global trade as they do not require strict storage conditions due to their low water activity ($a_w < 0.26$) which slows down microbial development. Despite the thermal processes applied in the production of dairy powders, heat-resistant endospores affect the microbial quality of dairy powders (Pal *et al*. 2016; Dettling *et al*. 2019). In areas of high temperatures, such as pasteurisation, UHT or spray drying, spores can germinate and grow excessively. Spore-forming vegetative cells form spore status if conditions become unfavourable. In addition, direct contact with processing surfaces or packaging material may cause the products to be contaminated with spores (Li *et al*. 2019).

Important quality and security indications for milk powders are bacterial endospores. They may be able to survive in a variety of settings due to their high temperature and pressure tolerance and stability to numerous environmental stressors, such as low pH and a_w (Miller *et al*. 2015). The most prevalent spores in raw milk tanks are mesophilic spores of *Bacillus licheniformis* and *Bacillus pumilus*, which can withstand environmental challenges including low pH, high temperature, cleansers and high pressure, among others.

Li *et al*.'s (2019) investigation in Ireland revealed that 68% of the isolates detected in the skim-milk powder were *B. licheniformis*, which can thrive in both mesophilic and thermophilic environments and is developed as a facultative anaerobe. These bacteria can be resourced from the agriculture environment. Conversely, dairy powders tend to contain more thermophilic spores. The three main spore-forming bacteria in milk powders are *B. licheniformis*, *Anoxybacillus* sp. and *Geobacillus* sp. In a study on milk powder processed in France, Delaunay *et al*. (2021) discovered 313 thermophilic isolates, of which 43.5% were *G. stearothermophilus*, 30% were *B. licheniformis* and 19.8% were *A. flavithermus*. However, *B. licheniformis*, *B. pumilus* and *B. cereus* predominate while *G. stearothermophilus* and *A. flavithermus* are infrequently isolated from raw milk tanks. The obligatory thermophiles *Anoxybacillus* sp. and *Geobacillus* sp. are frequently linked to environmental contamination during milk processing. The spore-forming bacteria might originate from the earth, animal waste, feed, silage, milking equipment and raw milk tanks.

Due to the lack of hygiene standards, contamination can also spread through infected equipment used to transport milk from the farm to the plant. Preventing contamination of raw milk is the first step to lower the number of spores in dairy powders (Te Giffel *et al*. 2002; Miller *et al*. 2015; Kent *et al*. 2015a; McHugh *et al*. 2017). Infant formula and medicinal baby meals may include no more than 5–50 cfu/g of *B. cereus* (European Commission 2005). Mesophilic and thermophilic spore counts in dairy powders used in infant formula are restricted by the United States Dairy Export Council to less than 1000 and 500 cfu/g, respectively (Watterson *et al*. 2014a).

In milk powders, anaerobic spore-forming bacteria like *C. botulinum* are less prevalent than their aerobic counterparts. This could occur as a result of the test criteria for spore-formers being optimised to identify aerobic spore-formers, in addition to the high level of aeration involved in the processing of milk powder or phenotype-based tests for specific groups of anaerobic species. On specific medium, certain *Clostridium* species can convert sulphite to sulphur under anaerobic conditions, which causes the growth of black colonies. The accuracy of these qualitative and quantitative approaches was previously reviewed (Doyle *et al*. 2015).

Sporulation can happen at many stages of the production of dairy powder. Raw milk is heated in the preheaters to 50°C as the first step in the procedure whereas, temperatures in the heat exchanger and evaporators are between 45 and 75°C. These temperatures are ideal for the growth and sporulation of thermophilic bacteria. Concentrated milk may be a favourable medium for *Geobacillus* sp. and *Anoxybacillus* sp. thermophilic spores under low a_w circumstances. Spores contained in biofilm formed on production line surfaces can cause cross-contamination of the new product feed. At 55 and 60°C, *A. flavithermus's* spores and biofilms may develop flow-resistant biofilms. However, some spores on the surface may be discharged as the feed moves, depending on how frequently the line is cleaned (Gauvry *et al*. 2017).

Common spores in whey powders

Bacillus sp.

Geobacillus stearothermophilus, *A. flavithermus* and *B. licheniformis* are three thermophilic bacilli found in dry milk powder products. These Gram-positive, aerobic bacteria can produce relatively heat-resistant spores at high temperatures (Yuan *et al*. 2012). Heat shock therapy is used to activate bacterial spores for a brief duration (5 min) at specific temperatures. The heat shock temperatures can differ depending on whether the bacterial spore is mesophilic or thermophilic. *Bacillus megaterium*, for example, may be heated to 60°C, whereas *Bacillus stearothermophilus* needs 115°C (Berg and Sandine 1970). Rod-shaped, thermophilic spore-forming bacteria (*Anoxybacillus* sp. and *Geobacillus* sp.) are frequently recovered from dairy plants (Govindasamy *et al*. 2010; Takami 2011). The types of these genera are defined as rod-shaped (typically round-ended), Gram-positive, spore-forming, obligatory or facultatively anaerobic, thermophilic, alkalyphiles or alkaline tolerant bacteria. *A. pushchinensis*, *A. flavithermus* (formerly *B. flavithermus*), *A. gonsis*, *A. ayderensis* and *A. kestanbolensis* are species of the genus. While *Anoxybacillus* sp. and *Geobacillus* sp. are thermophilic (grow at higher temperatures), *Bacillus* sp. are mainly mesophilic (optimum growth temperature of 10–30°C). *Anoxybacillus* sp. in dairy powder products are commonly observed and the probable source is raw milk. The presence of *Geobacillus* sp. may not be relevant to raw milk but may be related to whey and whey powder products (Miller *et al*. 2015).

In the studies to the present, the dominant flora forming spores in dairy powder products is *Anoxybacillus* sp. and *Geobacillus* sp., together with *Bacillus* sp. (Zain *et al*. 2016). The presence of these bacteria in raw milk also changes according to milking season. These spores are resistant to high temperature and other severe conditions, especially in case of biofilm (Ronimus *et al*. 2003; Gómez-Torres *et al*. 2014; Watterson *et al*. 2014a), causing economic losses to the dairy industry and causing food-related diseases. These microorganisms are known to have several mechanisms that have an impact on food safety and product quality. These include (i) toxin production; and (ii) creating enzymes that hydrolyse food components (De Jonghe *et al*. 2010). The spores of *B. cereus* can adhere to glass and steel surfaces, where they are difficult to remove, and also persist for months in dairy powders (Pavic *et al*. 2005). After pasteurisation, the development of such biofilms on surfaces also contributes to transmission of spores to the medium (Uraz and Gündüz 2013; Watterson *et al*. 2014a). It is well known that spores of thermophilic bacteria, like *B. cereus*, are a frequent contamination in dairy powder products. In the United States and Brazil, respectively, 62.5% and 80% of the dairy powder samples under investigation tested positive for *B. cereus*.

In WPC, the microflora of whey may vary depending on the type of whey (acid or sweet). Sweet whey may contain a higher number of mesophilic and thermophilic spores compared to acid whey (Watterson *et al*. 2014b). Dettling *et al*. (2019) found that the thermophilic spores in skim milk powder and whey powder produced in Germany was between 3 and 5 log cfu/g. It was determined that *A. flavithermus* was present in high abundance and *B. licheniformis* was present in low numbers. Zain *et al*. (2016) stated that high bacterial counts were found in the WPC80 samples with six *Bacillus* sp. identified including *B. licheniformis* (67%), *B. cereus* (19%), *Bacillus tansensis* (4%), *Bacillus subtilis* (4%), *B. pumilus* (4%) and *Paenibacillus. gluclanolyticus* (2%). In a study by Miller *et al*. (2015) to determine spore populations in raw milk tank and dairy powders produced thereof [SMP, acid whey powder (AWP), sweet whey powder (SWP) and whey protein concentrate (WPC-80)], *Bacillus* sp. (68.9%) and *Geobacillus* sp. (12.1%) were the most frequently isolated spore-forming bacteria. *B. licheniformis* was associated with both raw milk and powder, while *Geobacillus* and *Anoxybacillus* was only found in the final powder product. *Geobacillus* sp. were isolated from WPC and SWP, during processing and in final product. In the whey powders, *Geobacillus* sp. spores were related to contamination from the plant as well as whey. Kent *et al*. (2015a) stated that the contamination with *Geobacillus* sp. spores is derived primarily from whey. As components like fat, lactose, and protein may serve as a protective matrix for spores, the rise in dry matter with concentration may also be a contributing factor to the increase in heat-resistant spores. According to Marx and Kulozik (2018), a decrease in a_w and an increase in the amounts of lactose and WP both contribute significantly to the *B. subtilis* spores' enhanced heat resistance.

In dairy powders such as WP concentrate (WPC-80), sweet whey (SW) and acid whey (AW) powders, consistent production of high quality products can only be assured when spore numbers are low, although there are no regulatory limitations for spore numbers. In addition, it is important to develop preventive approaches to limit the spore contamination in these products and determine the spores in these powders. In summary, reduction of the contamination of raw milk tanks to a minimum level would have a significant impact in reducing the number of spores in powder products.

Clostridia

Clostridium botulinum

Clostridium sp. reported in dairy powders are *Clostridium halophilum*, *Clostridium septicum*, *Clostridium butyricum*, *Clostridium bifermentans*, *Clostridium sporogenes*, *Clostridium innocuum*, *Clostridium cochlearium*, *Clostridium tyrobutyricum*, *Clostridium perfringens*, *Clostridium lundense* (Barash *et al*. 2010; Burgess *et al*. 2013). Anaerobic *Clostridium* sp. generate gas during the cheese ripening and maturation. The sulphite-reducing Clostridia reference value for many foods is less than 100 cfu/mL and 1 endospore/mL. da Silva Duarte *et al*. (2020) evaluated the microbial composition of cheese whey in four Italian cheese manufacturers. None of the samples exceeded 10 cfu/mL, although five of the 24 samples included sulphite-reducing Clostridia that were over the technique's detection limit of 1 cfu/mL. Only three out of the 24 samples tested positive for spores and had fewer than 10 mL^{-1} spores. Since *C. tyrobutyricum* has been found in cheese, spores are probably also found in whey by-products (Klijn *et al*. 1995; Bassi *et al*. 2013).

Over the past 20 years, there have been additional reports of botulism outbreaks in cattle as a result of increased use of plastic-packaged silage. When *C. botulinum* grows in silage and the gastrointestinal tract of cattle, it has the potential to contaminate the farm environment, raw milk, and the entire dairy supply chain. In the dairy business, outbreaks are typically serious and result in significant financial losses.

The Group I–III *C. botulinum* can cause a botulism outbreak when nonacidic, plastic-wrapped silage is fed to cattle. Concerns about the risk of human botulism associated with the intake of dairy products arose as a result of the association between group I and group II pathogens and bovine botulism. Standard milk pasteurisation does not totally eliminate spores. Although they are uncommon, both domestic (home) and commercial (household) dairy products have been linked to many sizable botulism epidemics (Lindström *et al*. 2010). In a cow with a healthy mammary gland, it is expected that milk has a very low bacterial count, although milk contained in an infected udder with bacterial mastitis can have a high bacterial count. However, the counts of microorganisms increase based on the level of contamination during and after the milking process. In raw milk, the number of *Clostridium* sp. may be present at low level depending on the contamination level. However, toxins could be developed by any *C. botulinum* spores that survive normal pasteurisation procedures if the product is not kept refrigerated or at later stages of the dairy supply chain or in the domestic setting. Because they are high in protein, carbon and minerals and have a high a_w, milk and the majority of milk products provide ideal bacterial growth media (Lindström *et al*. 2010; Pal *et al*. 2016).

Spores, such as those produced by *C. botulinum*, are resistant to drying and pasteurisation and can last for a very long time in dairy powder products (Ronimus *et al*. 2006). Although not a frequent concern, *C. botulinum* may be present in dairy powder products. Research on the frequency, concentrations, or varieties of clostridial spores in dairy powder products is scarce. *C. botulinum* spores can cause infant botulism which is a toxico-infection by germination in the gastrointestinal system and downstream toxin production. This is particularly problematic as the powder infant formula is reconstituted in water in the absence of cooling. This causes the spores of *C. botulinum* to germinate, develop and produce neurotoxins if stored above refrigeration temperatures for extended periods of time. Both *C. botulinum* and *C. perfringens* spores are of concern in infant formula (Pal *et al*. 2016). In the United Kingdom, powdered milk used in infant formula was linked to a case of newborn botulism in 2001. In milk powder containers that had been opened from the homes of patients with newborn botulism, clinically isolated strains of *C. botulinum* were found (Johnson *et al*. 2005; Sobel 2005). Milk powders have never been confirmed to be the direct cause of newborn botulism cases (Brett *et al*. 2005; Johnson *et al*. 2005), despite the fact that numerous *Clostridium* species have been reported in milk powders (Barash *et al*. 2010; Buehner *et al*. 2015). Thirty-nine commercial infant formula samples (5 brands) were examined for the presence of anaerobic spores in the United States following an incidence of newborn botulism linked to infant formula in the United Kingdom. Although *C. botulinum* or other neurotoxigenic Clostridia were not discovered in any of the 12 samples, they did contain spores of *Clostridium* sp. (Doyle and Glass 2013).

Clostridium botulinum spores that may be present in whey concentrate raise questions about the potential risk and control of spores in dairy powder products. Therefore, spore populations in dairy powder products should be minimised by using high-quality milk, temperature control, sanitation of equipment and processing plants, and the use of new processing technologies such as membrane filtration.

Incidence of *C. botulinum in infant formula and sports drinks in 2013*

In New Zealand, the world's largest dairy exporter experienced an incident caused by whey protein concentrate (WPC-80) that was believed to be contaminated (three batches of 38.2 tonnes) with *C. botulinum* which was used in infant formula and sports drinks. Countries such as China, Russia Kazakhstan, Belarus and Sri Lanka have imposed a temporary ban on some dairy products from New Zealand. Vietnam also decided to stop the circulation of milk powder produced by this company after contamination. Singapore and Malaysia took measures for some infant dairy products produced by the company. While the ban has since been lifted in China, extra testing is being done with inspections at the New Zealand dairy border. The company suffered a 9% devaluation, but still recovered about the reduction after the incident was announced. The company confirmed almost a month later that the incident was a false alarm following an internal investigation and analysis. According to the supplier's website, WPC produced at a single production site in May 2012 and there were no toxins or active cells. In March 2013, after a test by the supplier revealed that sulphide-reducing Clostridia (SRC) had exceeded the limit, tests conducted by the Ministry of New Zealand (New Zealand's Ministry for Primary Industries) stated that 'There is absolutely no *C. botulinum* in the WPC powder and the whey concentrates used in infant formula'. In addition, the company announced that the affected product (WPC80) was never contaminated with *C. botulinum*, but instead contaminated with *Clostridium sporogenes*, which do not cause botulism in humans (GAIN Report NZ1314 2013; Stojkov 2016).

While the total financial loss is not known, the company estimated to have lost more than $60 million in the hours following the initial announcement. A French company, one of the largest global infant formula powder producers, received a Food Safety Alert that sparked product recall from eight different markets. This French company demanded ~ €200 million ($270 million) in compensation from the company from New Zealand to cover the costs (Hussain and Dawson 2013).

Factors affecting spore inactivation

Several factors need to be considered during milk and whey powder processing to ensure low spore count and high quality in the final powder. These include the properties of the concentrated liquid feed to be spray dried and cleanliness of equipment surfaces from fouling deposits of persisting strains which can accumulate and increase in numbers during processing through the application of an efficient CIP cleaning protocol. These factors are summarised in Figure 2 and detailed here below.

Feed concentration

Whey powder production ranks first among the indispensable by-products of the food industry with a wider range of uses. During the production, the parameters of the process are important as quality characteristics and determinants of shelf life in the final product. Within these parameters, heat treatment and spray drying are considered essential process steps in the production of whey powder. However, there is an intensive energy need during the heat treatment phase. Alternatively, whey concentrates can be preferred instead of the liquid native whey for energy saving. Membrane filtration methods can be defined as highly efficient processes in terms of producing whey concentrates at lower temperatures. Different membrane filtration technologies in dairy processing are outlined in Figure 3. However, a heat treatment application is needed to reduce a_w in concentrates produced by membrane filtration. The heat treatment applied at this stage should be effective in killing vegetative bacteria as well as bacterial spores. In determining the heat treatment norms to be applied in whey production, the conditions in which spores are inactivated are determined. In heat treatment applications, the pH value of the environment, a_w, and components of the heat-treated matrix (protein, fat, lactose, calcium, etc.) are important in inactivating bacterial spores. In this context, the concentration is determined in the final product according to the type of membrane to be applied in the production of whey concentrate. Reverse osmosis, nanofiltration and ultrafiltration applications are used in the production of whey concentrates, of which reverse osmosis application is only for the water removal. While nanofiltration allows minerals to be removed from the concentrate, the composition of the concentrate in ultrafiltration can be defined as the closest content to whey powder.

Although all the preferred membrane applications in milk processing allow the production of whey concentrate, the heat treatment norms to be applied in the next process stage are important. Because different membrane techniques

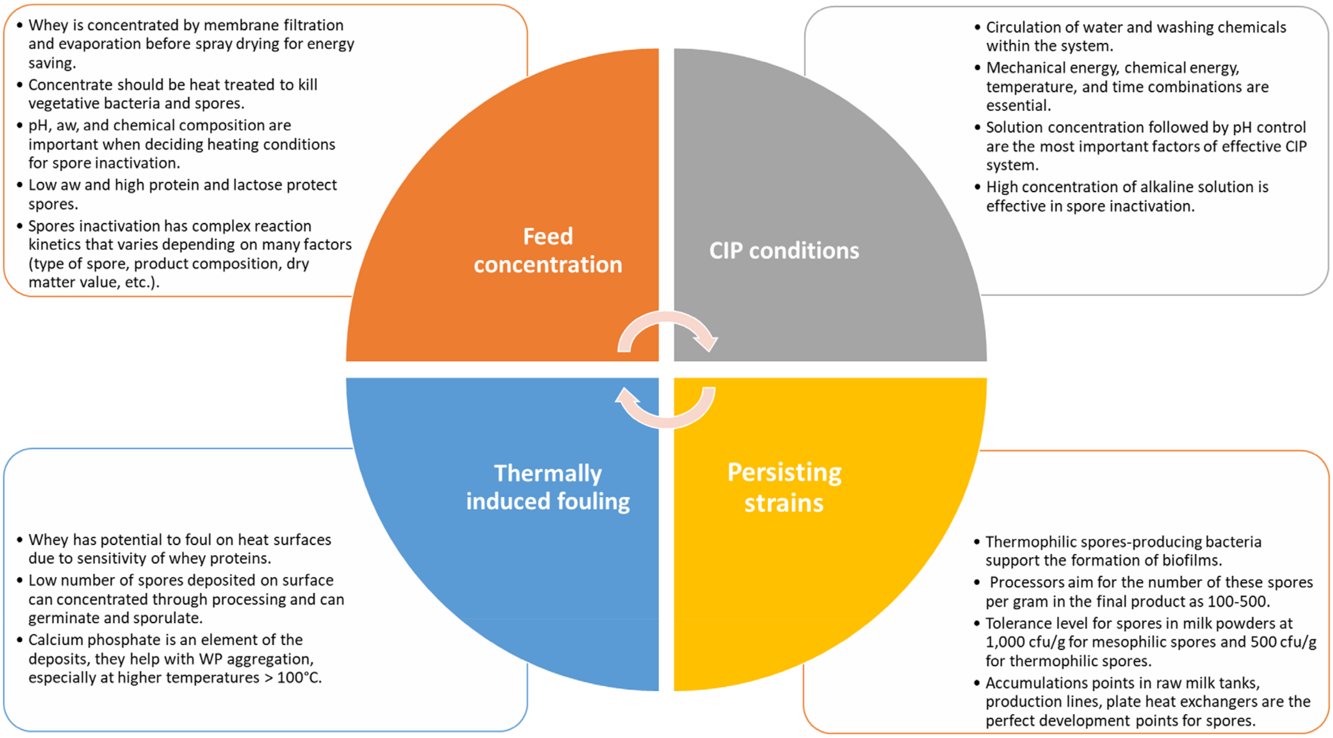

Figure 2 Factors that impact the spore numbers during milk and whey powder processing.

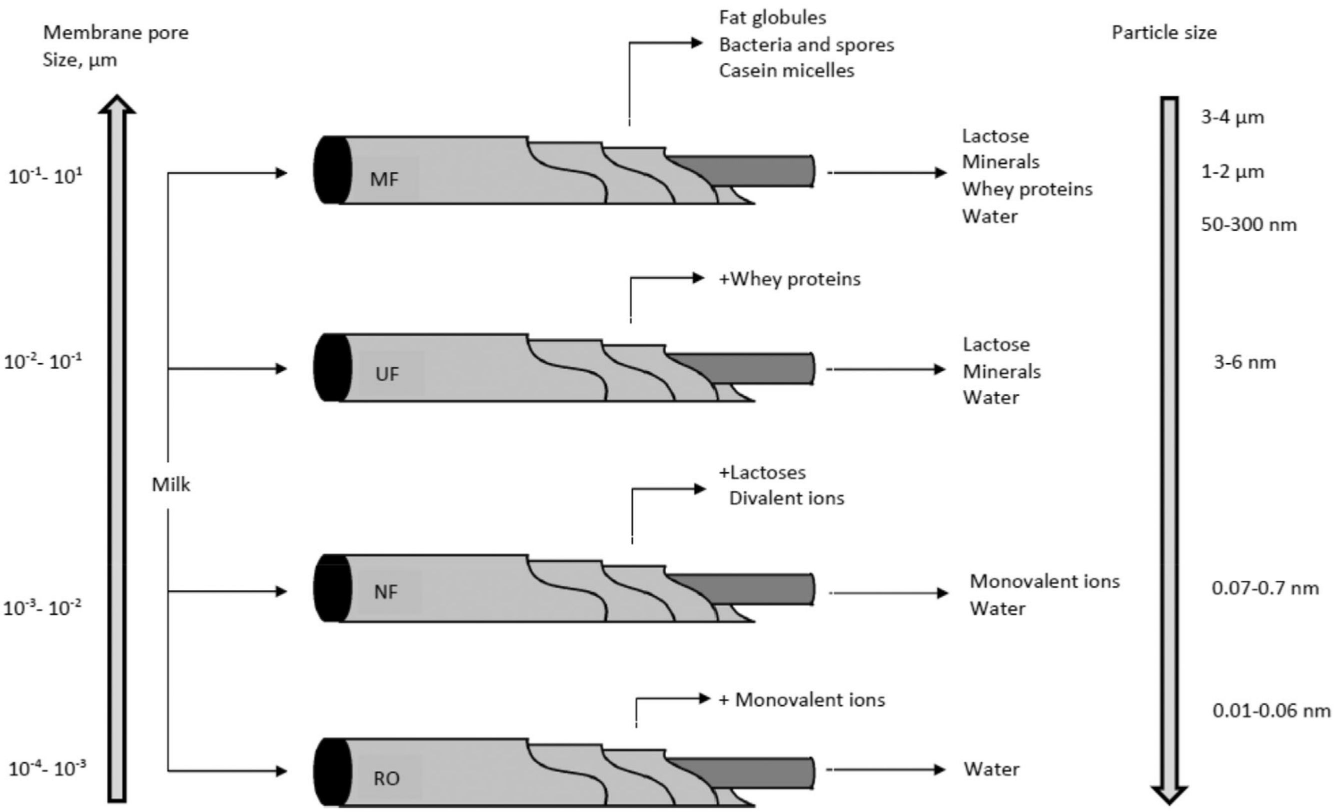

Figure 3 Membrane technology in milk processing applications (adapted and re-drawn from Lipnizki 2010). MF: microfiltration, UF: ultrafiltration, NF: nanofiltration and RO: reverse osmosis.

determine the final product composition, its effectiveness may increase or decrease depending on the heat process to be applied.

A study investigated the inactivation kinetics of *B. subtilis* spores in the temperature range of 75–140°C for different whey concentrates produced using different membrane processing technologies (Marx and Kulozik 2018). Examining the change in D value, the time required to kill *B. subtilis* spores at 110, 125 and 140°C was calculated. Ultrafiltration concentrate (DM ≤ 8.1%), nanofiltration concentrate (DM ≤ 24%) and reverse osmosis concentrate (DM ≤ 12%) were detected closely for this temperature norm. Decreased a_w, increased WPs and lactose concentration protect the spores from heat. The D-value detected in the range of 5.1–11.1 s at 125°C was reported as 0.8–3.2 s at 140°C. It should not be overlooked that the results obtained to produce a high-quality product with a long shelf life are product-specific. In addition, the spore flora of the product should be carefully evaluated when determining the optimum norms. Spore inactivation has complex reaction kinetics that varies depending on many factors (type of spore, product composition, dry matter value, etc.).

Cleaning-in-place (CIP) conditions

Integrated milk factories need a system that supplies the need for microbiologically adequate and effective cleaning in a shorter time. CIP systems have become an indispensable part of the food industry. The main feature of models operating on the CIP principle is that they ensure the circulation of water and washing chemicals within the system. Fully automated systems include chemical tanks in which chemicals are recovered and their efficiency are monitored using a conductivity meter device that is activated to accurately dispense and control chemical application. Based on mechanical energy, chemical energy, temperature and time combinations, the CIP system is effective in both microbiological disinfection and the removal of residues.

Recycling, which is important for the cost calculation of enterprises, requires rigorous control. For these systems, which provide an effective cleaning with multiple chemicals, the effectiveness of recovered chemicals is the decisive factor in the effectiveness of the cleaning applied. Reduced solution concentrations reduce the efficiency of the cleanliness and the efficiency of the system. Effective cleaning is ensured in CIP systems with solutions of acid and alkali chemicals at different concentrations. The effectiveness of acid–alkali or alkali–acid combinations can be increased by differing concentrations of chemicals and contact time. Although the details of commercially used formulations are unknown, some chemicals used and their functions are given in Table 4. Solution concentration followed by pH control are the most important factors of effective CIP system. Research on this subject reveals that thermophilic spores and milk residues in processed milk and dairy products are significantly affected by the concentration of the cleaning solution (Wedel *et al*. 2019). A significant decrease in spore concentrations has been reported when the concentration of alkaline cleaning solutions is gradually increased from 0.5% to 2% with constant heat treatment for 10 min at 65°C for thermophilic spore-forming microorganisms. While the concentration increases in the range of 0.5–2% in strains belonging to *the G. stearothermophilus* species decreased by 2.4 log, this value was reported as 1.8 log for *A. flavithermus* strains (Wedel *et al*. 2019).

Persisting strains in processing lines

Among the factors that determine the quality of the product in the production of milk powders and concentrates, the number of thermophilic spores in the raw material is important. Thermophilic spores-forming bacteria also support the formation of biofilms in milk and dairy processing lines thus the removal of spores is essential to plant hygiene as well as product quality. For processes involving long processing lines and large production tanks, the presence of such spores is an obstacle to the production of quality products. For this reason, food producers aim for the number of spores per gram in the final product as 100–500 thermophilic spores. This is associated with the U.S. Dairy Export Council setting the tolerance level for spores in milk powders at 1000 cfu/g for mesophilic spores and 500 cfu/g for thermophilic spores (Watterson *et al*. 2014b). Highlights in milk storage tanks and processing lines can be listed as *A. flavithermus*, *G. stearothermophilus*, and *B. licheniformis*.

There may be accumulations in raw milk tanks, production lines, plate heat exchangers, and especially at the points where pipeline joints. These populations, which can increase in quantity due to the intractability of the CIP system, are the perfect development points for spores. For a system where sanitation is fully achieved, it is important to use equipment designed for CIP applications. Mature spores can resist CIP applications or temperature applications below 100°C. Biofilm formation in the process can be easily seen during storage in tanks and processing on plate surfaces. This means that the number of spores at a low level at the beginning may pose a risk for the next processing step. Therefore, it is necessary to evaluate the effect of the processing steps and the processing scale during milk and whey powders production on the spore load in the production line for products with a known raw material load. In a study that investigated the impact of the increase in dry matter content during whey powder production process, the effect on the number increase of spores was reported as 1 log cfu/mL. This value was considered statistically insignificant in relation to the 16-fold increase in dry matter during whey concentration and drying (Dettling *et al*. 2020). Although there does not appear to be a significant increase in the product during the process, colonised spores can germinate and multiply rapidly. The most important parameters for the number of spores in the final product to be at the desired levels are: Optimum and efficient CIP cleaning and using raw materials with low initial spore concentration. Within the scope of these objectives, increasing the concentration of CIP chemicals, applying more frequent cleaning, ensuring product-specific optimisation of the cleaning plan may limit or prevent the colonisation of spores.

Thermally induced fouling (TIF) on heating surfaces and incomplete cleaning

The load of pathogenic microorganisms in dairy powders is normally very low. However, although they are not pathogens, the increased spores load significantly affects the quality of the product. Producers in various countries aim to minimise the spores load in powdered dairy products. The number of thermophilic microorganisms in dairy powders is defined as the most important quality parameter (Burgess *et al*. 2010; Hill and Smythe 2012; Sadiq *et al*. 2016; Reich *et al*. 2017). High spore loads can be a result of TIF and incomplete cleaning practices on heating surfaces. Although the initial number of spores is low, the number of spores can reach undesirable levels in the final product which is not preferable for both the manufacturer and the consumer. This might be because they become concentrated or they germinate and bacterial counts and subsequent spore counts increase. Although spores themselves cannot multiply, they are concentrated through processing and can germinate and sporulate culminating in high spore counts in the final product. For this reason, it is very important to keep the number of spores under control.

Whey is a product with a high potential to create thermally induced fouling (bacterial biofilm) in milk processing lines. Whey proteins are rich in necessary nutrients for microbial growth. Whey proteins, mostly β-lactoglobulin, begin to denature at 65–70°C (Visser and Jeurnink 1997), which falls within the scope of pasteurisation norms applied in milk powder processing. Therefore, TIF is inevitable in processes where milk and heat treatment norms converge. In addition to increasing the level of TIF in higher temperature norms, calcium phosphate deposits also appear as an element of the fouling material. These deposits, usually consist of 70–80% mineral, 15–20% protein and 4–8% fat (Bansal and Chen 2006). Studies indicate that the resulting biofilm layers are affected by acid/alkaline use and concentration change in cleaning applications.

Although the CIP process is aimed to prevent the formation of biofilms, this is not fully possible. In addition, biofilm structures provide the basis for the retention of spores and continuity in the system. Cleaning solutions used alternately in CIP systems also support the viability of spores in the process. In this context, researchers continue to examine the relation of biofilm structures and spores to cleanliness.

Wedel *et al*. (2020) established a pilot mechanism stablished to allow thermophilic spores to attach to biofilm structures. In this process, the effect of acid and alkaline cleaning applications on spores was taken into consideration. In addition, biofilm was evaluated on surfaces that could not be cleaned effectively by simulation of inadequate cleaning applications. The study emphasised that biofilm layers are holding points for thermophilic spores, and acid and alkaline cleaning applications are not the definitive solutions in removing spores from the environment. If there is no effective cleaning application, the spores in the environment can hold on to thermal deposits, germinate over time and create new spores when conditions allow (Figure 4).

Therefore, improper cleaning and removal of biofilm structures may result in high number of spores in the final product. TIF elements in whey powder production and the effective cleaning of these structures are the determinants of the spores concentration of powders. One of the measures to be taken is the monitoring of the concentration of cleaning solutions in CIP process used in the cleaning of milk processing lines. It should be noted that the cleaning effectiveness decreases after several times chemical solutions are reused. Chemical concentrations should be increased to counter the formation of biofilm structures if needed and this should be supported by effective rinsing application.

Mitigation strategies for spore inactivation

The dairy industry continues to face a sizable difficulty as a result of thermoduric spore-former contamination of milk (Li *et al*. 2019). Thirteen thermophilic bacilli were found to be the main milk powder contaminants according to Reich *et al*. (2017), which is a sign that the manufacturing facility's sanitary measures are deficient (Burgess *et al*. 2010). It has been claimed that HTST pasteurisation is insufficient to create a low-spore powder. Although harsh commercial sterilisation processes like UHT and retort sterilisation can kill spores, they may also have unfavourable effects such nutrient loss, denaturing of proteins, and the development of off flavours (Hill and Smythe 2012).

New applications are being evaluated to reduce contamination of food products with spores. Biofilm structures, which are an inevitable problem in dairy thermal processes, are the comforting shelters of spores. For this reason, in addition to the process optimisation and ensuring cleaning efficiency, additional application of other technologies, as partial or complete replacement of conventional heating, that will reduce the current intensity of spores are also important. Microwave, heat treatment, chemicals applied at different concentrations, membrane filtration, ultrasonication (US), intense pulsed light (IPL) application stand out among these approaches. In addition, the combination of different applications can be effective in reducing spores' intensity. Strategies for spores' inactivation are outlined in Figure 5.

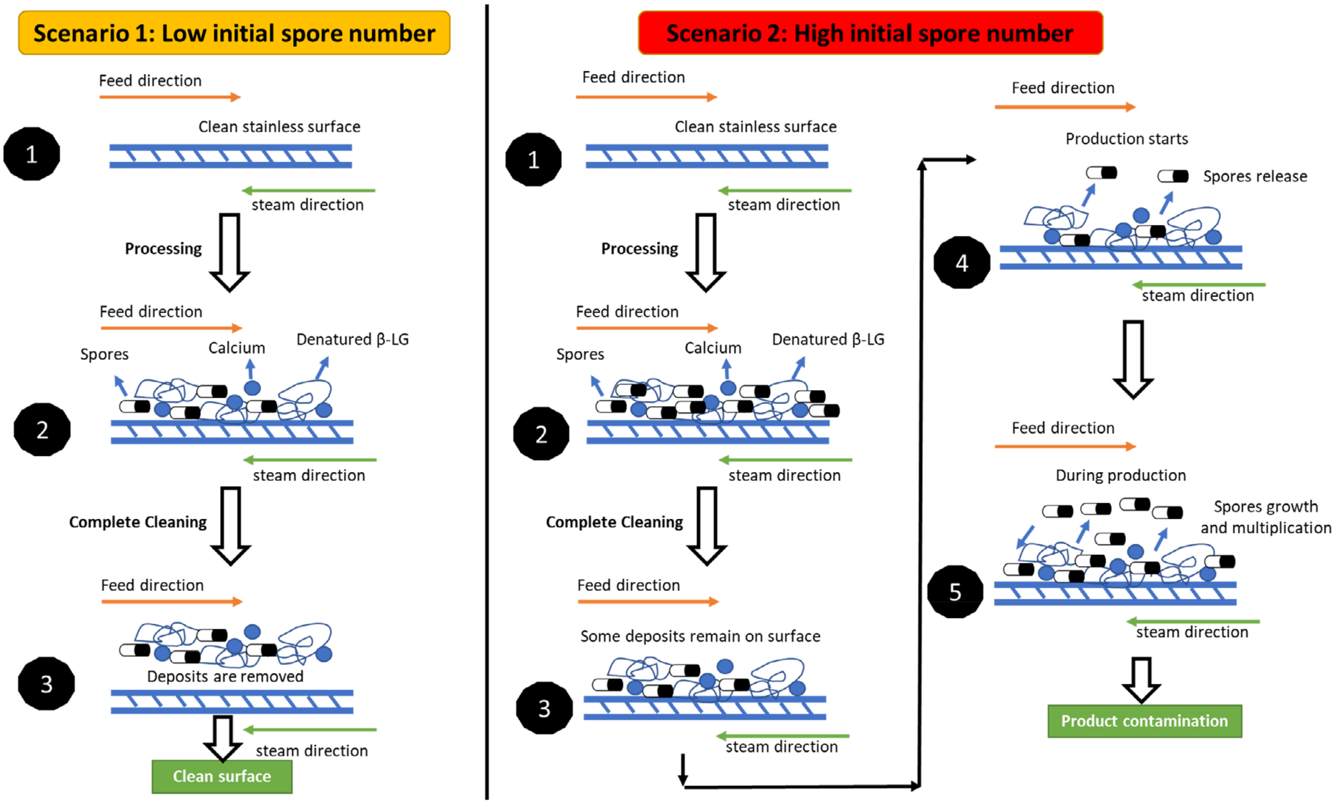

Figure 4 Effect of inadequate cleaning during powder processing (adapted from Wedel *et al*. 2020). 1–3 Low spore counts in milk powder at the end of the production (scenario 1, left); 1–5 high spore counts in milk powder at the end of the production due to recontamination (scenario 2, right).

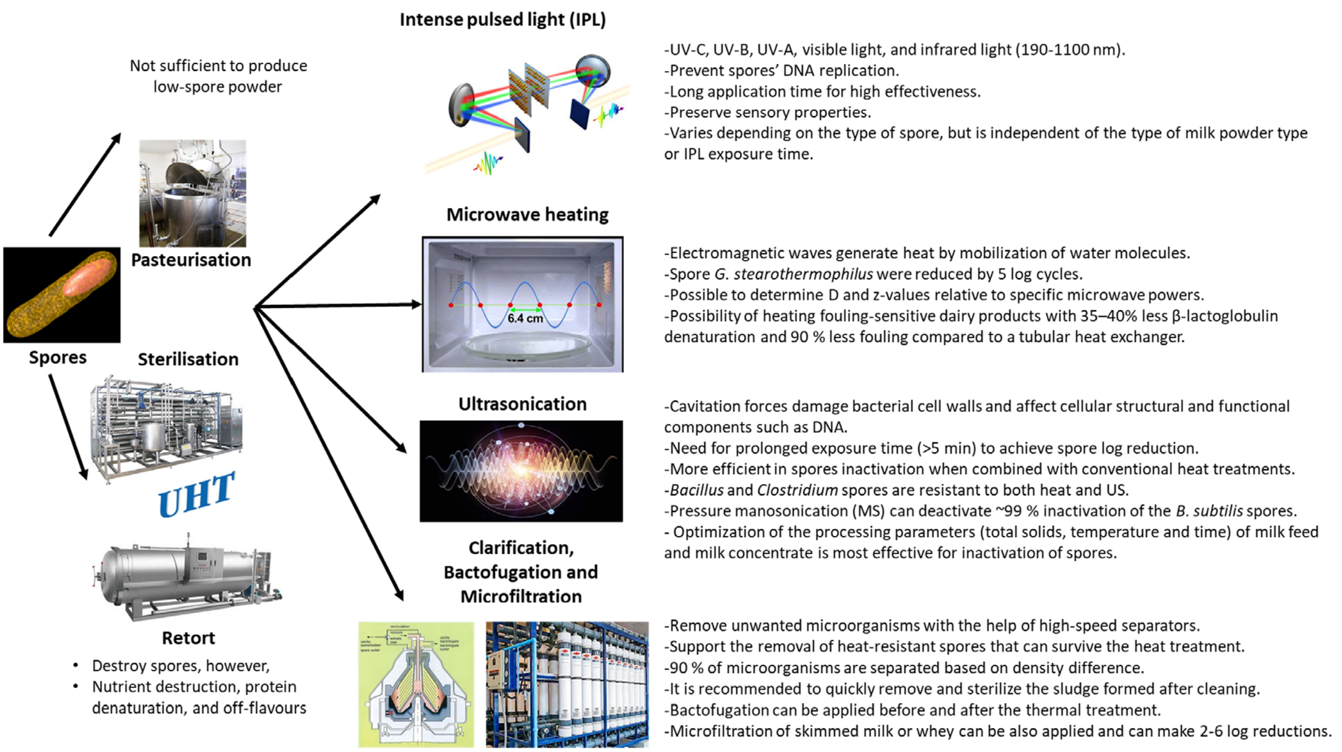

Figure 5 Summary of the different strategies to reduce numbers of spores in milk and whey powders.

Intense pulsed light

Intense pulsed light is a nonthermal technology that affects microorganisms by applying high-energy light pulses. IPL applications on the food matrix are common in the removal of pathogens from the environment (Gomez-Lopez *et al*. 2005; Setlow and Li 2015; Chen *et al*. 2018). This application covers light pulses consisting of ultraviolet (UV-C, UV-B, UV-A), visible light, and infrared light defined in the wavelength range of 190–1100 nm. UV and similar applications contribute to reducing spores intensity by preventing DNA replication of spores. However, for bacteria capable of creating endospores, the potential for germination and subsequent increase in numbers is an important consideration. In this context, IPL application with long application time and high effectiveness comes to the fore. Inactivation of spores that have switched to germination is also provided by light pulses. In addition, IPL is a nonthermal processing procedure in practice, it contributes to the preservation of the sensory properties of food.

Although IPL is highly effective in maintaining product quality and reducing the intensity of spores, IPL applications should be controlled for dairy powders. Unlike liquid dairy products, powdered dairy products provide convenience in storage and transportation. However, these products are also very open to moisture absorption, browning, collapse, and loss of quality. Therefore, parameters such as light intensity, frequency and a_w of whey powder and similar products should be optimised.

The ability of IPL to inactivate *B. cereus* and *B. licheniformis* spores was investigated in SMP, MPC and WPC. Results underlined the effectiveness of IPL to make a log reduction of spores. IPL application varies depending on the type of spore, but is independent of the type of milk powder type or IPL exposure time (Chung Le 2019). Although relatively little functional changes were observed in SMP, some undesirable changes in sensory characteristics were observed.

Microwave heating

Studies that provide effective inactivation with microwave heating (MH) in the inactivation of thermophilic spores are included in the literature (Martins *et al*. 2019; Graf *et al*. 2020, 2021). In microwave applications, heat is generated by electromagnetic waves as a result of the mobilisation of water molecules. Microwave applications are a method that maintains product quality in milk powders and can be an alternative to classical heating methods. In a study by Laguerre *et al*. (2011), The authors studied the effect of microwave treatment (2450 MHz) on sterilisation of nonpathogenic spore *G. stearothermophilus*. A 5 log reduction was obtained which was considered efficient.

In the literature, studies demonstrate efficient inactivation of thermophilic spores with MH (Martins *et al*. 2019; Graf *et al*. 2020, 2021). In microwave applications, the movement of water molecules causes electromagnetic radiation to produce heat. In milk powders, microwave application is a technique that preserves product quality and can be used in place of conventional heating techniques. Laguerre *et al*. (2011) examined the impact of microwave sterilisation (2450 MHz) on the nonpathogenic spore *G. stearothermophilus*. It was determined that a log decrease of 5 log cycles was effective. The authors reported that laws similar to those used for conventional heating could be used to express heat resistance in MH. Furthermore, it was possible to determine *D* and *z*-values relative to specific microwave powers.

To lower the high levels of thermophilic endospores in milk powders, skim milk concentrate or protein concentrate used for concentrated protein powders must be heated before powder production (Wedel *et al*. 2018). In a recent study, constant pilot-scale MH was employed to lower the high spore count in skim milk powders, which is thought to be caused by persistent thermophilic bacilli in the processing plants (*A. flavithermus* and *G. stearothermophilus*; N0 = 6 $\log_{10}$ cfu/mL). With *G. stearothermophilus* spores being more heat resistant than those of *A. flavithermus*, reductions of 1–3 $\log_{10}$ units were possible at 110–125°C for 5–270 s (Graf *et al*. 2021). According to the authors, the main benefit of utilising MH is the ability to heat dairy products that are susceptible to fouling with 35–40% less β-lactoglobulin denaturation and 90% less deposit formation (fouling) compared to heating in a tubular heat exchanger.

Ultrasonication

When used in conventional dairy operations, US offers a number of advantages, including but not limited to improved product qualities and energy savings. The cavitational effects of US, which can destroy bacterial cell walls and disrupt cellular structure and functional components including DNA, are the major method by which US deactivates microorganisms (Chandrapala *et al*. 2012).

The requirement for a lengthy exposure time (>5 min) to achieve log decrease limits the use of US when used alone to inactivate spores. Recent studies have demonstrated that the inactivation of vegetative and spore-forming bacterial populations is increased when high intensity ultrasound (HIU) or thermosonication (TS) is used in conjunction with traditional heat treatments (Herceg *et al*. 2012). Other methods include heat and pressure mano-thermo sonication (MTS) or pressure manosonication (MS). Heat and US do not harm *Bacillus* or *Clostridium* spores (Dehghani 2005). According to Raso *et al*.'s 1998 study, the *B. subtilis* spore population was 99% inactivated by the MS treatment (500 kPa, 117 m of amplitude, 12 min).

Another study (Beatty and Walsh 2016) investigated the inactivation of *G. stearothermophilus* vegetative cells and spores using HIU combined with conventional heating in the manufacturing of SMP. Response Surface Methodology and two polynomial models were created. Skimmed milk powder that had been reconstituted was processed at various total solids contents, temperatures, and durations. It was possible to optimise the process parameters to increase microbial reduction. In order to inactivate cells and spores during the processing of milk powder, it was found that optimising the processing parameters in the milk fed into the evaporator to be (9.2% TS, 75°C, and 10 s) and milk concentrate exiting the evaporator to be (50% TS, 60°C, and 10 s) was most successful.

Clarification, bactofugation and microfiltration

The dairy processing industry is particularly concerned about the development of homogeneous or heterogeneous multicellular bacterial communities on the surface of biofilm processing equipment because these communities, when present, can cause recurrent microbial contamination issues. Milk powder can become contaminated as biofilms that are difficult to remove from surfaces serve as a reservoir for bacterial spores (Faille *et al*. 2014; McHugh *et al*. 2017). The removal of heat-resistant spores that can withstand the heat treatment is supported by the development of the bactofugation method, which is used to remove undesired microorganisms with the use of high-speed separators (Gésan-Guiziou 2010). Since microorganisms have a higher density than milk, at least 90% of them are separated by the effect of centrifugal force in specialised bactofugators that operate at a higher speed. However, because the spores' cell plasmas have a larger density, more clearance can be accomplished. Bactofugation allows for the removal of 98–99% of anaerobic spores, 95% of aerobic spores, and 90–92% of lactobacilli. The sludge that forms after cleaning should be swiftly removed and sterilised, nevertheless (Juraga *et al*. 2021). Bactofugation can be used both before and after the thermal treatment if spore counts are high. Skimmed milk or whey can also be microfiltered, which can result in reductions of 2–6 logs (Gésan-Guiziou 2010).

LEGISLATION AND REGULATIONS GOVERNING MICROBIAL CONTAMINATION IN WHEY POWDERS

Except for newborn infant formula, the rules limiting the permissible concentrations and varieties of bacteria in milk and whey powders are not extremely detailed. The Food Safety Authority of Ireland (FSAI), Food Standards Australia New Zealand (FSANZ) and other governing agencies all define test parameters that differ from country to country. Table 3 summarises these test parameters. Commission Regulation (EC) No. 2073/2005 (European Commission 2005), imposes restrictions. According to the FSAI, milk powders should ideally less than 10^4 cfu/g of aerobic colonies (FSAI 2014). However, as characterisation of the isolated species will be necessary to assess product safety, this is not a legally mandated condition and does not imply that the food is unsafe.

The U.S. Department of Agriculture (USDA) sets microbiological limitations of 30 000 cfu/g for whey powder (USDA 2000) and acid casein powder (USDA 1968) based on the norm for plate counts. Governmental organisations in Australia and New Zealand enforce the restrictions imposed by FSANZ. Based on 1 g of milk powder and powdered infant formula including lactic acid bacteria, it is acceptable to have 100 cfu/g of *B. cereus* in 4/5 samples and 1000 cfu/g in 1/5 samples. Similar EN/ISO 7932 regulatory microbiological requirements are established by EC regulation (European Commission 2005), including a limit of 50 cfu/g putative *B. cereus* in 4/5 samples and 500 cfu/g in 1/5 analysed samples (Standards ISO 7932:2004).

A report titled 'Dried milk—Enumeration of specifically heat-resistant spores of thermophilic bacteria' was released by ISO and IDF in 2009. Despite a confirmed report of an infant botulism case in the United Kingdom (Brett *et al*. 2005; Johnson *et al*. 2005), the Advisory Committee for the Microbiological Safety of Food for the UK Food Standards Agency has not recommended routine testing for clostridial spores in powdered infant formula (PIF). A breast-fed, weaning 5-month-old infant was reported to the Public Health Laboratory Service (PHLS) in London, making this the sixth verified incidence of infant botulism in the United Kingdom; five other instances were previously confirmed in 1978, 1987, 1989, 1993, and 1994 (Johnson *et al*. 2005). According to reports, the case was brought on by close contact with a newly constructed house, disruption of the soil, and the creation of dust, all of which contributed to the spread of *C. botulinum* spores.

For any spore formers found in dried dairy products, infant cereals, and PIF, the International Commission for Food Microbiological Specifications (ICMSF) does not include finished product specifications. Instead, it is necessary to investigate aerobic bacteria, Enterobacteriaceae, *Salmonella* and *Cronobacter* sp. (ICMSF 2011). The ICMSF recently came to the conclusion that there is not enough scientific evidence to support the establishment of any specification for *C. botulinum* spores in dried milk products and does not advocate routine testing for *C. botulinum* in dried milk powders. The ICMSF also came to the conclusion that SRC limitations of 100 cfu/g in dry milk components used in PIF would be helpful as a sign of sanitary practices and environments that would favour anaerobic clostridia.

The only requirement for staphylococci food safety in Regulation (EC) No. 2073/2005 (as amended) is the absence of staphylococcal enterotoxins in milk powder

Table 3 Regulatory bodies governing, legislation and regulations governing microbial contamination in dairy powders

Regulatory body	Country	Product	Microbial limits
The Irish Food Safety Authority of Ireland (FSAI)	Republic of Ireland	Milk powder	Aerobic colony count (<10^4 cfu/g)
U.S. Department of Agriculture (USDA)	The United States of America	- Whey powder - Acid casein	Aerobic colony count (<30 000 cfu/g)
Food Standards Australian New Zealand Food Standards (FSANZ)	Australia and New Zealand	- Milk powder - Powdered infant formula containing lactic acid bacteria	- <100 cfu/g of *B. cereus* in 4/5 samples - <1000 cfu/g of *B. cereus* in 1/5 samples - It does not require it to be absent in five samples
European Commission (EC), according to EN/ISO 7932	Europe	Milk powder	- <50 cfu/g hypothetical *B. cereus* in 4/5 samples - <500 cfu/g in 1/5 analysed
European Commission (EC) No. 2073/2005	Europe	Milk and whey powder and cheese	- Absence of staphylococcal enterotoxins during shelf life. - Process hygiene criteria with limits of 10^1 to 10^5 coagulase-positive staphylococci/g - If values >10^5 cfu/g are detected, the batch should be tested for staphylococcal enterotoxins.
The International Commission for Food Microbiological Specifications (ICMSF)	International	Dried milk powders	- No finished product specifications for any spore formers in dried dairy products, infant cereals, and powdered infant formula (PIF). - Aerobic bacteria, *Enterobacteriaceae*, *Salmonella*; *Cronobacter* sp. need testing. - There is insufficient scientific support to initiate any specification for *C. botulinum* spores. - Routine testing for *C. botulinum* is not recommended. - 100 cfu/g is the limit for sulphide-reducing clostridia (SRC) in dried milk components used in PIF.
Turkish Food Codex Communiqué on Microbiological Criteria	Turkey	Whey powders Powdered baby foods	- *Salmonella* sp. is absent 25 g of in five samples. - *Enterobacteriaceae* count is less than 10^1 cfu/g. - Coagulase-positive staphylococci is 2- less than 10^2–10^3 cfu/g in three samples. - *Cronobacter sakazakii*, *Salmonella* sp. and *L. monocytogenes* are absent in 25 g. - *B. cereus* does not exceed 10^2 cfu/g in three samples and 10^1 cfu/g in two samples.

and whey powder sold during their shelf life. For cheese, milk, and whey powder during manufacture, this regulation sets limits of 10^1 to 10^5 coagulase-positive staphylococci/g; if levels greater than 10^5 cfu/g are found, the batch should be tested for staphylococcal enterotoxins. Public health measures should not be postponed while waiting for results, however, as enterotoxin detection tests are not quick, may be insensitive to specific food matrices, and do not identify all kinds of staphylococcal enterotoxins.

According to the Turkish Food Codex Communiqué on Microbiological Criteria valid in Turkey, while no sample is allowed to contain 25 g of *Salmonella* sp. in five samples of whey powder, Enterobacteriaceae count is less than 10^1 cfu/g, and the number of Coagulase-positive staphylococci is 2- less than 10^2–10^3 cfu/g in three samples is desired. In powdered baby foods, *Cronobacter sakazakii*, *Salmonella* sp. and *L. monocytogenes* number should not be at 25 g, for *B. cereus* this limit cannot exceed 10^2 cfu/g in three samples and 10^1 cfu/g in two samples.

Due to the fierce competition in the dairy products industry, specific customers frequently establish their own microbiological thresholds to maintain high standards. Whey and dairy milk powders are typically not further processed before being added to other products. For instance, to guarantee that a high microbiological quality is met and to set tight standards, powdered infant formula producers frequently maintain strong contacts with the milk powder provider (Kent *et al*. 2015b).

Table 4 Some chemicals in the CIP system and functions (Adapted from Vaughn, 2004).

Type	Chemical	Function	Agent
Surfactants	Anionics	Wetting and foam	Sodium dodecyl sulphate (SDS)
	Cationics	Disinfection, corrosion inhibition	Domiphen bromide
	Nonionics	Wetting and antifoam	2-Ethoxyethanol
Strong alkalis	Sodium hydroxide	Removal of fat and protein	
	Potassium hydroxide	Removal of fat and protein	
	Orthosilicate	Emulsification	
	Metasilicate	Corrosion inhibition	
Mild alkalis	Sodium triphosphate	Dispersion	
	Sodium carbonate	Alkalinity	
Chelating agent	Polyphosphate	Prevention of Ca deposits	Ethylenediaminetetraacetic acid tetrasodium salt hydrate
	Na EDTA	Dissolution of Ca deposits	Nitrilotriacetic acid trisodium salt
	Na NTA	Prevention of Ca deposits	
	Gluconate	Prevention of Ca deposits	
Threshold agents	Phosphonates	Prevention of Ca deposits	
	Polyacrylates	Prevention of Ca deposits	
Inorganic acids	Phosphoric acid		
	Nitric acid		
	Sulphamic acid		
Organic acids	Hydroxy-acetic acid	Removal of scale	
	Gluconic acid		
	Citric acid		
	Formic acid		

CONCLUSIONS

Good manufacturing practices, which ensure the quality of the milk to be processed, the training of dairy suppliers and factory workers, the temperature control, and the sanitation of equipment and processing facilities, include practices that can significantly reduce contamination with spores. Properly processed whey has a low risk of contamination with pathogens, as long as there are no problems with pasteurisation of milk and its products. Pasteurisation process kills pathogens such as *E. coli*, *L. monocytogenes*, *Salmonella* and *Shigella*, but there is always a risk of contamination of milk with spores due to the frequent occurrence of *Clostridium* and *Bacillus* spores in raw milk, pasteurised milk and tools and equipment used in production facilities. The presence of *C. botulinum* spores in milk and its products depends on long-term storage and processing methods and conditions. In the production of whey powder, the water in the pasteurised milk is evaporated and dried with air at high temperatures. During the drying phase, the temperature of the whey powder is not sufficient to destroy all bacterial spores. Outbreaks due to consumption of commercial dairy products have often occurred due to low or ineffective temperature practices, contamination before or after processing, inadequate food safety systems, or faulty formulation and deficiencies in storage conditions. Therefore, spore populations in dried dairy products should be minimised by using quality milk, temperature control, sanitation of equipment and processing facilities, and potentially other processing techniques. Some technological processes such as bactofugation are among the systems that must be used as they can eliminate spores and microorganisms that cause some deterioration. Heat pasteurisation efficiency to remove biofilms might be limited if used as a standalone decontamination technology and hence, other technologies are encouraged, as partial or complete replacement of conventional heating such as IPL, MH, US and bactofugation to enhance inactivation.

AUTHOR CONTRIBUTIONS

Essam Hebishy: Conceptualization; data curation; investigation; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing. **Oktay Yerlikaya:** Data curation; investigation; resources; supervision; writing – original draft; writing – review and editing. **Jennifer Mahony:** Data curation; investigation; supervision; writing – review and editing. **Asli Akpinar:** Data curation; investigation; writing – original draft; writing – review and editing. **Derya Saygili:** Data curation; investigation; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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