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Review

Towards low-spore milk powders: A review on microbiological challenges of dairy powder production with focus on aerobic mesophilic and thermophilic spores

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ABSTRACT

Powdered milk products, such as skim milk powder or whey protein powder, represent a large fraction of the dairy sector, especially with respect to export. In the past years, the contamination with aerobic endospore-forming bacteria has become one of the main factors to evaluate microbial powder quality. Besides mesophilic spore formers, thermophilic and thermoresistant species have been isolated all over the world. During production of powdered commodities, milk or intermediate products go through several process steps. This review highlights and discusses individual production steps and their effect on the microbiota and the spore count. The plant cleaning and its influence on spore resistance are discussed in detail, since this is the most important step in controlling recontamination and persistence. Finally, future technologies to reduce spore counts during powder production are presented. The contribution on 'non-thermal' and novel technologies towards low-spore milk powders are discussed critically.

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1. Introduction

Milk and milk-based powders are important dairy products. They are produced by the removal of free water from milk or a different intermediate product such as whey. One can distinguish

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between powders based on milk as a raw material (such as skim milk powder (SMP) and whole milk powder (WMP) or milk protein powder) or powders that are based on whey (e.g., sweet whey powder, WP, or whey protein isolate powder, WPI). Because of the high dry matter content and the low water activity (a_w)-value (moisture content of skim milk powder, 4.7%; Sharma, Jana, & Chavan, 2012), no growth of microorganisms can occur in dairy powders. They can be stored over a long period and are easy to ship (Rolls & Porter, 1973). This property makes dairy powders very suitable merchandise for export. From 2014 to 2019, the amount of exported skim milk powder (SMP) in the European Union increased from 650 to 962 thousand tons. The SMP export increased from 550 to 680 thousand tons between 2014 and 2018 (USDA, 2018). Data collected for the US market found that 114 thousand tons of whole milk powder was exported in 2020 (USDEC, 2020). The numbers increased steadily in the last years. This demonstrates that powdered milk products are traded worldwide and have a big impact on the global dairy market.

The application of powdered milk products besides their use by simple reconstitution and recombination is very extensive. Skim milk powder or casein/whey protein powders are used for protein fortification. One example of this is the use of powders for yoghurt manufacturing, where nutritional and functional properties can be enhanced (Karam, Gaiani, Hosri, Burgain, & Scher, 2013; Mistry, 2002). Also, powders are used for standardisation, for instance standardisation of protein content (Rattray & Jelen, 1996). Fortification with milk powders can be used for special nutritional requirements (Hoppe et al., 2008). Powdered milk products play a very important role in the feeding of infants and are distributed as powdered infant formula (PIF). One way to produce PIF is a dry-mix option, where the different ingredients are blended to a final composition that resembles human milk (Kent, Fitzgerald, Hill, Stanton, & Ross, 2015). Depending on the final application, the powders need to fulfil different properties such as emulsification, heat stability, flowability, hygroscopicity or foaming ability (Chegini & Taheri, 2013; Sharma et al., 2012). For PIF the microorganism *Cronobacter sakazakii* is the biggest threat since it causes severe illness in infants. We refer to the reviews that focused on this problem (Norberg et al., 2012; Yan et al., 2012) and will not discuss contamination with this species further.

The primary organisms of interest regarding powdered milk products are spore formers, especially those with an optimum growth temperature of 55–60 °C (Burgess, Lindsay, & Flint, 2010; Hill & Smythe, 2012; Reich et al., 2017). Thermophilic spore formers are not reported to cause any harm in humans, in contrast to the mesophilic representative *Bacillus cereus*. It is the only aerobic spore former that can cause a foodborne illness but is only rarely reported in powdered milk products (Kumari & Sarkar, 2014). The plate counts of aerobic spore forming microorganisms are regulated by several governmental authorities (e.g., US Dairy Export Council, Food Safety Authority of Ireland, China and others listed by Sadiq, Flint, & He, 2018, Table 1). High counts of spore forming bacilli are undesirable because of the spoilage potential after reconstitution (Hill & Smythe, 2012; Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz, 2013). After powder is reconstituted with water, the environment changes to favourable conditions for spores, which starts to exhibit outgrowth. Enzymes such as lipases and proteases are produced and can lead to defects of the milk product (De Jonghe et al., 2010; Sadiq et al., 2016b).

The objective of this review is to discuss each process step during production starting from the raw materials to the final milk and whey powders with respect to their significance towards spore levels. The resulting contamination levels with mesophilic and thermophilic spore formers are given. Alternative technologies to reduce the spore load during production are reviewed critically, considering the process temperature as one important factor for powder quality.

2. Levels of aerobic spore-formers in raw ingredients, intermediate products and powders

During the production time of powders, the microbiota undergoes different changes not only in the total cell count but also in the composition of microbial groups. The composition of the microbiota in final dairy powders was reviewed recently and will be not part of this work (McHugh, Feehily, Hill, & Cotter, 2017; Sadiq et al., 2018). In these studies, many different species were reported to be found in milk powders belonging to the group of aerobic spore forming bacteria. However, the most dominant species were reported to be the thermophilic species *Anoxybacillus flavithermus* and *Geobacillus stearothermophilus,* and the mesophilic specie *Bacillus licheniformis* (Dettling et al., 2019; Ronimus et al., 2003; Sadiq et al., 2016a; Yuan et al., 2012).

There is evidence that the amount of thermophilic spore formers might have been underestimated in previous years. Recent findings showed that, for enumeration of thermophilic spores, temperatures above 80 °C already reduced the spore count in milk powder samples (Dettling et al., 2019). The need for an accurate and harmonised spore quantification method is very important for the future because the applied methodologies are different (Kent, Chauhan, Boor, Wiedmann, & Martin, 2016; McHugh et al., 2017). Since in the final powdered product the amount of spore formers is crucial for import restrictions (see Table 1), we focus in this section on the different levels of spore formers during powder production. Many authors have investigated the contamination of powdered products and intermediate products (concentrate, skim milk) in the past (see Table 2). While thermophilic spore formers are seen as the most dominant group of contaminants, their presence in raw milk is minor. In the processed product, however (e.g., whey or skim milk), the counts are higher. This is a strong indicator, that microbial load and abundance changes already in the first process steps. Overall, the thermophilic and mesophilic spore levels in the different powder products range from low levels (log 1 cfu g^{-1}) to high contaminations of more than 10^4 cfu g^{-1}.

Although thermophilic spore counts are especially of interest in the final powdered products, thermophilic spore formers are not reported in significant numbers in raw milk (Dettling et al., 2020; Scott, Brooks, Rakonjac, Walker, & Flint, 2007). The increasing numbers of spore formers during processing of the raw material (up to 5 log cfu g^{-1}, Dettling et al., 2019) cannot be explained by

Table 1
Microbiological quality standards and specification for spore content.[a]

Spores	Spore levels (cfu g^{-1})	Powder type	Reference
Thermophilic spores	$<5 \times 10^2$	Skim milk powder, whole milk powder, infant formula powder	US Dairy Export Council (USDEC)
Mesophilic spores	$<10^3$		
Aerobic spore count	$<10^4$	Milk powder	Food Safety Authority of Ireland (FSAI)
Aerobic spore count	$<10^3$	Infant formula powder	National Standards on Food Safety of P. R. China

[a] Based on Sadiq et al. (2018).

Table 2
Range of thermophilic and mesophilic spore counts in raw materials and powders.[a]

Milk product	Spore counts(cfu mL^{-1} or g^{-1})	Groupaffiliation	Reference
Raw milk	<10	Thermophilic	Scott et al. (2007)
	<1–10^2	Mesophilic	te Giffel, Wagendorp, Herrewegh, and Driehuis (2002)
	5.5×10^3	Mesophilic	Scheldeman, Pil, Herman, De Vos, and Heyndrickx (2005)
	<10	Mesophilic	Martinez, Stratton, and Bianchini (2017)
	<10–10^2	Thermophilic	Dettling et al. (2020)
Sweet whey	2.7×10^3	Thermophilic	Watterson, Kent, Boor, Wiedmann, and Martin (2014)
	1.5×10^1	Mesophilic	Watterson et al. (2014)
	2.5×10^1–1.2×10^4	Thermophilic	Dettling et al. (2019)
Skim milk	<10	Thermophilic	Scott et al. (2007)
Pasteurised milk	7.9×10^2–5.0×10^2	Mesophilic	Martinez et al. (2017)
Whole milk concentrate	10^2–10^4	Thermophilic	Scott et al. (2007)
Whole milk powder	10^2–10^3	Thermophilic	Scott et al. (2007)
	5.5×10^1–2.2×10^4	Thermophilic	Yuan et al. (2012)
	1.0×10^1–1.3×10^3	Thermophilic	Sadiq et al. (2016a)
	1.6×10^1–1.4×10^4	Mesophilic	Sadiq et al. (2016a)
Skim milk powder	1.0×10^2–2.0×10^4	Thermophilic	Sadiq et al. (2016a)
	4.0×10^2–2.5×10^5	Thermophilic	Dettling et al. (2019)
Nonfat dry milk	7.9×10^2–3.1×10^3	Thermophilic	Buehner, Anand, and Djira (2015)
	1.9×10^2	Mesophilic	Buehner et al. (2015)
IFM powders	1.5×10^2–3.9×10^4	Thermophilic	Yuan et al. (2012)
	7.3×10^1–1.2×10^4	Thermophilic	Sadiq et al. (2016a)
	1.2×10^2–1.4×10^4	Mesophilic	Sadiq et al. (2016a)
WP powder	2.5×10^2	Thermophilic	Watterson et al. (2014)
	3.1×10^2	Mesophilic	Watterson et al. (2014)
Whey powder	1.5×10^4	Thermophilic	Watterson et al. (2014)
	<10–3.5×10^3	Mesophilic	Sithole, McDaniel, and Goddik (2006)
	7.9×10^2–3.1×10^4	Thermophilic	Dettling et al. (2019)

[a] Abbreviations are: IFM, infant formula milk; WP, whey protein. Mesophilic growth temperature 20–37 °C; thermophilic growth temperature >40 °C.

concentrating factors alone. The removal of free water leads to a concentration of the dry matter content by approximately 10-fold (from 9% dry matter in skim milk to 97% in skim milk powder). By this assumption, only 1-log level could be explained by the effect of concentration. Therefore, spore formers appear in the production plant, either through recontamination and/or through an initial contamination by the raw material. These organisms are capable of adhering on stainless steel surfaces and can grow into biofilms (Sadiq et al., 2017). Thus, the number of spore formers through outgrowing and multiplication increases by the length of the production cycle and the operation conditions (e.g., temperatures). In the following section, we will have a closer look at how the different process steps of a powdered product impact aerobic spore formers.

3. Processing of dairy powders

3.1. Powder production of milk-based powders

The processing of raw milk into milk powders can result in milk powders with differing ratios of fat and protein (e.g., whole milk, skim milk, and milk protein powders). Fig. 1 shows the processing steps for the production of dairy powders based on raw milk. Coorevits et al., 2008 found that the microbial diversity (e.g., number of thermotolerant or mesophilic spore formers) varies depending on the farming strategy (organic versus conventional). In conventional dairy farms, slightly higher numbers of thermotolerant organisms were found. Moreover, it was hypothesised that mesophilic and thermophilic spore levels in raw milk could be managed by the herd size, bedding practice for the animals and the milking routine (Miller, Kent, Boor, Martin, & Wiedmann, 2015). Thus, the raw milk that enters the powder production site can vary a lot not only in the composition regarding mesophilic, thermoresistant and thermophilic spore formers but also in the total numbers. The variety of thermophilic aerobic spore formers in bulk tank raw milk was shown to differ enormously compared with milk powder products. In raw milk 38 different species were identified compared visa 1–4 species in semi-concentrates (Dettling et al., 2020). Although a producing company could control from which farm the raw milk is obtained at this point of production the manufacturer is not able to influence milk quality. The spore levels are routinely not checked in the microbiology routine work. Although, the total numbers of spore counts (Table 2) are generally very low in the raw product (<10 cfu g^{-1}), eliminating some supplementary feeds that contain high levels of spores may be one approach to reduce the contamination of raw milk.

For the separation of cream from raw milk the difference in the density between fat globules and milk serum based on Stokes' Law is applied (Kessler, 2002). Typical separation temperatures range between 50 and 55 °C. Therefore, separation marks the first control point in powder processing with respect to the growth temperature of thermophilic and thermoresistant spore formers (Burgess et al., 2010; Dettling et al., 2020; Reich et al., 2017). Some laboratory work showed that a reduction in temperature (to 48 °C) prevented the formation spores (Burgess, Brooks, Rakonjac, Walker, & Flint, 2009). Through the adaption of the temperature or novel approaches such as two-step centrifugation, the number of discs or the diameter of the fat-globule size can be managed (Dhungana, Truong, Palmer, Bansal, & Bhandari, 2017). The separated cream fraction is further used in a different product stream (e.g., production of butter) if a skim milk product is produced. For whole milk powder, skim milk and cream are standardised to a defined fat content.

The next processing step (with skim milk or whole milk) is the thermal treatment. The temperature and heating time are crucial for the classification of powders. Typically, the so-called WPNI (whey protein nitrogen index) is used. The values are expressed as milligrams whey protein nitrogen in g powder. Therefore, this parameter gives the native whey protein content and thus the powders can be classified in high, medium or low heat powders, for which the pre-heat treatments were 70 °C for 15 s, 90–105 °C for 30 s and 120 °C for 1–2 min, respectively (WPNIs of ≥6, 1.5–5.9, ≤1.5; Patel, Anema, Holroyd, Singh, & Creamer, 2007). For whole

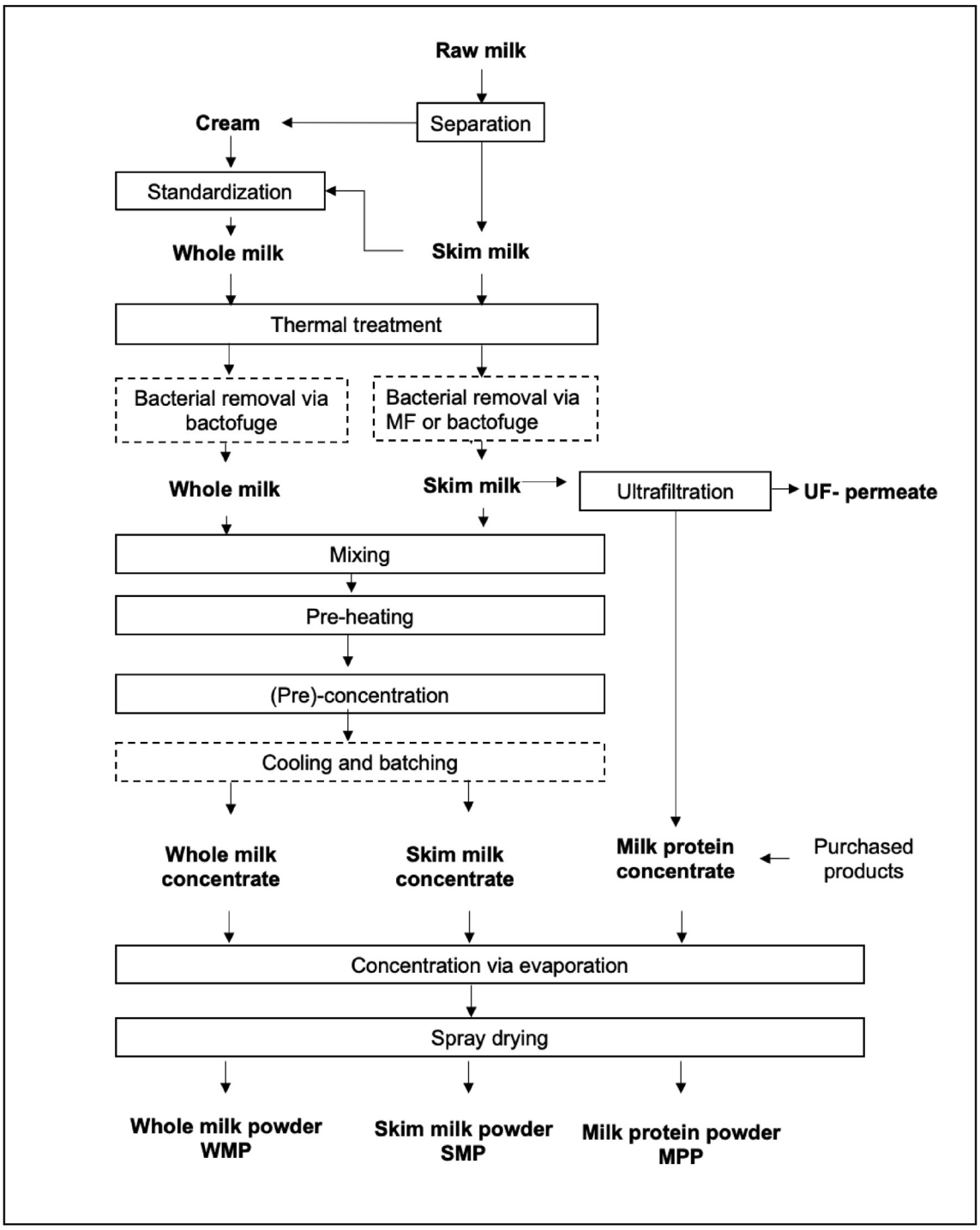

Fig. 1. Processing steps for the production of dairy powders based on raw milk. Optional operations are shown in dashed boxes. Data are from an unpublished survey of German powder producers.

milk powders, the inactivation of lipases is necessary and at least a medium-heat product is generally required. This lowers the lipid oxidation in the powder and reduces potential off-flavours (Páez et al., 2006). Depending on the fat content, the heat load is adjusted. There is an obligatory pasteurisation step which aims to inactivate any present pathogenic bacteria. By the standard high-temperature-short-time treatment of 72–75 °C for at least 15 s (HTST; Kessler, 2002), milk-borne vegetative cells of the pathogens are completely inactivated but not the spores of the pathogens. Alternatively to HTST, the low-temperature-long-time (LTLT) pasteurisation at 62 °C for at least 30 min can be applied (Ryser, 2011). While the latter pasteurisation step is very critical regarding the optimal temperature for growth and survival of thermophilic spore formers, the HTST pasteurisation is seen as a suitable tool to inactivate vegetative, thermophilic cells. Recent work showed that some strains belonging to the group of thermophilic spore formers are able to withstand the thermal treatment (73 °C, 20 s) not only as a spore but in their vegetative form (Reich et al., 2017). Moreover,

after the actual heat treatment the next place for the growth of bacteria is the cooling section. Through bacterial adhesion on heat-exchangers, mesophilic and thermophilic spore formers are able to contaminate the product flow at this early stage of production (Jindal, Anand, Metzger, & Amamcharla, 2018).

The two most common ways are the microfiltration I (with a filter size of approx. 1.4 μm) or a centrifugation (often called bactofugation) (Gésan-Guiziou, 2010; Sant'Ana, 2014). The principle of the separation step is the difference between the densities of the cells (spore, bacterial or somatic cells) and the milk serum. Bactofugation is either used before the thermal treatment or afterwards. Due to the possibility of higher spore counts in milk after the pasteurisation step a physical removal of spores via a separation step after the thermal treatment would be of high interest (see Fig. 1). The log-reduction of spores obtained by commonly used centrifuges are in the range of 1–2, whereas the microfiltration can reduce the bacterial population from 2 to 6 log (Gésan-Guiziou, 2010) depending on the cell or spore size (mean spore length of spore range between 1.07 and 1.74 μm; Carrera, Zandomeni, Fitzgibbon, & Sagripanti, 2007); microorganisms group or species. The reduction of thermophilic spores via bactofugation during a powder production process are demonstrated to be 1-log (Dettling et al., 2020). However, the use of microfiltration for the production of whole milk powders is not possible. Pore sizes usually used for bacterial removal also lead to a reduction in the fat content by retaining fat globules (Michalski et al., 2006). It was recently shown that the removal of spores might be even possible with bigger membrane pore sizes because of the spore's surface characteristics. If the spores are hydrophobic, they may form clusters and can be retained at pore sizes of 1.4 μm (Griep, Cheng, & Moraru, 2018).

After the thermal treatment and the optional microfiltration step either evaporation begins or an ultrafiltration (UF) is integrated depending on the type of produced milk powder. For example, milk protein powder (MPC powders) has an higher amount of protein (caseins and whey protein) whereas the lactose and mineral contents are depleted proportionally (Mistry, 2002). Besides UF, a diafiltration (DF) step can be applied to reduce the lactose content (not indicated in Fig. 1). For MPC powders with protein contents of 80% (MPC80), the evaporation of the concentrate is not always necessary and a direct spray drying could be applied. This has to be evaluated regarding energy consumption (high for direct spray drying) and functional properties of the powder (shift in calcium concentration) (Rupp, Molitor, & Lucey, 2018).

During UF, the membrane pore size is small enough to retain all cells and spores. This also can result in biofouling where the growth of spore forming bacteria can occur. Filtrations are typically conducted at either cold (approximately 10–15 °C) or warm conditions (50 °C). The bacterial genera shifts towards the genera *Bacillus* if the operation temperature is warm (Chamberland et al., 2019). Therefore, cold filtration and short operation times are important to ensure low mesophilic and/or thermophilic spore counts in the final powder.

One step before spray drying, evaporation takes place. In principle, different process options besides evaporation are known but, if used at all, reverse osmosis or cryoconcentration are conducted as a pre-concentration step. Falling-film evaporators are the most commonly used types in dairy industry. The basic principle of these evaporators is the application of a vacuum resulting in the evaporation of water at temperatures of 40–80 °C (Zhang, Munir, Udugama, Yu, & Young, 2018). A schematic illustration is given in Fig. 2. Multi-level stage evaporation is commonly used, where the temperature decreases stepwise through the single levels. The temperatures of evaporation are again unable to inactivate the total load of mesophilic and thermophilic spore formers. This moreover provides an environment where the spores can undergo outgrowth and the population increases. This was demonstrated for semi-skim-milk-concentrate (total solids of 36.5%) during skim milk powder production (Dettling et al., 2020). At some point of concentration, the osmotic pressure (due to the concentration of salts, lactose and minerals) reaches a level where no growth can occur and the spore and vegetative cell content is conserved in the milk

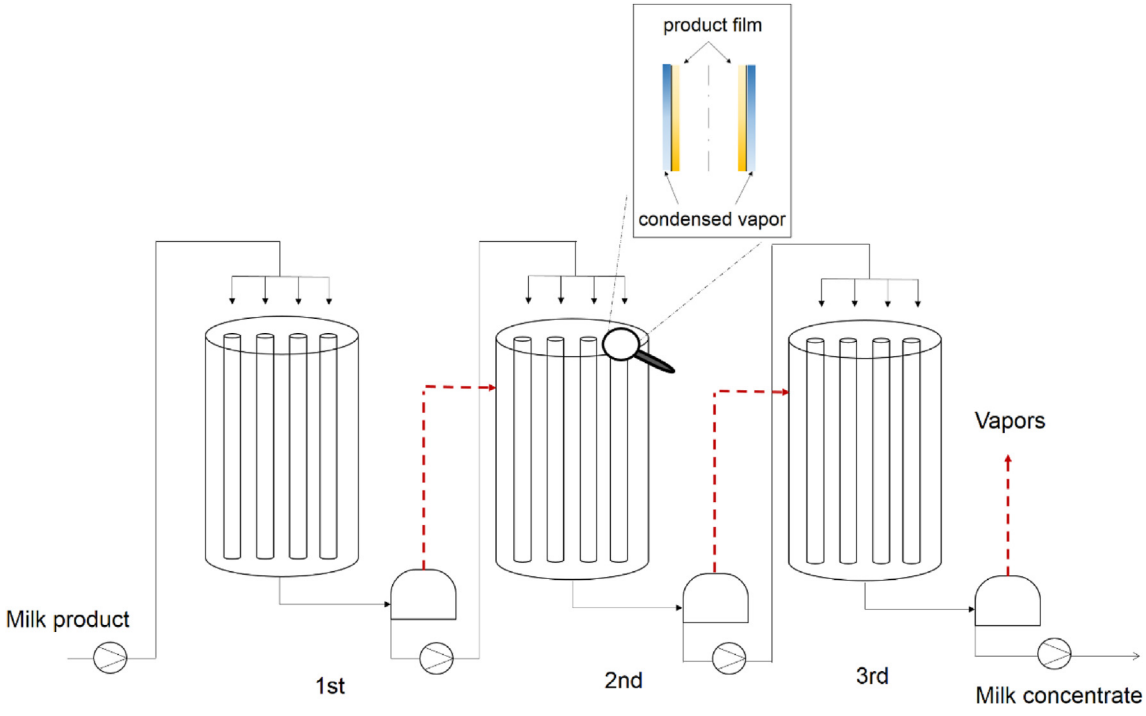

Fig. 2. Schematic of a multi-level falling film evaporator.

concentrate (Dettling et al., 2020). The spore levels at this point stays stable over a long period during the storage. A heat-treatment with milk concentrate could be a possible process option and can reduce spore loads significantly (Wedel et al., 2018). Until now, it is not used in dairies on an industrial scale.

The final step in the processing is the spray drying. The liquid stream needs to be divided into small particles, the atomisation process, to achieve the drying by hot air. Most common atomiser technologies are rotary wheels, pressure nozzles and pneumatic nozzles (O'Sullivan, Norwood, O'Mahony, & Kelly, 2019). The inlet air temperature of a spray drying unit ranges from 170 to 230 °C (Ozmen & Langrish, 2003). The composition of the feed must be considered for the inlet air temperature. The viability of bacterial endospores during spray drying was discussed in literature for spore formers. For *Bacillus thuringiensis* spores, an inactivation effect during spray drying could be observed for inlet air temperatures ranging from 170 to 250 °C. Still, the log-reduction was comparatively small with under 1-log value (Zhou, Dong, Gao, & Yu, 2008). However, for *B. cereus* (mesophilic) much higher log-reduction values are observed at inlet temperatures from 150 to 190 °C. Depending on the microorganism strain and media (skim milk or whole milk) log-reductions of 0.1 up to 4.4 were noted (Alvarenga et al., 2018b). The strain dependency on the resistance was noted as well in another study where 12 *B. cereus* strains were processed with spray drying in whole milk. Log-reductions of 1–4.7 were observed (Alvarenga et al., 2018a). The air outlet temperature was 110 °C. It is also important to mention here that the actual particle temperatures are much lower than the inlet or outlet temperatures of the spray dryer. The question is that whether for mesophilic spore formers spray drying could be seen as a tool to reduce the final spore count. It is strongly dependent on species affiliation and heat resistance of the respective strains. The results are obtained on a laboratory scale; detailed studies on the spore reduction during spray drying under industrial conditions are missing so far.

3.2. Powder production based on whey

The process of dairy powders originating from whey as a raw material is highlighted in this section. The different steps are illustrated in Fig. 3. The most crucial differences to milk-based powders is that the raw material is already a processed product. While the raw milk microbiota is affected by environmental factors of the farms (see section 3.1), the raw whey is an intermediate product and has already undergone different process steps before the powder processing begins. Depending on the produced cheese from which the whey was a by-product, such as raw milk, soft or hard cheese, the steps may vary. However, they have all the following procedures in common: (i) Acidification of milk by either addition of starter culture or organic acids, (ii) rennet-induced coagulation, (iii) cutting the curd and finally (iv) draining of whey. Besides the whey proteins, the majority of the lactose remains in the cheese whey (Prazeres, Carvalho, & Rivas, 2012). For the production of whey powder, the differentiation between acid (pH 4.5) and sweet whey (pH 6.5) is crucial since sweet whey is used preferably for powders. If acid whey is used, this can cause process problems because of the different composition (higher amount of lactic acid, different pH, higher concentration of ions). Acid whey is generated during production of dairy products such as fresh cheese or Greek-style yoghurt (Chandrapala et al., 2015).

Since the differences in the pre-treatment of the raw material (from milk to whey) are huge, the contamination with spore formers fluctuates and is higher than the contamination levels for raw milk (Dettling et al., 2020). Before the whey is further processed, so-called 'cheese-fines' and remaining fat are removed from whey. As illustrated in Fig. 3, this can be conducted via filtration and/or separation via a decanter while again temperatures for the growth of thermophilic and mesophilic spore formers of ~50 °C are achieved (Kessler, 2002). As highlighted in section 3.1, the following thermal treatment affects the microbiota in the whey product depending on the chosen temperature-/time-combination.

Before evaporation takes place, the whey can further undergo a filtration step to enrich the whey protein amount in the concentrate phase. Ultrafiltration is used for this. As it was reported recently for whey ultrafiltration processing, a cold filtration (~10 °C) is preferable (Steinhauer, Hanély, Bogendörfer, & Kulozik, 2015). This is not only to avoid growth of thermophilic spore forming bacteria but also to limit the membrane fouling. Falling film evaporation of the liquid stream is mostly applied before spray drying to increase the dry matter (see Fig. 2). The last step is the spray drying where the mesophilic microbiota might be reduced but not the thermophilic spores (see section 3.1).

3.3. Cleaning of the production process

In this section, we focus on the cleaning of the production process as one important factor in controlling the microbiological quality of milk powders. First, the commonly used cleaning strategies are highlighted. Second, the importance of cleaning in regards to the microbial composition is discussed. All modern dairies use 'cleaning-in-place' (CIP) operations nowadays. The plant does not need to be dismantled and cleaning solutions are pumped through the whole plant, starting from tanks with the concentrated cleaning solutions. The first step of the cleaning is always rinsing the plant with water. For removal of proteins the alkaline treatment is typically conducted with NaOH (concentrations of 0.5–2%) at temperatures of 65–70 °C for a given time (depending on the plant size) followed by rinsing. Then, the acidic cleaning takes place (0.5–1%). In pasteurisation plants, the acidic cleaning is not always conducted after each production run. Nitric acid is mostly used as an acidic chemical and temperatures of 60–70 °C are applied (Kessler, 2002). Between the cleaning steps, rinsing of the plant with water for at least 5 min is carried out to discharge the previous liquid. It is very common to use 'ready-to-use' solutions for industrial cleaning. They contain additives, such as emulsifiers or complexing agents.

It is important to have a closer look at the survival of aerobic spore formers towards these typical cleaning conditions stated above. The resistance of *Bacillus* spores (biofilm-derived) against standard alkaline CIP agents (ready-to-use) was tested for mesophilic strains (*Bacillus subtilis, B. licheniformis* and *Bacillus paralicheniformis*) recently. The spores could withstand the treatments (10 min, 50 °C, concentration 0.5%) and were only reduced in their initial counts by around 1 log (Ostrov, Paz, & Shemesh, 2019). Similar results were demonstrated for *Bacillus subilits* for different caustic (NaOH based, 0.5%) agents at 50 °C (Ostrov, Harel, Bernstein, Steinberg, & Shemesh, 2016). For thermophilic strains isolated from dairy environments, results were obtained for alkaline cleaning solutions that showed as well small reduction numbers. An acidic treatment (HNO_3) showed lethal effect on the spores (Wedel, Wenning, Dettling, Scherer, & Hinrichs, 2019). At 0.5% HNO_3, only three of the eleven tested strains were able to survive the acid treatment (initial counts were around 7 log units). Two of the three strains survived with values < 2 log. One showed the highest resistance and a reduction of 4.4 log was observed.

The use of sanitisers such as peracetic acid is discussed as a suitable source for the inactivation of spores (e.g., *Bacillus*) (Ding & Yang, 2013). However, if the biofilm is not completely removed from the wall the sanitiser will react with the remaining microorganisms but might not inactivate all of them. Thus, the next run can

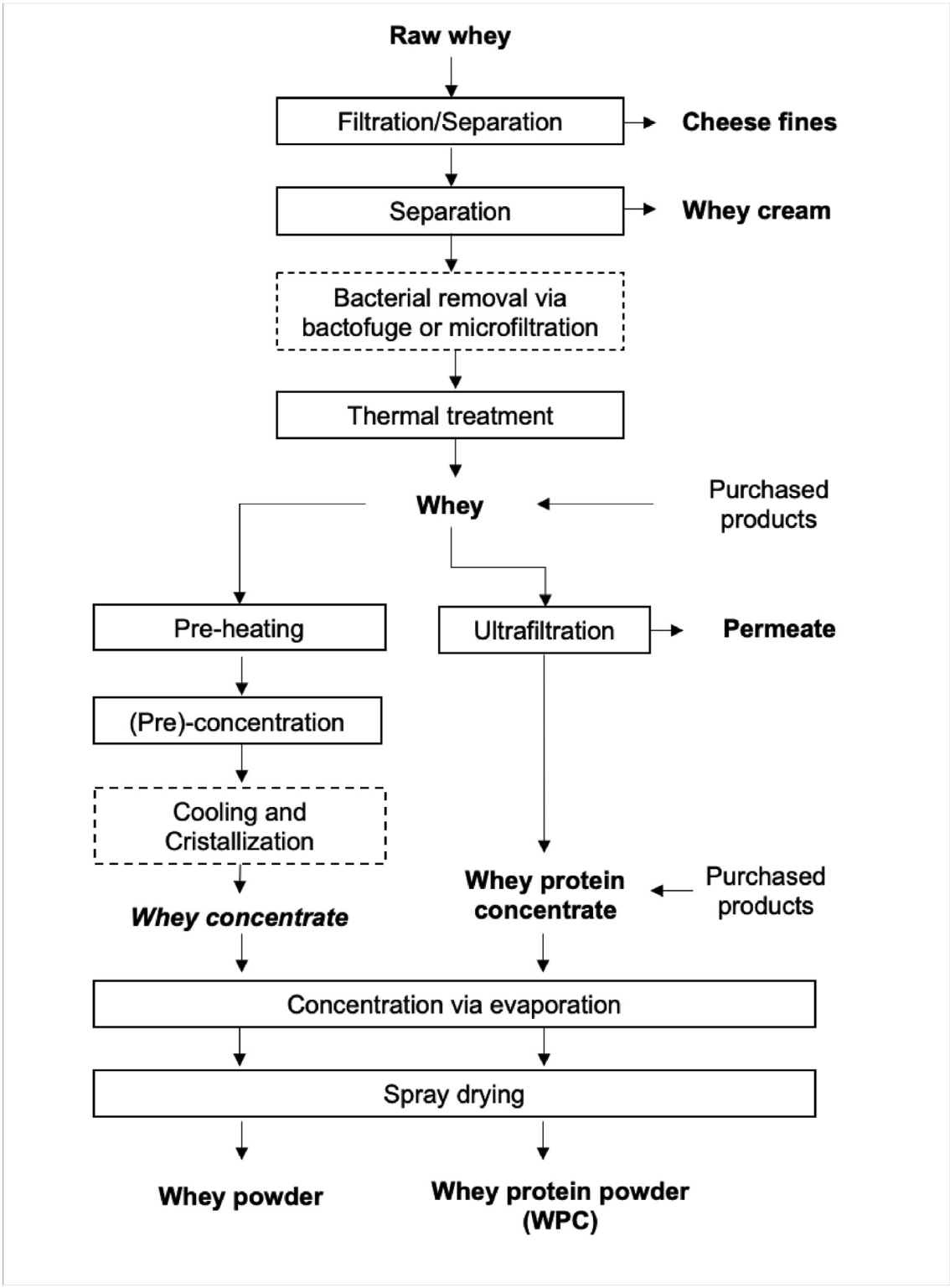

Fig. 3. Processing steps for the production of dairy powders based on whey as a raw material. Optional operations are shown in dashed boxes. Data are from an unpublished survey of German powder producers.

be contaminated easily. Persisting strains that are found over month and years in the same powder production plants, support the hypothesis that the cleaning does not sufficiently remove spores (manufacturers criteria: 100–500 thermophilic spores per gram powder at maximum) (Dettling et al., 2020). The spores' resistance towards the used chemicals is mainly strain specific and can vary within a microorganisms group (Faille et al., 2013). Another problem is the ability of spores to re-attach on stainless steel after a cleaning step. This can happen if the spores are not inactivated and are suspended in the cleaning solution. The

solution is pumped through the plant and the spores can re-attach at different parts. This was reported at laboratory scale especially for *B. cereus*, a mesophilic spore former (Faille et al., 2010; Le Gentil, Sylla, & Faille, 2010). It was hypothesised that the caustic treatment changes the surface characteristic of the spore and enhances a better attachment ability. The use of cleaning solutions (applications of 1% sodium hydroxide and 1.0% nitric acid) with caustic additives (e.g., Eliminator) was demonstrated to enhance the removal of biofilms on stainless steel (Bremer, Fillery, & McQuillan, 2006).

Besides the sole attachment of spore formers on pristine stainless steel, another mechanism takes place: the so-called fouling formation (see Fig. 4). The deposit or fouling mainly consists of protein and salts, depending on the outer-wall temperature (Visser & Jeurnink, 1997). More precisely, the β-lactoglobulin in the whey protein fraction plays the crucial part in the fouling formation (Petit, Six, Moreau, Ronse, & Delaplace, 2013). During heat treatment and evaporation of milk, fouling formation can co-occur to biofilm attachment of cells and spores. Fouling was found to be another important factor in protecting the spores form the CIP procedure (Hinton, Trinh, Brooks, & Manderson, 2002). Fouling was observed to harbor high amounts of thermophilic spores and thus seems to be very important in managing the problem of spores in dairy powders (Scott et al., 2007). The protective effect of thermally-induced fouling on a thermophilic spore formers was confirmed recently for alkaline and acidic treatment and a disinfection agent. The transmission of spores from an insufficiently cleaned wall was discussed (Wedel et al., 2020). Fouling and biofilm formation may occur simultaneously during powder production and intensify each other. An illustration is given in Fig. 4.

As we discussed in sections 3.1 and 3.2 filtration is not only an important technology to remove bacterial cells and spores from the product stream but is used to change the composition of the media (e.g., protein concentration) as well. In fact, the use of membrane filtration techniques for the production of whey powders (whey protein or whey protein isolates) is often required. Therefore, it is very important to have a closer look on the cleaning strategies of the membranes, since they can harbor contaminations as well (Chamberland, Lessard, Doyen, Labrie, & Pouliot, 2017; D'Souza & Mawson, 2005). Depending on the filtration aim, membranes are divided in two groups: (i) spiral-wound membranes (organic) and (ii) tubular ceramic membranes (inorganic). Inorganic membranes offer higher resistance towards elevated temperatures and thus can be sterilised or treated with chemicals and disinfectants (Daufin, Merin, Labbé, Quémerais, & Kerhervé, 1991). Besides the membrane material, the membrane fouling is one important factor for cleaning efficiency. The deposit that forms on membrane surfaces consists mainly of proteins and salts. It is classified as organic fouling (Shi, Tal, Hankins, & Gitis, 2014). For organic materials, a too aggressive approach can cause a deterioration of the membrane (D'Souza & Mawson, 2005). Because of this, the cleaning regime is often conducted at low temperatures and an enzymatic cleaning solution is used additionally (Argüello, Álvarez, Riera, & Álvarez, 2002). Backwashing of membranes (tubular ceramic) is seen as one tool to enhance the cleaning efficiency (Head & Bird, 2013). The cleaning efficiency of membranes is measured often indirectly by changes in the water flux. Membrane fouling and therefore the filtration step must be seen as a significant point during powder processing. Research on membrane fouling microbiota showed that of the 25 most abundant genera classified via 16S rRNA sequencing bacilli, the genera *Bacillus* and *Anoxybacillus* are the dominant

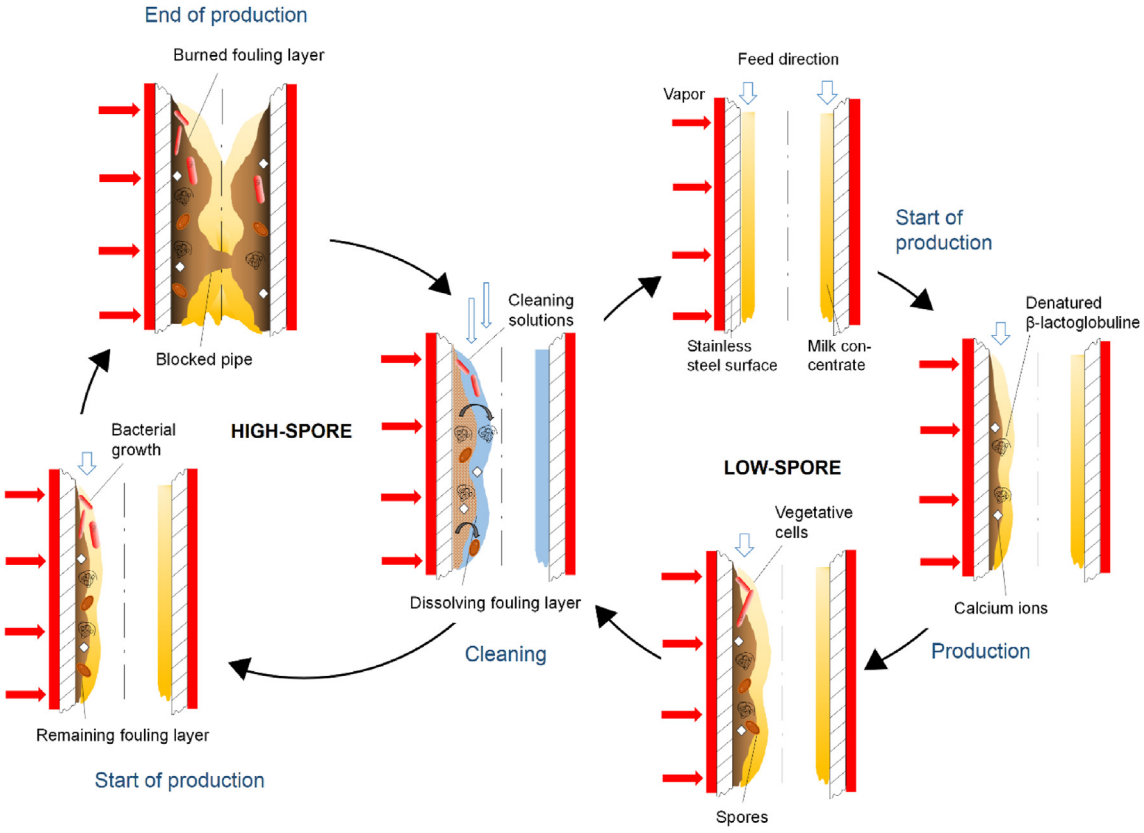

Fig. 4. Illustration of proposed mechanisms during powder production with combined fouling and biofilm formation in a falling-film evaporator. The low–spore cycle shows an efficient cleaning strategy with a pristine stainless steel surface at the beginning of production. The high–spore cycle shows an insufficient cleaning with a blocked pipe at the end of production (modified after Wedel et al., 2020).

group (Chamberland et al., 2017). This highlights that insufficiently cleaned membrane surfaces can harbor bacterial spores that are later of concern in the powdered product.

4. Critical evaluation of alternative technologies during manufacturing of dairy powders

Towards a low-spore powder processing there are alternative technologies besides heat treatment to reduce spores in different intermediate products. Heat-treatment is especially not preferable if the powder producers aim for a "low-heat" product. In this section, alternative technologies are reviewed for mesophilic and thermophilic spore reduction. Important to consider for alternative technologies is that whether the advantages they provide are greater when compared to the well-known and well-established heat-treatment technologies such as plate or tubular heat exchangers or direct steam injection.

Many different technologies were investigated in the past, based on physical stress factors to the bacteria. As a key factor, the final temperature reached in the product has to be evaluated (Table 3). If possible, the temperature differences are given to compare the alternative technology with a heat treatment over conventional heating methods.

The first reviewed technology is ultrasonication (US). Current can be converted into ultrasonic waves and those waves can be applied to liquid food samples. The effect of ultrasonication on the microorganisms is based on cavitation and leads to a breakdown of the cell wall (Ross, Griffiths, Mittal, & Deeth, 2003). The use of US to reduce bacterial spores is unsuitable, even if high intensities are used (see Table 3). An application would be only of use for the inactivation of vegetative cells.

Pulsed-electric field (PEF) is a well-studied technology. Its principle is based on very high voltages for short pulses of a few microseconds. The effect on the microbial cell is the electroporation of the membrane and thus an enhanced permeability (Rifna, Singh, Chakraborty, & Dwivedi, 2019). In most of the published data in the past, the focus was the inactivation of vegetative bacterial cells. Therefore, the aim was to achieve comparable results as milk pasteurisation (inactivation of all pathogenic vegetative microorganism). For the application of low-spore powders, the treatment needs to reduce the bacterial endospores since they are present in the final product in high numbers. As given in Table 3, it is possible to reduce thermophilic spore formers with PEF but an additional temperature effect of >115 °C is needed (Reineke, Schottroff, Meneses, & Knorr, 2015). The authors simulated a synergistic effect of temperature and PEF on the *G. stearothermophilus* inactivation of 2.4–3.2 log levels compared to heat alone. PEF might serve as a future technology for reducing the spore load in concentrates but not at low temperatures. For mesophilic spore formers and in particular *B. cereus,* it was stated that PEF technology is not able to reduce the spore load by more than 6 log below temperatures of 60 °C (Soni, Oey, Silcock, & Bremer, 2016). Therefore, the use of PEF may be applied for powders classified as 'high-heat'.

Ultra-high pressure homogenisation (UHPH) is a continuous technology that uses a pressure level up to 400 MPa. The killing effect of the UHPH is not only due to the temperature but also because of cavitation phenomena and high shear stress and disruption of cell fragments (Dumay et al., 2013). UHPH was studied for consumer liquid milk and was seen as a possible alternative (Pereda, Ferragut, Quevedo, Guamis, & Trujillo, 2007). Nonetheless, depending on the applied pressure, an increase in temperature takes place (as given in Table 3) up to 60 K. Thus, it is not possible to work with UHPH in a temperature range that does not affect the temperature and pressure sensitive whey protein fraction (Hinrichs, Rademacher, & Kessler, 1996). For milk powder processing, a treatment of the milk concentrates may be a preferable option. Still, the final product would be medium or high-heat treated. Data for UHPH treatments of milk concentrates are of high interest since the alteration in viscosity due to the heating (gelation of concentrate) may be managed better.

Ohmic heating (OH) is a thermal method and the main killing effect is due to the increase in the temperature. However, the principle of the heating itself differs. A current (mostly alternating current) passes through a conductive food matrix and causes a temperature increase (Tian, Yu, Wu, & Dai, 2018). The advantages are that no temperature differences between the outer wall and the fluid exist. Thus, the problem of fouling formation during the heat treatment of concentrates might be reduced. Although the studies on bacterial spores showed a slight, still significantly enhanced killing effect compared with conventional heating (D-values at 105 °C for ohmic heating at 60Hz and conventional heating for *Bacillus coagulans* spores were 0.91 and 1.32 min, respectively; Somavat, Mohamed, Chung, Yousef, & Sastry, 2012, 2013), the observed differences are small. In a different study, higher additional inactivation effects of up to 2.4 log are observed for mesophilic *B. subtilis* strains (Schottroff et al., 2019). A mechanistic hypothesis for the findings was that the spore's core was affected by targeting the small acid soluble proteins' (SASPs) DNA complex by electric fields which leads, in combination with heat, to a combined killing effect. The technology might be used for dairy concentrates because of the short times for heating up but experiments in this direction are lacking. Moreover, it might be useful to reduce the

Table 3
Alternative technologies to reduce spores in different media.[a]

Media	Reduction principle	Experiment mode, energy input	Log reduction	Temperature increase (K)	Species	Reference
Non-fat milk	HIUS	Discontinuous, 63.5–94.2 W	< 1	23–34	*B. licheniformis, B. coagulans G. stearothermophilus*	Khanal, Anand, and Muthukumarappan (2014)
Skim milk	HT- PEF	Continuous, 60.9–257.1 kJ kg^{-1}	up to 3.6	15–25	*B. subtilis*	Reineke et al. (2015)
Skim milk	HT- PEF	Continuous, 60.9–257.1 kJ kg^{-1}	up to 3.5	25	*G. stearothermophilus*	Reineke et al. (2015)
Ringers solution	PEF	Continuous, up to 350 kJ kg^{-1}	>4.5	50–94	*B. subtilis*	Siemer, Toepfl, and Heinz (2014)
Low fat milk	UHPH	Continuous, 200–370 MPa	up to 4.5	30–40	*B. amlyoliquefaciens*	Dong, Georget, Aganovic, Heinz, and Mathys (2015)
Full fat milk	UHPH	Continuous, 300 MPa	up to 6.3	54–58	*B. licheniformis*	Amador Espejo, Hernández-Herrero, Juan, and Trujillo (2014)
Full fat milk	UHPH	Continuous, 300 MPa	up to 5.3	54–58	*G. stearothermophilus*	Amador Espejo et al. (2014)
Deionised water	OH	Discontinuous, 60 Hz, 10 kHz	up to 3.8	–	*G. stearothermophilus*	Somavat et al. (2012)
Tomato juice	OH	Discontinuous, 60 Hz, 10 kHz	up to 4	–	*B. coagulans*	Somavat, Mohamed, and Sastry (2013)
NaCl solutions	OH	Discontinuous, 20, 40 and 60 kHz	>5	–	*B. subtilis*	Murashita, Kawamura, and Koseki (2017)

[a] Abbreviations are: HIUS, high intensity ultrasonication; PEF, pulsed-electric field; HT-PEF, high temperature PEF; UHPH, ultra-high pressure homogenisation; OH, ohmic heating; *B*, *Bacillus*; *G*, *Geobacillus*. The log-reduction and the temperature increase caused by the treatment is shown.

spore load but would still not be suitable for powders that are classified as “low-heat”.

The reviewed data demonstrate that it is possible to reduce bacterial spores in an order of magnitude that would result in a low-spore powder (log values < 1). However, none of those technologies operates at low temperatures when aiming to reduce bacterial endospores. It is very likely that some of the reviewed technologies provide advantages over conventional heat treatments in terms of product properties, heating-up time or energy efficiency. Still, it is always necessary to apply thermal energy on an intermediate milk product in order to reduce the spore load.

5. Conclusion

Dairy powders are widely used and valuable products. Literature data of the past years showed that regarding the quality of milk and whey powders the count of aerobic spore-forming bacteria (ranges between 10 to 10^6 cfu g^{-1}), especially mesophilic and thermophilic groups are of major concern for producers worldwide. During the production of powdered milk products, many process steps take place. Some of them, such as heat treatment, mild or high-heat, are mandatory while others are optional. From a process point of view, there are several options to maintain the spore level as low as possible:

i. use of membrane filtration (with a filter size of approx. 1.4 μm) or bactofugation to reduce spore load in the ingoing product stream (up to 6 reductions with membrane filtration or 2 log reductions with bactofugation)
ii. use validation of cleaning protocols to avoid recontamination
iii. sterilisation of heating equipment
iv. development of evaporators that are to be sterilised
v. ensure the plant design is hygienic
vi. avoid long production cycles for temperature-sensitive steps, i.e., separation, pasteurisation and evaporation

Non-thermal technologies such as US, PEF and UHPH to reduce microorganisms and/or enzymes have been extensively studied in the past. For the inactivation of spores, however, these technologies need to be combined with high temperatures. Future technologies might serve as better tools to treat milk and whey concentrates before evaporation and thus reduce the spore load in the final products. Scale-up experiments for proofing the suitability of the technologies are missing. Nowadays, the biggest opportunity for an improvement of milk powder processing is the quality control of cleaning systems.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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