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Microbiological aspects in whey powder production: What is the relevance of enterotoxigenic *Staphylococcus aureus*?

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Invited Review

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Abstract

Whey, a greenish-yellow liquid resulting from curd separation in cheese manufacturing, was historically considered economically insignificant in the dairy industry and often discarded into the environment without proper oversight. However, recognizing its high nutritional value, whey has become a valuable ingredient in the food industry. Unprocessed whey (raw material) is highly susceptible to contamination, as it can serve as a substrate for the multiplication of a range of microorganisms, including spoilage, spore forming, pathogenic and toxin producing bacteria, particularly if stored at inappropriate temperatures. *Staphylococcus aureus* is one of these potential pathogenic bacteria often associated to dairy, that can also persist in the environment through biofilm formation and, once reaching the food matrix, can grow and produce enterotoxins. During the processing of whey powder production, there are points where *S. aureus* might find its way into the final product. Here we demonstrate critical contamination steps, and we highlight the need for more research to assess the microbiological integrity of whey powder, especially in Brazil, where its production has been growing in recent years. Considering the increasing use of whey powder as an ingredient for various formulations, continuous surveillance for the presence of spoilage microbiota and potentially pathogens, including *S. aureus* and associated enterotoxins is indispensable to prevent food poisoning outbreaks.

Introduction

Bovine milk stands as one of the most versatile foods for humans and is used for various dairy products, such as cheese, yogurt, fermented milk, butter, milk powder and dried dairy powders. In 2020, Europe witnessed a substantial milk production of nearly 158 million tonnes, with almost 50% dedicated to cheese production, resulting in 54.8 million tons of liquid whey (Eurostat, 2020). In Brazil, whey processing presents challenges due to process technologies, often requiring complex industrial facilities and considerable financial investment (Alves *et al.*, 2014; Costa *et al.*, 2022). Much of the whey generated in Brazil, for example, originates from small and medium-sized cheese companies and despite the potential for developing new products based on whey, this co-product is often overlooked (Tabelini *et al.*, 2023).

Unsatisfactory hygienic conditions during cheese production and improper whey storage can compromise microbiological quality of this co-product (Santos *et al.*, 2017; Duarte *et al.*, 2023). In this context, the presence of *Staphylococcus aureus* is an important concern. The natural habitat of this pathogen includes the skin and mucous membranes of both humans and animals (Thomson *et al.*, 2022; Howden *et al.*, 2023) and poor hygienic practices during food production and processing can lead to contamination and possible production of staphylococcal enterotoxins (SEs) in food matrix (Deddefo *et al.*, 2023; Souza *et al.*, 2024). SEs are water-soluble, resistant to proteolytic enzymes in the digestive system, and can survive thermal treatments like pasteurization and ultra-pasteurization (Landgraf and Destro, 2013; Guanhong *et al.*, 2023). As *S. aureus* can grow in various food types, there are numerous potential sources of staphylococcal food poisoning, such as milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings (Grispoldi *et al.*, 2021).

Indeed, the presence of *S. aureus* in dairy products, specifically in cheese, has been reported by several studies (Gonzalez *et al.*, 2017; Nunes and Caldas, 2017; Alves *et al.*, 2018; da Silva Cândido *et al.*, 2020; Silva *et al.*, 2021). These studies have found *S. aureus* concentrations higher than 10^5 cfu/g and potentially intoxicating levels of SEs. During the 1950s, contamination of milk powder with SEs posed a significant issue, often due to *S. aureus* multiplication and enterotoxin production in raw milk before heat treatment or in concentrated milk prior to drying. Hygiene improvements and temperature control before drying resolved this problem (Anderson and Stone, 1955; Armijo *et al.*, 1957). However, in 2000, an outbreak linked to the consumption of reconstituted skimmed milk powder was reported in Japan. While sample analysis did not show viable *S. aureus*, heat-stable SEs were detected at concentrations sufficient to induce illness (Asao *et al.*, 2003).

Whey is a co-product that can serve as a substrate for *S. aureus*, particularly when stored at temperatures favorable for its growth (Miller and Ledford, 1977; Halpin-Dohnalek and Marth, 1989; Mehli *et al.*, 2017). Nonetheless, studies addressing the presence of SEs in whey powder (WP) remains scarce. Within this context, this review aims to underscore the significance of *S. aureus* in WP processing and identify possible critical contamination steps during production. This knowledge will allow the implementation of strategies to avoid the presence of *S. aureus* and its associated enterotoxins in WP.

Whey

Whey can be defined as the liquid milk derivative obtained during the production of cheese, casein, or similar products by separation from the curd following the milk's coagulation, and/or products derived from milk. The coagulation process primarily involves the activity of enzymes, especially those of the rennet type (Codex Alimentarius, 1995) and the whey can present in liquid, concentrated or powder forms. Approximately 80–90% of milk processed in cheese manufacturing facilities becomes whey (Buchanan *et al.*, 2023). Globally, the annual production of whey is estimated to be around 206 million tonnes (Tebbouche *et al.*, 2024), with a yearly growth rate of approximately 1-2% (Buchanan *et al.*, 2023).

In terms of classification, sweet whey originates from enzymatic milk coagulation employing enzymes of microbial, vegetable or animal origin (such as chymosin), and presents a pH between 6.0 and 6.8. Acid whey is obtained during cheese making after acid-induced milk coagulation using lactic, acetic, or citric acids, or lactic yeast, and presents a pH below 6.0 (Brazil, 2020a). The two coagulation processes yield whey types with distinct compositions, as presented in Table 1 (Papademas and Kotsaki, 2019). Acid whey exhibits lower lactose concentration due to the fermentation process that converts a fraction of lactose into lactic acid during the formation of the curd. Conversely, acid whey contains higher levels of calcium and phosphorus compared to sweet whey, attributed to the solubilization of the calcium-phosphorus complex in casein micelles at acidic pH (Alves *et al.*, 2014).

In Brazil, production is mainly represented by sweet whey, derived from cheese manufacturing through enzymatic coagulation, since cheeses such as Mozzarella, Prato and Minas Frescal are the most commercialized varieties in the country (Santos *et al.*, 2017; Trindade *et al.*, 2019). According to Trindade *et al.* (2019), who applied a questionnaire survey to 100 dairy industries from Southeast and South Brazil (most of them subjected to Federal Inspection System), these industries use whey mainly for production of dairy beverages (60%), ricotta (20%), whey concentrate (15%), and milk blends (5%), while 27% discard the whey in the effluent treatment system or use for animal feed. Hebishy *et al.* (2023) performed a systematic literature review and identified and examined 17 competitive factors that influence the production and use of whey by the Brazilian dairy industry. Four of these factors were referenced by more than ten authors: nutritional composition of products improvement, reduction of the environmental impact, sensorial aspects of products improvement and new production technologies.

Table 1. Typical composition of sweet and acid whey (Papademas and Kotsaki, 2019)

Component	Sweet whey	Acid whey
Total solids (g/L)	63.0–70.0	63.0–70.0
Lactose (g/L)	46.0–52.0	44.0–47.0
Total protein (g/L)	6.50–6.60	6.10–6.20
Milk fat (g/L)	0.20–0.50	0.30
Minerals (ash)	5.00–5.20	7.50–7.90
Phosphate (g/L)	1.0–3.0	2.0–4.5
Lactate (g/L)	2.0	6.4
Chloride (g/L)	1.1	1.1

Whey can also be obtained through microfiltration systems (Kelly, 2019; Reig *et al.*, 2021). Membrane technology is a key processing tool in agro-food industries for the treatment of food products, by-products and food waste (Castro-Muñoz *et al.*, 2020; Reig *et al.*, 2021). The global market for microfiltration membranes reached approximately USD 3.9 billion in 2022 and should reach USD 6 billion by 2027, with a compound annual growth rate of 8.8% during the forecast period of 2022-2027 (BCC Research, 2023). Membrane techniques possess the ability to physically separate fat and proteins from lactose and mineral salts, including monovalent and divalent ions (Rice *et al.*, 2005; Baldasso *et al.*, 2022). The resulting solution, known as permeate, contains particles smaller in size than the membrane pores and typically comprises around 4.5% lactose. On the other hand, substances of larger size, such as proteins and fats, are retained by the membrane, forming a solution referred to as concentrate (Rice *et al.*, 2005; Bhushan and Etzel, 2009; Baldasso *et al.*, 2022).

Ultrafiltration is one of the most prevalent techniques employed in producing whey protein concentrate (WPC) and whey protein isolate (WPI). In this procedure, low molecular weight components like water, salts and lactose permeate the membrane, while proteins, due to their larger molecular weight and size, are retained (Kravtsov *et al.*, 2021). While the dairy industry initially adopted this technology to find solutions for whey utilization (Price, 2019), its use remains relatively nascent in Brazil. In addition to ultrafiltration, a variety of advanced techniques are used for whey processing, such as reverse osmosis, drying, chromatographic methods, ion exchange, electrodialysis, fermentation and chemical processes, offering a spectrum of pathways for creating transformed products (Kilara and Vaghela, 2018; Kelly, 2019). Ohmic Heating has been considered as a potential technology for sweet whey processing, as it provides fast and uniform heating, minimizing the generation of off-flavor compounds and providing minor changes in nutrients, when compared to the traditional heat treatment (Costa *et al.*, 2018).

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Whey contains a wide range of components, some holding significant nutritional value and biological activities that are intensifying interest in exploring this co-product (Kilara and Vaghela, 2018). High whey protein fractions, in particular, have drawn attention for their attractive properties, leading to their integration into food formulations. Indeed, the attraction for whey has gained ground within the dairy industry (Ballatore *et al.*, 2020; Tsermoula *et al.*, 2021; Elkot *et al.*, 2024). Ingredients derived from whey exhibit the most rapid market growth among all dairy ingredients, with the market valued at $53.8 billion in 2019 and projected to reach $81.4 billion by 2025 (Tsermoula *et al.*, 2021). An important technological innovation in the milk production chain involves the conversion of whey into a valuable input. Producers are increasingly considering environmental responsibility, which is gaining prominence in the industry. This shift not only introduces a fresh income source for the industrial sector but also repurposes whey into a raw material to produce other items, primarily due to its high content of lactose and protein. This transformation enhances the economic efficiency of the dairy chain (Lavelli and Beccalli, 2022; Al-Tayawi *et al.*, 2023).

Contributions from consumer demand for healthier, innovative, safer and more convenient products have fueled the expansion of dairy beverage production (Guiné *et al.*, 2020). In this way, dairy beverages are an alternative for the use of whey due to its cost-effectiveness, ease of processing, and compatibility with existing industry equipment (Alves *et al.*, 2020; Kurnick *et al.*, 2024). Further alternatives encompass the manufacture of ricotta, protein isolates and the production of WP, that can be used in different formulations (Pires *et al.*, 2021). There has been also a growing emphasis on the manufacturing of dried dairy powders, to create new products and enhance their shelf life. These powdered forms are applied in diverse product categories, including confectionery, infant formula, sports dietary supplements and supplements for health recovery (O'Donoghue and Murphy, 2023). For example, the increase in infant formula consumption drives a global need for lactose, thereby amplifying interest in whey as a primary source of this sugar (Tuler Perrone *et al.*, 2016).

Whey powder production

According to Božanić *et al.* (2014), the main industrial processing of whey is drying to produce WP, which is 70% of the annual production of whey. As a main advantage, this process does not produce residues that should be treated, but on the other hand it demands a high initial investment to purchase the equipment, and consume a lot of energy during production. Furthermore, WP has a relatively low selling price when compared to WPC.

When fluid whey is obtained from other companies (smaller cheese manufacturers, for example), fluid whey may be subjected to pasteurization when arrives at the powder processing facility. Then, it is subjected to a sequence of procedures involving evaporation, lactose crystallization and drying, as shown in Fig. 1. The membrane separation process can also be used. These steps are designed to prevent microbial growth, by eliminating or deactivating microorganisms (Ziuzina *et al.*, 2018). To preserve the freshly acquired whey and control microbial growth until heat treatment, it should be immediately refrigerated at 4 °C. The reduction in temperature causes an extension of the latency time of microorganisms already present in the whey (Kilara, 2015). Hence, it is crucial to emphasize that during the steps involving heat treatment, the binomial time and temperature needs to be carefully respected.

Concentration of pasteurized fluid whey solids is achieved through vacuum evaporation, which incurs a significantly lower energy cost per kilogram of evaporated water compared to spray drying. This procedure allows concentration levels of milk solids ranging from 50% to 60% (m/m), carried out within temperatures of 45 to 70 °C (Carić *et al.*, 2009; Schuck, 2009). However, the temperature range used in the evaporators is not sufficient to ensure microbiological safety, and it is necessary to apply prior pasteurization to the fluid whey. Once concentrated, the solution proceeds to the crystallizer where the lactose undergoes crystallization. This stage aims to reduce the hygroscopicity of the powdered product, thus avoiding technological problems such as product adherence and agglomeration (Schuck, 2009). From a microbial perspective, the lactose crystallization process plays a critical role in the processing of powdered whey, as the product remains at room temperature for extended periods (4 to 24 hours) under constant agitation at temperatures close to 25 °C (Simeão *et al.*, 2018). After crystallization, spray drying is conducted with inlet air temperatures ranging from approximately 165 to 180 °C and outlet air temperatures around 85 to 90 °C. The fresh powder leaves the spray dryer at around 85 to 90 °C, is cooled down to reach approximately 25 to 28 °C and is then packaged. The spray drying phase significantly impacts both the yield and quality of WP. Challenges include the potential loss of powder yield due to high lactic acid and galactose content, leading to particle adhesion, and an elevated amorphous lactose content causing clumping during storage, impacting overall powder quality and shelf life (Ozel *et al.*, 2022).

Numerous surfaces come into contact with the product throughout WP processing, including equipment, utensils, and accessories. Consequently, the risk of pathogen cross-contamination cannot be disregarded (Martin *et al.*, 2016; Andritsos and Mataragas, 2023). To avoid this, the industry employs a strategy of cleaning and sanitization of all equipment and utensils that come into contact with the product during processing. This hygiene procedure can be performed with the use of chemicals, such as detergents and sanitizers (Martin *et al.*, 2016; Wang *et al.*, 2020).

Microbiological aspects relevant for whey powder production

Fluid whey is considered to be a favorable medium for microorganism growth, due to factors such as pH (6.0-6.8), high water activity (0.92%) and significant amounts of nutrients (Table 1: Pescuma *et al.*, 2015; Pires *et al.*, 2021). This co-product has a diverse microbiota generally composed of both beneficial bacteria with significant technological importance, but also spoilage microorganisms or even pathogens (Andrighetto *et al.*, 2004; Walsh *et al.*, 2012; Morandi *et al.*, 2019, 2022). The whey microbial diversity can be influenced by factors like dairy herd health, milking procedures, environmental hygiene, milk processing and factors related to whey handling, storage, and processing (Murphy *et al.*, 2016). In addition to beneficial bacteria (such as acid lactic bacteria – LAB), fluid whey can be contaminated by aerobes mesophiles, coliforms, enterobacteria and microorganisms originating from diseased animals (*Mycobacterium bovis, Brucella abortus, S. aureus, Coxiella burnetii, Listeria monocytogenes, Salmonella, Streptococcus agalactiae, Streptococcus uberis*, and *Escherichia coli*) or from biofilms attached on equipment, utensils and processing environments (*Pseudomonas, Flavobacterium, Enterobacter, Cronobacter, Klebsiella, Acinetobacter, Aeromonas, Alcaligenes, Achromobacter, Corynebacterium, Microbacterium, Micrococcus*)

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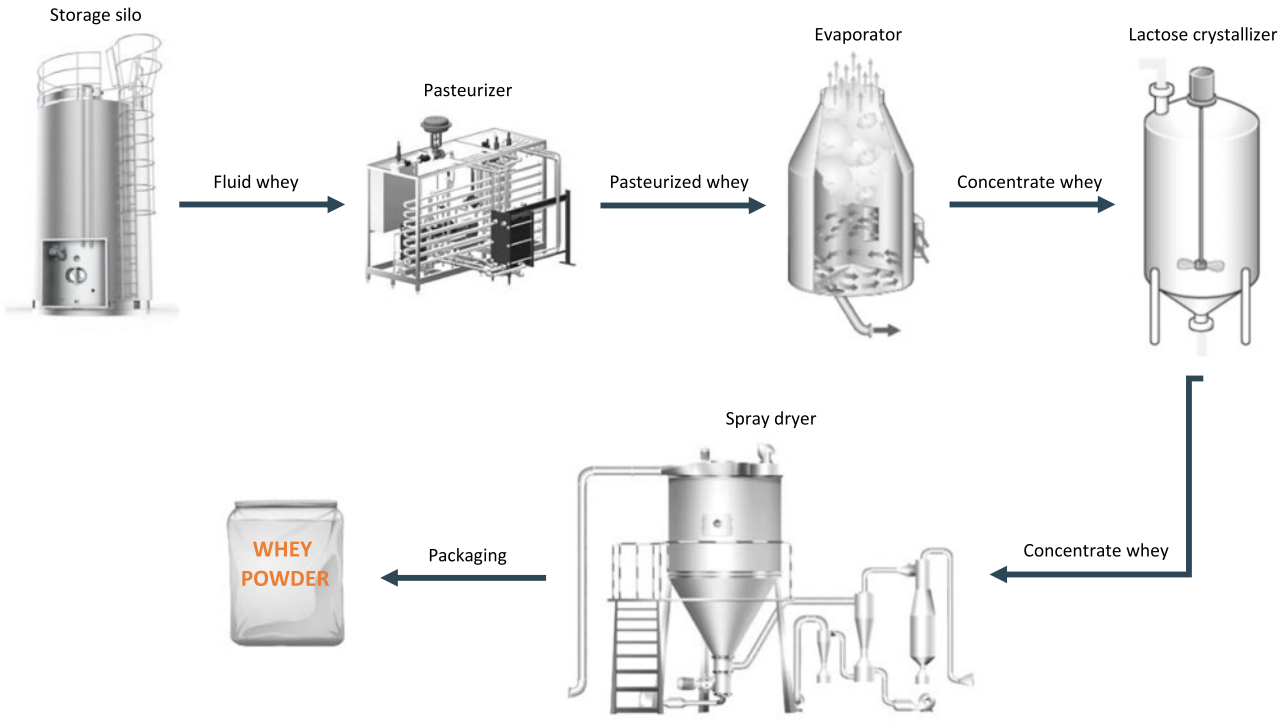

Figure 1. Main steps in whey powder production.

(Andrighetto *et al.*, 2004; Vissers and Driehuis, 2008; Walsh *et al.*, 2012; Morandi *et al.*, 2019).

Smaller cheese manufacturers who supply fluid whey to industries sometimes do not achieve adequate refrigeration, which may jeopardize the microbiological quality and safety of the whey. Thus, the co-product is subjected to pasteurization to inactivate pathogenic microorganisms, spoilage agents, and enzymes (Lewis and Deeth, 2008; Kilara, 2015). Most vegetative cells are typically destroyed by high-temperature short-time (HTST) treatment from 72 to 75 °C for 15 to 20 seconds, followed by cooling to 5°C. If whey is immediately processed through drying or evaporation, refrigeration may be omitted (Brazil, 2020a; Hebishy *et al.*, 2023). However, some microorganisms may persist in the whey, such those from the groups of thermoduric and thermophilic, represented mainly by *Alcaligenes, Microbacterium, Micrococcus, Enterococcus, Streptococcus, Lactobacillus* and *Corynebacterium*, as well as spore-forming bacteria such *Bacillus, Geobacillus* and *Clostridium*, which has relevance for the quality and safety of dairy products (Touch and Deeth, 2008). Despite not being able to multiply in dried dairy products, such bacteria can stay viable in dried products for long periods and upon reconstitution they may grow or their respective spores can germinate (Pal *et al.*, 2016) representing risks for the consumers, mainly for infants.

Walsh *et al.* (2012), described the presence of thermoduric bacterial isolates in an Irish WPC process. *B. licheniformis, Mic. lacticum, S. warneri, E. durans* and *B. subtilis* were recorded as the predominant microorganisms in the process line, while *Mic. phyllosphaerae, Neisseria subflava, Rothia aeria* and *Strep. mitis* were observed in dairy produce or indeed in any food product. Most recently, the *rpoB* and 16S rRNA genes have emerged as tools to characterize the spore populations of psychrophilic, mesophilic, and thermophilic contaminants present in sweet whey, WPC, non-fat dry milk and acid WPs. Through this approach, researchers identified spores from at least 12 genera, 44 species, and 216 rpoB allelic types, with *Bacillus licheniformis, Geobacillus* spp. and *Anoxybacillus* spp. standing out as the predominant ones. Moreover, when comparing spore species obtained from raw materials and the final powdered products, it was observed that while certain species, like *B. licheniformis*, were present in both the initial materials and the finished powders, other species such as *Geobacillus* spp. and *Anoxybacillus* spp. were more commonly detected in the final powdered products (Miller *et al.*, 2015). By using metagenomic sequencing, McHugh *et al.* (2018) also identified different groups of mesophilic sporeformers (namely, *Bacillus cereus, Bacillus licheniformis, Bacillus paralicheniformis*, and a heterogeneous group containing *Brevibacillus brevis*) in WP produced over 1 year in Ireland.

Anoxybacillus sp. and *Geobacillus* sp., together with *Bacillus* sp. are recognized as dominant spores identified in dairy powder products. These Gram-positive, aerobic bacteria produce relatively heat-resistant spores that can be activated by heat shock (Yuan *et al.*, 2012; Zain *et al.*, 2016). The occurrence of such spores in WP may represent spoilage potential, but presence of *B. cereus* can also be considered a concern in terms of safety since such isolates might be capable of causing emetic and enterotoxin food poisoning. Research on clostridial spores in dairy powder products are scarcer, although *Clostridium* species were already reported in milk powders (Barash *et al.*, 2010; Buehner *et al.*, 2015), and the presence of pathogenic *C. botulinum* and *C. perfringens* in milk powder is also considered a concern (Pal *et al.*, 2016). Other microorganisms potentially present in fluid whey, such as *Pseudomonas*, can lead to the deterioration of the raw material (Vissers and Driehuis, 2008) or potentially cause foodborne outbreaks, as in the case of *Salmonella, L. monocytogenes, C. sakazakii*, and *S. aureus* (Ünüvar, 2018). A common factor among these microorganisms is their vulnerability to heat treatments applied

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during fluid whey processing (Al-Holy *et al.*, 2012; Necidová *et al.*, 2019). However, the presence of *S. aureus* in fluid whey is a concern, given its ability to produce heat-resistant enterotoxins (Necidová *et al.*, 2019).

According to Schwabe *et al.* (1990), the thermal inactivation of SEs varies based on the food matrix and pH. Assessing the impact of heat treatment on enterotoxin activity is challenging, as it depends on the type of enterotoxin and the concentration in the food matrix. European law establishes criteria for coagulase-positive staphylococci cheeses, milk powder and whey powder. If the counts exceed 10^5 cfu/g, the batch must be tested for the presence of SEs, which must be absent in 25 g of the products (European Commission, 2005). Brazilian Ministry of Agriculture and Livestock Production (in Portuguese, Ministério da Agricultura e Pecuária) also establishes microbiological criteria for WP production, including the counting of viable mesophilic aerobes ($\leq 10^5$ cfu/g), total coliforms at 30-35 °C ($\leq$ 100 cfu/g), thermotolerant coliforms at 45 °C ($\leq$ 10 cfu/g), coagulase-positive staphylococci ($\leq$ 100 cfu/g), *Salmonella* spp. (absence/25 g) (Brazil, 2020a). In addition, once available for commercialization, WP must meet the microbiological standards established by National Health Surveillance Agency (in Portuguese: Agência Nacional de Vigilância Sanitária: Brazil, 2022) which determines the counting of viable mesophilic aerobes ($\leq 10^5$ cfu/g), coagulase-positive staphylococci ($\leq$ 100 cfu/g), and absence of *Salmonella* spp., Enterobacteriaceae and SEs.

The potential risk of *S. aureus* and SEs in whey and whey powder

Powdered milk is not sterile and has the potential to harbor diverse bacteria, including toxin producing pathogens (Bogdanovičová *et al.*, 2017). *S. aureus* is a Gram-positive bacteria, mesophilic cocci with a multiplication temperature in the range of 7 to 48 °C. The general survive features of this bacteria are pH limits between 4.2 and 9.3, minimum water activity of 0.85, and ability to survive in environments with up to 25% NaCl concentration. These immobile bacteria exhibit a diameter ranging from 0.5 to 1.5 μm and do not produce spores. They can be found in isolation, pairs, short chains, or clusters (Bennett *et al.*, 2013; Bhunia, 2018). *S. aureus* is a pathogen of great concern in food due to the ability of certain strains to produce heat-stable SEs that, once ingested, can lead to symptoms of gastrointestinal disorders, such as vomiting, nausea and abdominal cramps (Bennett *et al.*, 2013; Landgraf and Destro, 2013). Despite notable improvements in food safety procedures, SEs still figure as one of the main causes of foodborne outbreaks worldwide (Grispoldi *et al.*, 2021; Hu *et al.*, 2021).

From 2013 to 2022, *S. aureus* was listed as the main agent of reported outbreaks of food poisoning in Brazil, ranking third among pathogenic bacteria, behind *E. coli* and *Salmonella* spp. During this period, the Health Surveillance Secretariat reported a total of 6,523 outbreaks of foodborne diseases, affecting 107,513 individuals and resulting in 112 fatalities. Of these outbreaks, in 1,571 (24.08%) it was possible to specify the causative agents and *Staphylococcus* spp. was identified as the etiological agent in 170 (10.8%) outbreaks (Brazil, 2023). In a more detailed report, from 2016 to 2019, 2,504 outbreaks of foodborne diseases were identified, and information was present regarding the type of food involved for 894 cases (35.7%), with milk and dairy products responsible for 81 (9.06%) of these cases (Brazil, 2020b). However, the actual number could be significantly higher due to the underreporting of isolated cases.

Food poisoning caused by *S. aureus* typically occurs shortly after the ingestion of food contaminated with preformed toxins at concentration above 1 ng/g of food (Landgraf and Destro, 2013; Bhunia, 2018). In a study conducted by Bogdanovičová *et al.* (2017), the possibility of survival and growth of *S. aureus* in milk powder after its reconstitution was evaluated. Powdered milk was inoculated with 10^2 to 10^5 cfu/g of enterotoxigenic *S. aureus* and the reconstituted milk was stored for 48 h at 4, 15 and 25 °C. The multiplication of *S. aureus* as well as the production of SEA, SEB and SEC enterotoxins was regularly detected, and at the higher temperature and inoculum concentration, pathogen growth and enterotoxin detection were greater (Bogdanovičová *et al.*, 2017), demonstrating the risk that WP contamination could pose to consumers. The presence of staphylococci in a WP plant was examined and *S. aureus* was identified, originating from three powder residue samples from the working floor near filling devices. On the other hand, *S. aureus* was not present in air samples, but *S. saprophyticus* and *S. xylosus* were isolated. In addition, before the study was started, it was discovered that the WP contained a small number of *S. aureus* of a specific phage type, distinct from any isolates detected within the production line or its environment. Eight months later, *S. aureus* isolates from a site previously found to be contaminated, were of different phage types (Kleiss *et al.*, 1994).

Wang *et al.* (2012) evaluated samples of powdered infant formula milk and showed that 11.2% of them were positive for enterotoxigenic *S. aureus*. Even if these enterotoxigenic *S. aureus*-contaminated infant foods are reconstituted at low temperatures and subsequently stored at room temperature for extended durations, they still pose a health risk to children. Asao *et al.* (2003) documented a significant outbreak in Japan linked to the ingestion of reconstituted milk tainted with staphylococcal enterotoxin A. On the other hand, Cho *et al.* (2018) conducted a study of the microbial composition of powdered infant formula along the manufacturing processes and no presumptive colony for *Cronobacter* spp., *Salmonella* spp., and *S. aureus* was detected. *B. cereus* was the only detected bacteria, which also represents a concern.

In general, SEs exhibit resistance to freezing, drying and heat treatments, such as 100 °C, 110 °C, and 121 °C for 3 min. This suggests that conventional thermal processing techniques might not be sufficient to entirely eliminate the threat posed by *S. aureus* in milk and dairy products (Guanhong *et al.*, 2023). Necidová *et al.* (2012) demonstrated that the multiplication rates of *S. aureus* and the production of SEs in brain heart infusion culture medium and milk sources differ significantly. The culture medium often showed higher *S. aureus* counts and increased SE production compared to the food matrix. This raises the question of how the food matrix might impact the thermal stability and inactivation of enterotoxins. To investigate this further, Necidová *et al.* (2019) assessed the effect of heat treatment on the activity of SEs of type A, B, and C in milk. A total of 38 *S. aureus* strains capable of producing SEA, SEB, or SEC were subjected to heat treatment at 100, 110 and 121 °C for 3 min. Prior to heat treatment, all samples were positive for SE production. However, after heat treatment, positive rates of decrease were obtained, being 36.8%, 34.2%, and 31.6% at 100, 110 and 121°C, respectively. The reduction in positive samples differed according to the type of enterotoxin, with SEA being detected in higher amounts before and after heat treatment. Notably, the SE concentrations decreased significantly after heat treatment, leading to the conclusion that the success of SE inactivation depends on the initial concentration present before heat treatment.

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Among the foods that cause outbreaks and staphylococcal intoxications, raw milk, pasteurized milk and cheese are noteworthy, with *S. aureus* frequently associated in epidemiological investigations (Kümmel *et al.*, 2016; Hu *et al.*, 2018; Gajewska *et al.*, 2022; Zhang *et al.*, 2022). Considering that the fluid whey used in WP production predominantly originates from cheese manufacturing, where *S. aureus* and SE are recurrent, and considering that such microbiological criteria are applied to WP, monitoring *S. aureus* and associated SEs must be performed in WP, in order to avoid food poisoning outbreaks involving food that include WP in their formulations.

S. aureus can multiply within temperatures ranging from 7 to 48ºC, with optimum growth at 35-37 ºC, demanding adequate refrigeration when the whey is subjected to storage before processing in order to inhibit its multiplication and subsequent production of SEs (Hu *et al.*, 2018). In a study performed by Halpin-Dohnalek and Marth (1989), fresh cheddar cheese whey was inoculated with *S. aureus* at 10^6 cfu/ml and held at 4, 25 and 37 ºC for 48 h; the number of staphylococci increased in whey at 25 and 37 ºC and decreased or remained constant in whey at 4 ºC, revealing the importance of low temperature before the processing of this co-product.

In addition to avoiding growth of *S. aureus* in the whey, if the concentrated mixture becomes recontaminated by *S. aureus* at any post-pasteurization phase, the conditions of crystallization can inadvertently lead to an increase in the pathogen population and potentially production of SEs, although studies are needed to clearly demonstrate this. This is important because *S. aureus* is well known for its ability to produce biofilms and persist within food processing facilities, which may allow cross-contamination to foods and turn sanitization procedures ineffective, as chemicals may not reach inside the biofilm at necessary concentrations (Lee *et al.*, 2014; Idrees *et al.*, 2021). Toté *et al.* (2010) evaluated the inhibitory effect of different biocides on the biofilm of *S. aureus*. Among those analyzed, sodium hypochlorite and peracetic acid were the most effective in reducing the viability of both the biofilm matrix and the viable mass. Furthermore, Meira *et al.* (2012) explored the adhesion, kinetics of detachment, and development of biofilms by *S. aureus* isolates from food service surfaces on both stainless steel and polypropylene surfaces, as well as the effectiveness of peracetic acid (30 mg/L) and sodium hypochlorite (250 mg/L) in removing bacterial cells from the preformed biofilm matrix. The isolates showed high ability to adhere and form biofilm on the tested surfaces when immersed in a food-based broth at 7 and 28 °C. While both disinfectants reduced the number of adhered cells, they were not entirely successful in completely removing the viable cells of *S. aureus* forming a mature biofilm.

The removal of biofilms from within the crystallizer holds special significance, as adhered *S. aureus* cells can detach and reintroduce contamination to the concentrated mixture. Once the lactose crystallization stage is completed, the product is dehydrated using spray drying, a process that involves the dispersion of the mixture into small droplets under a stream of hot air at 160 to 200 ºC (da Silva *et al.*, 2017). The extensive surface area of the droplets and the elevated temperatures of the drying air result in rapid dehydration (Schuck, 2009). Despite the high temperature of the drying air, the droplets themselves reach relatively low temperatures (around 40 ºC). Consequently, spray drying is not a procedure that can be associated with the thermal elimination of microorganisms. Therefore, the microbial population within the concentrate containing crystallized lactose may not be substantially reduced, remaining potentially viable in powder form. In addition, thermoresistant SEs can persist despite the spray drying process (Tatini, 1976; Emiliano *et al.*, 2022). In this way, the microbial quality of the initial fluid whey (raw material) and the management of recontamination risks during processing are essential to ensure the safety of WP.

The microbiological quality of WPs is intricately linked to the microbial content initially present in the raw material, as well as to the possibility of contamination during and processing. This review highlights the need for more research to assess the microbiological integrity of WP, especially in Brazil, where the production of WP has been growing in recent years (Lopes *et al.*, 2023). Given the increasing use of WP as an ingredient for various food products and its global economic significance, continuous surveillance for the presence of spoilage microbiota and potential pathogens, including *S. aureus* and associated SEs, is indispensable to prevent potential outbreaks linked to the consumption of products containing WP in their formulation.

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