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Assessment of the development of *Staphylococcus aureus* during the production of soro on a laboratory scale

Avaliação do desenvolvimento de *Staphylococcus aureus* durante a produção de soro em pó em escala de laborátorio

Evaluación del desarrollo de *Staphylococcus aureus* durante la producción de suero en polvo a escala de laboratorio

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Abstract
The aim of this study was to evaluate the multiplication of *S. aureus* during whey powder production in laboratory scale. Whey was inoculated with 10^6 CFU· mL^{-1} of the *S. aureus* mixed culture at the beginning of the processing and the persistence of the microorganism after pasteurization, vacuum evaporation and drying was evaluated. The recontamination of whey by biofilms was also evaluated. Population of 10^2 CFU·mL^{-1} of *S. aureus* was found after vacuum evaporation. The microorganism was able to multiply in concentrated whey during the crystallization of lactose, which infers that it is possible to form a biofilm during this stage. Confocal microscopy analysis confirmed the presence of adhered cells after 4 h of process (10^3 CFU·cm^2). After 12 h of incubation, the number of adhered cells was approximately 10^5 CFU·cm^2. Sessile cells of *S. aureus* in stainless steel were able to return to the planktonic phase. After spray drying, it was verified that the *S. aureus* population in the powder was 10^3 CFU·g^{-1}. The results obtained in laboratory scale points to the need of quality assurance systems to control the steps of industrial whey powder production.
Keywords: Whey; Evaporation; Crystallization; Drying; Biofilm.

Resumo
O objetivo deste estudo foi avaliar a multiplicação de *S. aureus* durante a produção de soro de leite em pó em escala de laboratório. O soro foi inoculado com 10^6 UFC · mL^{-1} da cultura mista de *S. aureus* no início do processamento e foi avaliada a persistência do microrganismo após pasteurização, evaporação a vácuo e secagem. A recontaminação do soro por biofilmes também foi avaliada. População de 10^2 UFC · mL^{-1} de *S. aureus* foi encontrada após evaporação a vácuo. O microrganismo foi capaz de se multiplicar em soro concentrado durante a cristalização da lactose, o que infere ser possível a formação de um biofilme nessa etapa. A análise de microscopia confocal confirmou a presença de células aderidas após 4 h de processo (10^3 UFC · cm^2). Após 12 h de incubação, o número de células aderidas era de aproximadamente 10^5 UFC · cm^2. As células sésseis de *S. aureus* em aço inoxidável foram capazes de retornar à fase planctônica. Após a atomização, verificou-se que a população de *S. aureus* no pó era de 10^3 UFC · g^{-1}. Os resultados obtidos em escala laboratorial apontam para a necessidade de sistemas de garantia da qualidade para controle das etapas de produção industrial do soro de leite.
Palavras-chave: Soro de leite; Evaporação; Cristalização; Secagem; Biofilme.

Resumen
El objetivo de este estudio fue evaluar la multiplicación de *S. aureus* durante la producción de suero en polvo a escala de laboratorio. El suero se inoculó con 10^6 UFC · mL^{-1} del cultivo mixto de *S. aureus* al inicio del procesamiento y se evaluó la persistencia del microorganismo después de la pasteurización, evaporación al vacío y secado. También se evaluó la recontaminación del suero por biopelículas. Se encontró una población de 10^2 UFC · mL^{-1} de *S. aureus* después de la evaporación al vacío. El microorganismo pudo multiplicarse en el suero concentrado durante la cristalización de la lactosa, lo que infiere que es posible formar una biopelícula durante esta etapa. El análisis de microscopía confocal confirmó la presencia de células adheridas después de 4 h de proceso (10^3 UFC · cm^2). Después de 12 h de incubación, el número de células adheridas fue de aproximadamente 10^5 UFC · cm^2. Las células sésiles de *S. aureus* en acero inoxidable pudieron volver a la fase planctónica. Después del secado por aspersión, se verificó que la población de *S. aureus* en el polvo era de 10^3 UFC · g^{-1}. Los resultados obtenidos a escala de laboratorio apuntan a la necesidad de sistemas de aseguramiento de la calidad para controlar los pasos de la producción industrial de suero en polvo.
Palabras clave: Suero; Evaporación; Cristalización; Secado; Biopelícula.

1. Introduction

In 2017 a major recall of food products containing whey contaminated with *Salmonella* alerted the regulatory agencies and industry to a more rigorously control of the microbiological quality of whey powder (FDA, 2018). Pathogenic microorganisms frequently found in cheesemaking environments such as *Salmonella* sp., *Listeria* sp., *Escherichia coli* and *Staphylococcus aureus* may also be present in the fluid whey (Perin et al., 2017). *S. aureus* is a microorganism normally found in the skin of the animal, hands of manipulators and in the processing environment in the form of biofilms (Hamadi et al., 2014). Despite the flaws in epidemiological data from Brazil, *S. aureus* was the third pathogen described as the cause of foodborne diseases outbreaks and highly associated with dairy products (Brazil, 2019b; SVS, 2019). Although the microorganism does not cause any disease when ingested in its vegetative form, counts greater than 10^5 CFU·mL^{-1} in whey can produce heat-resistant enterotoxins that cause food poisoning (Necidová et al., 2012; da Silva Cândido et al., 2020). A systematic efficacy evaluation of the whey powder processing in reducing the *S. aureus* population has not yet been described in previous studies. Considering the potential risk of this pathogen to public health, the objective of this work was to simulate laboratory scale different conditions of whey powder processing considering *S. aureus* as the target microorganism.

2. Methodology

2.1 Microorganisms and preparation of the mixed culture

After preparing the bacterial stock, the cultures of *S. aureus* (Table 1) were stored in ultra-freezer at -62 °C in Eppendorf tubes containing brain and heart infusion broth (Sigma Aldrich®, Germany) and 60 % glycerol ($v.v^{-1}$).

Cultures of *S. aureus* were inoculated by introducing 0.1 ml of each culture into 10 mL of brain and heart infusion broth and incubated at 35 ° C ± 2 ° C for 24 h to obtain a culture with approximately 10^8 CFU·mL^{-1}. Subsequently, the mixed culture was standardized for approximately 10^6 CFU·mL^{-1} of *S. aureus*.

Table 1. Microorganisms used and their respective origin.

Bacteria	Origin	Enterotoxin produced	Origin
S. aureus ATCC 6538	Human injury	SEK	ATCC *
S. aureus ATCC 25923	Clinical isolate	SEA, SEB, SEC	ATCC *
S. aureus ATCC 700698	Patient with pneumonia	SEA	ATCC *
S. aureus ATCC 43300	Clinical isolate	SEA, SEB , SEC, SED	ATCC *

Being*=*American Type Culture Collection*. Source: Authors.

The *S. aureus* strains described in Table 1 were selected considering their biofilm formation capacity, staphylococcal enterotoxin production and reports of its presence in dairy products (Zurita et al., 2010; Feitosa, et al., 2017; Al-Nabulsi et al., 2020; Yu et al., 2021).

2.2 Simulation of whey powder production with contamination of *S. aureus* in raw material

Whey fluid was prepared aseptically by dilution of 135 g of whey powder (Germinal®) in 165 ml of autoclaved distilled water. After complete solubilization of the powder, the reconstituted whey powder was heat treated (72 °C for 20 s), cooled to room temperature and inoculated under sterile conditions with the mixed culture of *S. aureus* standardized to 10^6 CFU·mL^{-1}. Was predicted an initial *S. aureus* population of 10^6 CFU·mL^{-1} in the reconstituted whey powder based on works describing a population of *S. aureus* between 10^3 and 10^8 CFU·g^{-1} in cheeses (Borelli et al., 2011; Jakobsen et al., 2011; Verraes et al., 2015; da Silva Cândido et al., 2020; De Leon et al., 2020).

In order to evaluate the bactericidal potential of the pasteurization, vacuum evaporation concentration and spray drying steps, reconstituted whey powder inoculated with the *S. aureus* mixed culture was pasteurized at 72 ºC for 20 s in a water-bath (Figure 1). Then, the fluid whey was concentrated at 65 °C for 20 min on the rotary evaporator Marconi®(model MA 120/2057, Brasil) up to 50 °Brix, determined in a digital refractometer Biobrix® (model 2WAJ-D – Ideal Lab, Brasil).

The crystallization of lactose in the concentrated whey occurred immediately after the end of the vacuum evaporation according to protocol defined by Simeão et al. (2017). Approximately 300 mL of concentrated whey was packed in glass beaker (diameter 37.0 cm, height 12.5 cm), cooled to 25 °C and kept under stirring for 12 h with the aid of a magnetic bar at 500 rpm. The concentrated whey was heated to 40 °C and injected at 1.0 kg·h^{-1} in a single-stage laboratory *spray dryer* with a MSDi 1.0 (Labmaq do Brasil, Brazil) nozzle spray. The drying parameters were: inlet air temperature 170 °C and outlet air 100 ºC. The high temperature of the outlet air aimed to provide drying in an extreme condition in order to enhance the heat treatment suffered by *S. aureus*. The powdered whey collected in the cyclone was packaged under sterile conditions.

The experiment was conducted with three replicates and after the steps of pasteurization, vacuum concentration, lactose crystallization and spray drying, samples were collected for *S. aureus* enumeration.

2.3 Simulation of whey whey production with whey contamination during the lactose crystallization step

The crystallization of lactose (Figure 1) is a critical step in the processing of whey powder since the product is kept at room temperature for long periods. Thus, recontamination with *S. aureus* during this step may compromise the microbiological safety of whey powder.

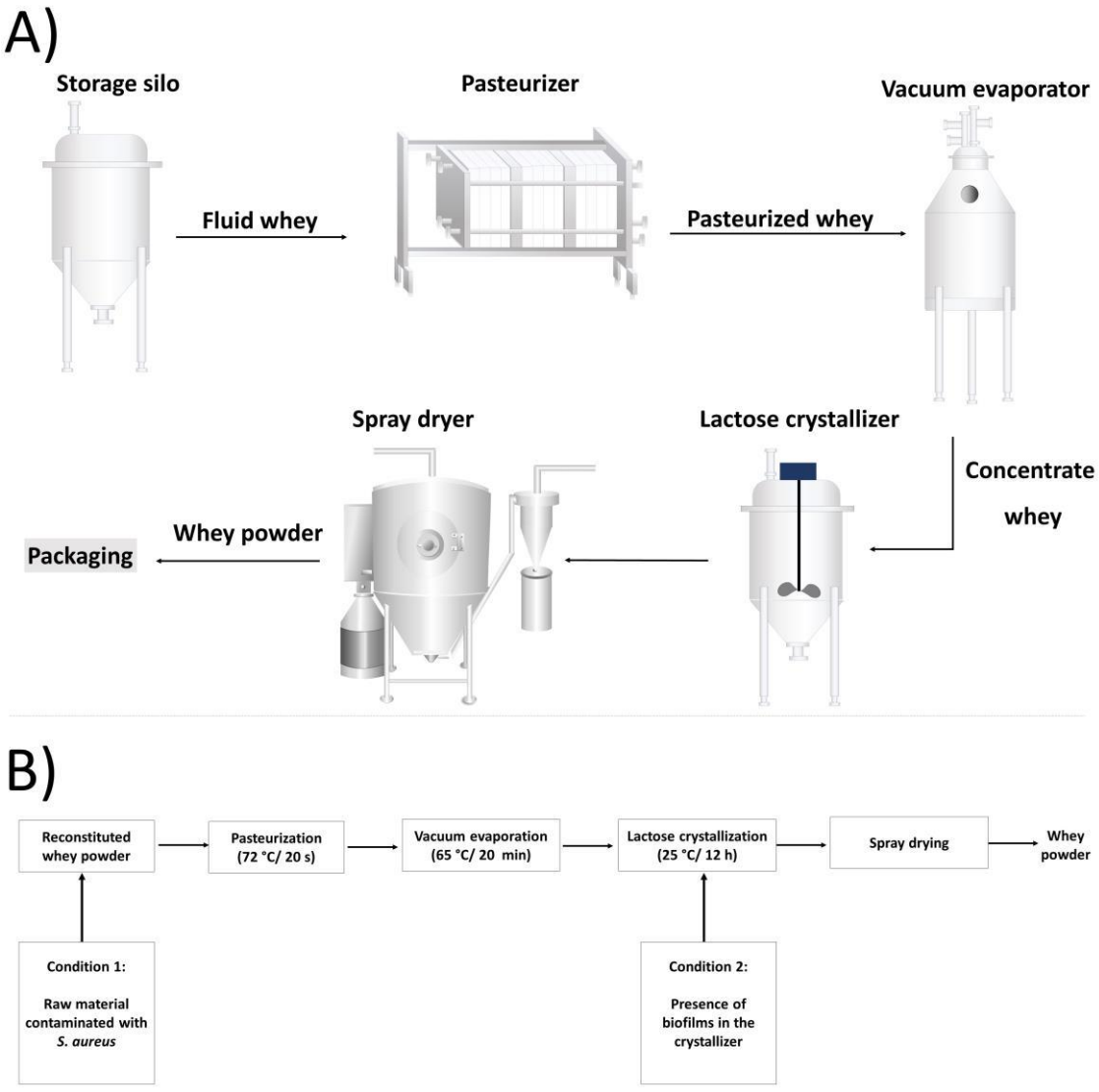
In order to evaluate the multiplication of *S. aureus* during the crystallization of lactose, reconstituted whey powder was processed according to the flowchart shown in figure 1 with intentional contamination of the concentrated whey with a

population of approximately 10^3 CFU·mL^{-1} of the mixed culture of *S. aureus*. During the crystallization step, aliquots of the concentrated whey were periodically collected for the determination of the viable cells of *S. aureus.*

To evaluate the ability of biofilm formation by *S. aureus* during the crystallization of lactose, stainless steel AISI 304 coupons (2 cm x 2 cm x1 mm) previously sanitized and sterilized according to Rossoni and Gaylarde (2000) were added to the concentrated whey. Throughout the crystallization the coupons were removed aseptically and washed with 0.85 % saline solution (m·v^{-1}) for the removal of not adhered cells.

For the biofilms observation by scanning confocal microscopy, the coupons were washed twice by immersion in phosphate-buffered saline-PBS (0.2 M, pH 7.2) and incubated in the absence of light for 15 min in a mixture of 20 µg·mL^{-1} of propidiumiodide (PI) (Sigma Aldrich, Germany) and 2 µg·mL^{-1} of fluorescein isothiocyanate (FITC) (Sigma Aldrich, Germany) in PBS (0.2 M, pH 7.2) prepared immediately before the use. After incubation, the coupons were washed by immersion in PBS and analyzed in confocal laser scanning microscope model LSM 510 META (Zeiss, Germany) using the Argon laser with wave-length of 458 and 514 nm (Wang et al., 2012).

Figure 1 - Flowchart of whey powder production. Production of whey powder on an industrial scale (A). Production of whey powder in laboratory scale (B).



Source: Authors.

2.4 Determination of *S. aureus*

The enumeration of *S. aureus* for both simulations (sections 2.2 and 2.3) was performed using the surface plating technique as recommended by ISO 6888-2 (ISO, 1999). Briefly, 1 mL of reconstituted whey powder, 1 mL of reconstituted concentrated whey or 1 g of whey powder were diluted in 9 mL of peptone water 0.1 % ($m \cdot v^{-1}$) and serial decimal dilutions were performed using the same diluent. 20 µL of the appropriate dilutions were deposited on the ágar surface *Baird Parker*-BP (Acumedia®,Furlab, Brasil) by microdroplet technique (Morton, 2001). Plates were incubated at 35 ± 2 ºC for 24-48 h and drops with colonies numbers between 8 and 80 were considered for counting.

The *S. aureus* enumeration was expressed in $CFU \cdot mL^{-1}$ or $CFU \cdot g^{-1}$ according to equation 1:

Eq. 1 $$CFU \cdot mL^{-1} or\ CFU \cdot g^{-1} = \frac{M.D}{a}$$

Where:

M: mean plate count (CFU)

D: dilution held

a: volume or mass of the aliquot used in the plating (mL or g)

In order to quantify the adherent cells in the form of biofilm coupons was added in 300 mL of concentrated whey and sonicated for 20 min using ultrasound (Branson® 150, Model UC-6, EUA) 40 kHz and power of 160 W (Malheiros et al., 2010).

Coupons were withdrawn under sterile conditions and serial dilutions containing peptone saline solution 0.1 % ($m \cdot v^{-1}$) as diluent and deposited on agar BP surface as described above. The counting of the viable cells in biofilm was expressed in log $CFU \cdot cm^2$ as indicated by equation 2 (Careli et al., 2009):

Eq. 2 $$CFU \cdot cm^2 = \frac{(V_A - V_D).M.D}{A}$$

Being:
V_D: volume of the diluent used in the rinsing (mL)
V_A: volume of the aliquot used in the plating (mL)
M: average count obtained on the plates (CFU)
D: dilution performed
A: coupon area (cm2)

To evaluate the biotransferential potential of the cells adhered to the coupons for the concentrated whey, the methodology described by Boari et al. (2009) with adaptations was used.

After 12 h of lactose crystallization, a coupon was withdrawn and immersed in a new sterile milk whey. Plankton cell counting was performed to determine the biotransfer potential from the cells adhered to the coupon to the environment. For this purpose, aliquots were removed and serially diluted in peptone water 0.1 % ($m \cdot v^{-1}$), plated by the micro agarose technique on agar BP and incubated at 35 ºC ± 2 ºC for 24-48 h. The results were expressed in log $CFU \cdot mL^{-1}$.

The evaluation of the transfer / detachment of cells adhered to the surface to the whey was determined by the results obtained during 12 h of lactose crystallization. Thus, the ability of sessile cells to release and contaminate the sterilized substrate is considered as a potential for biotransfer (Oliveira et al., 2010).

2.5 Data Analysis

The experiment was performed in three replicates. Descriptive data statistical analyses were performed as well as arithmetic mean and standard deviation with the aid of the Microsoft Excel® program.

3. Results and Discussion

Whey powder production was simulated on a laboratorial scale respecting the time/temperature binomials (Figure 1) commonly used by the dairy industry. The stages of *S. aureus* inoculation into the whey were defined in two conditions possible to occur in an industrial processing line:

1) Raw material (fluid whey) contaminated at the beginning of processing;
2) Recontamination of fluid whey after the steps involving heating (pasteurization and vacuum evaporation).

Considering a whey provenient from the production of cheeses contaminated with *S. aureus*, the condition 1 simulates a feedstock with an initial charge of 10^6 CFU·mL^{-1} of the pathogen, which is reasonable based on previous studies that indicate that the *S. aureus* population in cheeses can reach 10^8 CFU·g^{-1} (Freitas et al., 2013; Borges et al., 2003).

Condition 2 simulates the recontamination of concentrated whey during the crystallization stage of lactose in which the whey remains at room temperature for long periods (Figure 1). Accidental contamination of concentrated whey with *S. aureus* may occur in this step due to the release of biofilms formed in tubing or on the crystallizer surface itself.

3.1 Condition 1: Whey contaminated with S. aureus

On an industrial scale, the fluid whey stored under refrigeration is pasteurized in the plate heat exchanger (72 °C for 20 s) and then concentrated to 50 °Brix in a vacuum evaporator. The concentrated whey is destined to the crystallization tank with controlled agitation for prior crystallization of lactose before spray drying (Masters, 2002).

Pasteurization refers to the heat treatment that aims to eliminate all the pathogenic microbiota and part of the deteriorating microorganisms present in the raw material. Since *S. aureus is* a mesophilic microorganism and not described in the literature as thermoduric (Gleeson et al., 2013), there is evidence that pasteurization is efficient to eliminate this pathogen.

To simulate the laboratory-scale pasteurization step, reconstituted whey powder inoculated with 10^6 CFU·mL^{-1} of the mixed culture of *S. aureus* was added in an Eppendorf tube 1 mL and heated at 72 ° C for 20 s in a water bath. Upon completion of the heat treatment, the tube was cooled in an ice bath and the enumeration of *S. aureus* was performed.

After pasteurization, the *S. aureus* population in the whey was 10^3 CFU·mL$^{-1,}$ which proves that the binomial 72 ° C for 20s was not efficient to guarantee the innocuity of the raw material (Figure 2). This result is in agreement with other studies that demonstrated that pasteurization may not be effective in completely eliminating the *S. aureus* population; being the efficiency of this thermal treatment conditioned to factors such as type of food matrix, population density of the pathogen, genetic variations of the microorganism (Huck, Sonnen, & Boor, 2008).In studies conducted in the state of São Paulo by Queirós (1995) and by Badini et al. (1996), ware verified the counts of *S. aureus* in pasteurized milk in 40 % and 50 % of the samples, respectively.

Following processing, the whey is concentrated in a vacuum evaporator and can reach temperatures of 65 ° C for 20 minutes. Considering that this temperature can also lead to the death of *S. aureus* cells, such cumulative heat treatment may be effective in eliminating the population of the pathogen remaining from pasteurization.

After concentration of whey under controlled laboratory conditions, it was confirmed that a *S. aureus* population of 10^2 CFU·mL^{-1} remained present in the concentrated whey (Figure 2). Although this result indicates failures in the safety of the product and point to the need for more severe thermal treatments in the processing of whey powder, a population of *S. aureus* around 10^{2} CFU· mL^{-1} does not pose a risk to consumer health.

As described on literature reports, the production of staphylococcal enterotoxins occurs in food matrices with populations of *S. aureus* larger than 10^5 CFU.mL^{-1} or UFC· g^{-1} (Necidová et al.,2012). Asao et al. (2003) verified that staphylococcal enterotoxin type A was only detected in reconstituted powdered milk when the *S. aureus* population reached levels of 10^5 CFU·mL^{-1}.

Anunciação et al. (1994) inoculated enterotoxin producer *S. aureus* strains at levels of 10^2 and 10^4 CFU· mL^{-1} in pasteurized milk for cheese production. The enterotoxin was only detected in the cheese prepared with milk inoculated with 10^4 CFU· mL^{-1}, after being maintained for 5 hours at 27 ° C. When inoculating 10^4, 10^5 and 10^6 CFU·mL^{-1} of *S. aureus* in goat's milk for the manufacture of cheese, Rozand et al. (1996) obtained enterotoxin B production from the 5th and 12th days, only in the treatments with 10^5 and 10^6 CFU· mL^{-1}.

Although *S. aureus* levels after vacuum evaporation not posing a risk to public health, this microorganism can multiply during the crystallization of lactose reaching potential for staphylococcal enterotoxin production. The multiplication of pathogen in food matrices such as milk, cheese cakes, creams, meats, is well characterized in previous studies, but the development of *S. aureus* in concentrated whey has not yet been documented (Peresi et al.,2004; Xing et al., 2016).

To verify if *S. aureus* is capable of using whey as a substrate, a growth curve was constructed employing the conditions used for the crystallization of lactose (Figure 3). Note that in the culture incubated at 25 °C for 12 h, the growth curve shows the lag, logarithmic and stationary phases defined (Figure 3). However, the presence of lag and stationary phases was not observed in the culture of *S. aureus* in concentrated whey (Figure 3).

The growth rates of mixed culture *S. aureus* in BHI and whey were 0.42 and 0.04 (log CFU·mL^{-1}·h^{-1}), respectively (Figure 3). After 12 h of incubation under stirring conditions simulating the industrial crystallization of lactose, a population of 10^6 CFU· mL^{-1} was found in the whey. As previously described, this population is worrisome because it presents a risk of production of thermoresistant enterotoxins that may persist in the final product (Necidová et al., 2012).

Following processing, concentrated whey with crystallized lactose is subjected to spray drying. In industrial processes, the whey is injected into the drying chamber through a spray nozzle where it is dispersed in small droplets. The mist formed by the concentrated whey is brought into contact with a stream of heated air which promotes the instantaneous removal of water (Schuck, 2009). The consequence of this is the formation of a particulate material (whey powder) with low water activity (~ 0.200) (Schuck, 2002).

Although the drying process using of high inlet air temperatures (170 ºC), the whey dehydration process occurs in a short period of time (30 to 90 s) (Schuck, 2002). Thus, evaporation of water occurs rapidly at reduced temperatures (~ 45 ° C) and does not cause significant thermal stresses on the microorganism (Cebrián et al., 2017).

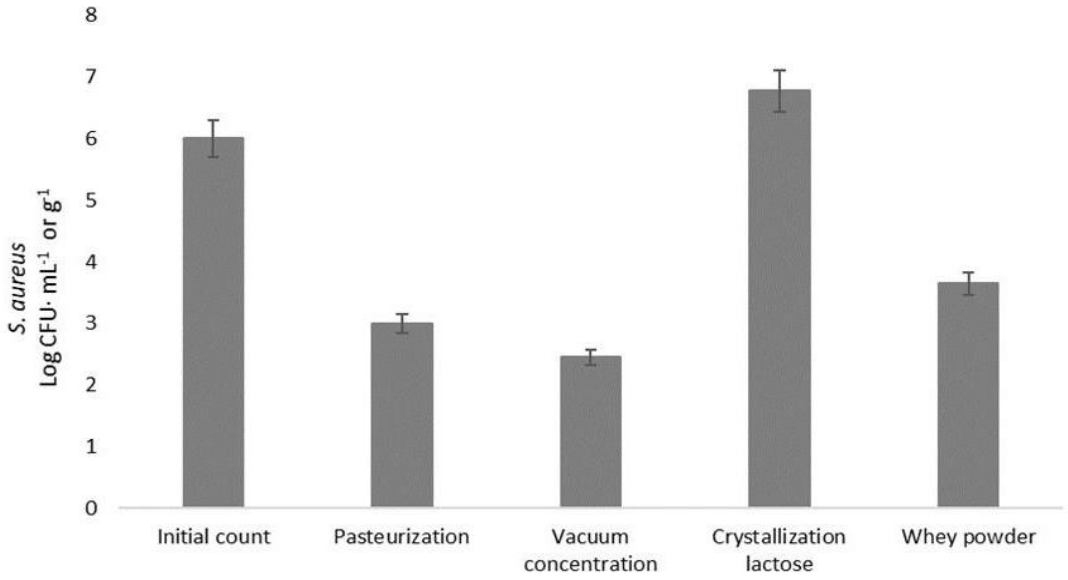
Taking into account the concentrated whey presents solids between 50 and 60% and after drying solids between 97 and 99 %, the final population in the whey powder is expected to be 10^2 CFU·g^{-1}. However, after drying, it was found that the *S. aureus* population in the whey powder was 10^3 CFU·g^{-1} (Figure 2).

Although *S. aureus* being normally resistant to the temperatures assumed by the whey particle during drying, other stressing conditions to the microorganism may occur during the process resulting in its loss of viability. According to Wolf et al. (2008) and Holy (2001), *S. aureus* may be sensitive to osmotic and oxidative stresses which helps explain the result found after spray drying.

The levels of *S. aureus* found in the final product (10^3 CFU·g^{-1}) do not present any risks to the consumer mainly due to the low Aw which limits the multiplication of the pathogen and consequent production of enterotoxin. However evaluating the processing history, the *S. aureus* population reached counts of 10^6 CFU·mL^{-1} (Figure 2) presenting a real risk of enterotoxin production.

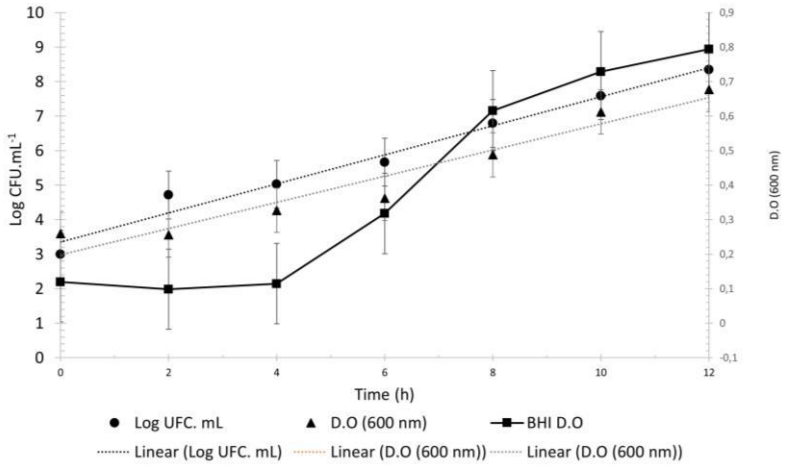
Inspite of the relatively low counts of whey powder, especially considering the high contamination load of the raw material, the product is outside the microbiological standards recommended by Regulation 2073/2005 (maximum of 10^2 CFU·g^{-1}).

Figure 2. Number of viable cells (log CFU· mL^{-1} or g^{-1}) during stages of whey powder production.



Source: Authors.

Figure 3. Growth curve of *S. aureus* at 25 ºC/ 12 h. Growth curve in whey in log CFU· mL^{-1} (●).Growth curve by optical density at 600 $_{nm}$ (▲).Growth curve in BHI broth by optical density at 600_{nm} (-■-).



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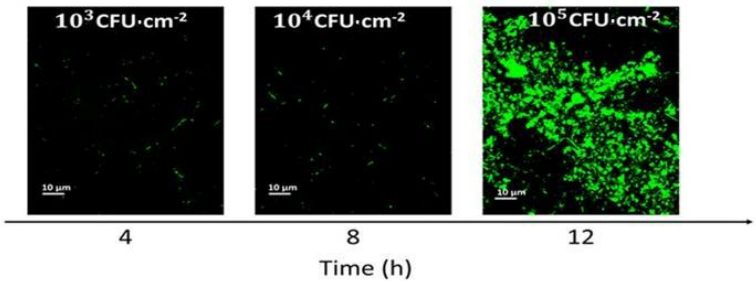
3.2 Condition 2: recontamination of the fluid milk by whey biofilms

Various studies points *S. aureus* as a biofilm forming microorganism in stainless steel surfaces (Herrera et al., 2007; Rosmaninho et al., 2007; Zouagh et al., 2019) suggesting that this pathogen may be present in food processing environments. The formation of *S. aureus* biofilms in stainless steel using milk matrix has already been reported (Allignet et al., 2001; Boari 2009; Hamadi et al., 2014). However, the capacity of biofilm formation in environments containing whey has not yet been investigated.

In the previous section, it was demonstrated that *S. aureus* is capable of multiplying in concentrated whey during the lactose crystallization, which justifies inferring that during this stage the formation of biofilm is also viable. To evaluate *S. aureus* adhesion ability during the lactose crystallization, concentrated whey at 50 °Brix and inoculated with approximately 10^3 CFU·mL^{-1} of *S. aureus* was added to the stainless steel coupons and maintained at 25 °C for 12 h under stirring.

Through the confocal microscopy analysis it was possible to observe the presence of adhered cells after 4 h of process (10^3 CFU· cm^2) (Figure 4). At 12 h of incubation the number of cells adhered was approximately 10^5 CFU·cm^2 (Figure 4) which can be characterized as a biofilm (Ronner & Wong 1993; Wirtanen et al., 1996). This results indicates that during the crystallization step in industrial scale, *S. aureus* can adhere to the surface of the crystallizer and the biofilm formed and act as focus of the raw material recontamination.

Figure 4. Adhesion and biofilm formation during lactose crystallization step.



Source: Authors.

In a process condition in which the crystallizer has not been sanitized from one batch to another or has been precariously sanitized, the cells of the biofilm may be peeled off and contaminated with the new whey free from To simulate this scenario, 2 stainless steel coupons containing *S. aureus* biofilm formed at 25 °C for 12 h were inoculated into 300 ml of concentrated whey. The whey added of the coupons was maintained under lactose crystallization conditions and after 12 h of process an aliquot was collected to determine the biotransfer potential of the pathogen.

It was found that *S. aureus* cells were able to migrate from the stainless steel surface to the concentrated whey and a final population of 10^6 CFU·mL^{-1} was reached at the end of the crystallization.

In the study by Rodrigues et al. (2017), where they evaluated the effect of essential oils on biofilms from *S. aureus* food contact surfaces, similar values were found for this work, three isolates of *S. aureus* exhibited biofilm cell counts greater than 10^5 CFU·mL^{-1} for the potential of biotransfer.

Concentrated whey recontaminated with *S. aureus* by biotransference was dried in spray dryer and the resulting powder had a count of 10^3 CFU·g^{-1} of *S. aureus*. Again, the final product is outside the microbiological parameters allowed by Regulation 2073/2005 and the high population density of the pathogen during the crystallization of lactose enables the production of enterotoxins in the product.

4. Conclusion

In this study it was evidenced that concentrated whey has nutritional composition favorable to the multiplication of *S. aureus*, which had not yet been documented in previous studies. Due to the ease with which this pathogen develops in the raw material, a high contamination of the fluid whey or faults in the hygiene of equipment can allow the pathogen multiplication during the whey powder processing. The consequence of this is the obtaining of a product with marginal microbiological quality and with risks of vehicular staphylococcal enterotoxins. This pioneering study presented two possible conditions to occur in an industrial production line of whey powder and our results point to the need to control the raw material and adequate hygiene of the processing plant. Therefore, this study may serve as a basis for the identification of critical control points in quality assurance programs applied to whey industries.

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