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Controle de NSLAB na Linha de Produção de Lácteos Fermentados Através da Avaliação dos Protocolos do Sistema CIP

Control of NSLAB in a Fermented Dairy Products Production line Through the Evaluation of CIP System Protocols

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Resumo

Em uma indústria beneficiadora, foram observados em produtos lácteos fermentados defeitos como redução acelerada do pH durante a fermentação, perda de coloração e viscosidade, sabor amargo e formação de gás. A partir das contagens de bactérias láticas não iniciadoras (NSLAB) e de bactérias fermentadoras de citrato, decidiu-se por avaliar os protocolos de higienização (CIP-clean in place) dos equipamentos no setor de produtos lácteos fermentados. Para comparação da eficiência dos CIPs intermediário e completo, três pontos da produção foram avaliados através do monitoramento do pH: pasteurizador de base láctea (A); tanque de fermentação antes (B1) e após (B2) a adição da cultura láctea. Amostras foram coletadas após a realização das CIPs intermediário (amostra I) e completo (amostra II). Em seguida, as amostras foram coletadas após 12 horas de produção dos lácteos fermentados e antes do CIP completo (amostra III) e intermediário (amostra IV). Estas amostras, dos pontos A e B1, mostraram redução do pH em 1 escala em 8h e 6h depois do CIP intermediário e 9 h e 8h depois do CIP, respectivamente. Apesar do CIP completo ter se mostrado mais eficaz, sua eficiência não apresentou a duração necessária determinada pelo controle interno da indústria. Foi proposta uma alteração no processo de limpeza no ponto A, mantendo apenas o CIP completo a cada 10 horas e solução de limpeza alcalina em maior concentração (2,0 a 2,5%), o que acarretou adequação no tempo de fermentação dos produtos e ausência dos defeitos observados.

Palavras-chave: Higienização. Biofilme. Bactérias Ácido Lácticas. Defeitos Tecnológicos. pH.

Abstract

In a milk-processing industry, defects such as accelerated pH reduction during fermentation, loss of color and viscosity, bitter taste and gas formation were observed in fermented dairy products. Observing non-starter lactic acid bacteria (NSLAB) and citrate fermenting bacteria counts, it was decided to evaluate the hygiene protocols (CIP-clean in place) of the equipment in the fermented dairy products sector. To compare the efficiency of intermediate and complete CIPs, three checking points of fermented dairy products production were evaluated by monitoring the pH: milk pasteurizer (A), and fermentation tank before (B1) and after (B2) the addition of the dairy culture. Samples were collected after performing the intermediate (sample I) and complete (sample II) CIPs. Then, samples were collected after 12 hours of fermented dairy products production and before the complete (sample III) and intermediate (sample IV). These samples from point A and point B1, showed a reduction of 1 pH scale in a period of 8h and 6h after the intermediate CIP, and 9 h and 8h after the complete CIP, respectively. Although the full CIP proved to be more effective, its efficiency did not show the required duration determined by the internal control of the industry. A change was proposed in the cleaning process at point A, keeping only the complete CIP every 10 hours and using alkaline cleaning solution in higher concentration (2.0 to 2.5%), which led to the adequacy in the fermentation time of the products and in the absence of the observed defects.

Keywords: Hygiene. Biofilm. Lactic Acid Bacteria. Technological Defects. pH.

1 Introduction

Fermented milks have been an important component of nutrition and diet and there are numerous types of them manufactured in different parts of the world (SURONO; HOSONO, 2011). In Brazil, fermented milks can be added from other food ingredients and are obtained by coagulation and pH reduction of milk or reconstituted milk, added or not of other dairy products. Lactic fermentation is done using specific microorganisms (lactic acid bacteria - LAB) that must be viable, active, and abundant in the product during its shelf life (RAMON; SILVA, 2018; ZHENG *et al.*, 2020).

Among LAB, there is a group of bacteria that are not part of the starter culture and therefore, are called non-starter lactic acid bacteria (NSLAB). It is composed of four main groups of bacteria (mesophilic lactobacilli, pediococci, enterococci and Leuconostoc spp.) *(BERESFORD, 2003;* MCSWEENEY; FOX, 2004) and are associated with different technological and sensory characteristics desirable of dairy products, such as those present in different types of cheeses (WOUTERS *et al.*, 2002; PEREIRA *et al.*, 2020). However, these microorganisms may be present as a secondary microbiota that develops spontaneously in dairy products such as yogurts and cheeses (HANSEN, 2017). They come from milk, milking environment or industry; some of them

are thermoresistant, can form biofilms and resist cleaning and disinfection treatments, contaminating dairy products at the end of technological processing (SACCO, 2013; COSTA *et al.*, 2017; RAMON; SILVA, 2018;).

It is estimated that most defects in fermented dairy products are related to NSLAB (HANSEN, 2017). Slow fermentation at low temperatures, loss of viscosity and staining, syneresis and taste change are defects associated with the presence of NSLAB (FERREIRA, 2020; HANSEN, 2017; SACCO, 2013). Those NSLAB that are capable of fermenting citrate produce diacetyl, acetate, acetoin, and carbon dioxide, causing defects such as gas formation. Proteases and lipases mischaracterize the texture of dairy products, besides forming aromatic compounds that promote strange odors and bitter or very acidic flavors (COSTA *et al.*, 2017; MCSWEENEY; FOX, 2004).

Em uma indústria de laticínios da região de Londrina, PR, Brasil, problemas tecnológicos e sensoriais foram verificados nos produtos lácteos fermentados elaborados, além da presença de NSLAB e de bactérias fermentadoras de citrato em amostras previamente coletadas. Desta maneira, o objetivo deste trabalho foi avaliar a eficiência do sistema de limpeza e sanitização utilizado na empresa, em três diferentes pontos da linha de produção, após CIP intermediário e completo, através do monitoramento do pH de amostras coletadas.

In a dairy industry in Brazil, technological and sensory problems were verified in the fermented dairy products, in addition to the presence of NSLAB and citrate fermenting bacteria in previously collected samples. Thus, the objective of this work was to evaluate the efficiency of the cleaning and sanitization system used in the company, at three different points of the production line, after intermediate and complete CIPs, by monitoring the pH of samples collected.

2 Material and Methods

2.1 Initial analysis

In a milk-processing industry, technological defects associated with NSLAB contamination and already described in the literature (FERREIRA, 2020; HANSEN, 201SACCO, 20137;) as accelerated pH reduction during fermentation (from 4-5 hours to 3.5 hours), loss of color and viscosity, bitter taste, and gas formation in final products were observed in different batches of fermented dairy products.

The other physical, chemical, and microbiological parameters of fermented dairy products were controlled and were within the standards and limits determined by the quality control of the industry and by the competent supervisory body of the Ministry of Agriculture, Livestock and Supply.

The cleaning of the fermented dairy products production line in the dairy plant was carried out with the Clean in Place (CIP) system in two stages: a) intermediate CIP, for 40 minutes, consisting of pre-rinse, alkaline cleaning and rinse and b) complete CIP, with pre-rinse, alkaline cleaning, acid cleaning, sanitization, and rinse, lasting 1 hour and 20 minutes. Based on the technological problems reported, a pilot study (SOUZA, 2020) was conducted with the research of NSLAB (MRD diluent and MRS Agar/ anaerobiosis/ 22 °C/72 h) and citrate fermenting bacteria (Peptoned water diluent, Leesment agar/ anaerobiosis, 22 °C/72 h) in 10 samples of fermented dairy products (HANSEN, 2017). The mean counts of NSLAB and citrate fermenting bacteria were 8,5 x 10^7 CFU /g (min. 2 x 10^6 CFU /g and max. 3,7 x 10^8 CFU /g) and 2,4 x 10^8 CFU/g (min. 1,5 x 10^6 CFU /g and max. 3,3 x 10^8 CFU /g), respectively (SOUZA, 2020). Parameters for NSLAB and citrate fermenting bacteria in fermented dairy products do not exist in the literature, but cheeses produced with low populations of mesophilic microorganisms, coliforms, staphylococci and molds and yeasts have an initial NSLAB count of 10^2 CFU/g (COSTA *et al.*, 2017).

2.2 Fermented milk production

After the analyses for quality control of refrigerated raw milk were performed and the results were in accordance with the established standards (BRASIL, 2018), milk was pasteurized at temperatures from 72ºC to 75ºC for 15 to 20 seconds and stored to produce the fermented dairy products.

To produce fermented dairy products, a milk base is prepared, consisting of a mixture of milk, whey, starch, gelatin, sugar, and milk protein powder. This milk base is heated in plate pasteurizer (base pasteurizer), where it is heated from 87 °C to 95 °C for 6 minutes, followed by cooling between 40°C and 45°C. Then, this base is pumped through stainless steel pipe to the isothermal fermentation tank, where the addition of the dairy culture and the fermentation are carried out until pH 4.4 to 4.6 is reached, which occurs around 5 hours. After this step, fermented dairy products is pumped and textured through stainless steel pipe by a cooling plate, stored in lung tanks and added of fruit preparation. After complete homogenization, products are packed, accommodated in cardboard boxes, and stored in cold chambers with a maximum temperature of 10ºC for distribution.

2.3 Cleaning system

In the pasteurization equipment, the intermediate CIP (Table 1) was performed every 12 h and the complete CIP (Table 1), once a day, at the end of the process. In the pipes and fermentation tanks, a complete CIP was performed (Table 1) at the end of each process, ranging from 4 to 6 hours, according to the fermentation time of the product (protocol 1).

Table 1 - Stages of CIP (Clean in Place) (Protocol 1) of equipment to produce fermented dairy products

Milk Base Pasteurizer for Fermented Dairy Product (Protocol 1)		
Stages of intermediate CIP	**Time; Temperature; Solution concentration**	**Active Agent**
Pre-rinse	5 min/40 ºC	water recovered from the CIP system
Alkaline cleaning	30 min; 60 to 65ºC; 2,0-2,5%	Sodium hydroxide
Rinse	5 minutos; 90ºC	Clean water
Stages of complete CIP	**Time; Temperature; Solution concentration**	**Active Agent**
Pre-rinse	5 min/ 40ºC	water recovered from the CIP system
Alkaline cleaning	30 min; 75 to 85ºC; 2,0-2,5%	Sodium hydroxide
Acid cleaning	30 min 65 to 75ºC; 1,0-1,5%	Nitric acid
Sanitization	10 min; 25 to 40ºC 0,20-0,25%	Peracetic acid
Rinse	5 min; 90ºC	Clean water
Pipes and Fermentation Tanks of Fermented Dairy Products (Protocol 1)		
Pre-rinse	5 min/40 ºC	water recovered from the CIP system
Alkaline cleaning	30 min; 80 to 85ºC 1,5-2,0%	Sodium hydroxide
Acid cleaning	20 min; 60 to 65ºC; 1,0-1,5%	Nitric acid
Sanitization	10 min; 25 to 40ºC; 0,20-0,25%	Peracetic acid
Rinse	5 min; 90ºC	Clean water

Source: resource data.

2.4 Sample collection points

To compare the efficiency of intermediate and complete hygiene processes (CIP) (Protocol 1), three checking points (A, B1 and B2) (Figure 1) of the fermented dairy products production process were evaluated. To verify the efficiency of the CIP of the pasteurization system, pasteurized milk base (A) samples were collected in the milk base pasteurizer. In the fermentation tank, samples were collected before (B1) and after (B2) the addition of the dairy culture. The samples from point B1 aimed to evaluate the efficiency of the cleaning of the pipes, from the pasteurizer outlet to the fermentation silo; B2 samples aimed to evaluate the fermentation time of the milk base.

The samples from the three checking points of the fermented dairy products producing line were collected in duplicates and at 4 different times. Initially, samples were collected after performing the intermediate (sample I) and complete (sample II) CIPs, and after 10 minutes of the heating process. Then, samples were collected after 12 hours of fermented dairy products production and before the complete (sample III) and intermediate (sample IV) CIPs. All samples were collected in sterile plastic bottles (200 mL) from equipment collection taps.

Figure 1 - Sampling points of the fermented dairy production line: Point A: milk base pasteurizer; Point B: fermentation tank of the milk base (before and after culture addition)

Point A Points B1 and B2

Source: the authors.

2.5 Evaluation of CIP system efficiency

To evaluate the efficiency of the CIP system, the pH of the samples (I to IV) collected at the three checking points (A, B1 and B2) of the fermented dairy products production process was performed. For each analysis, 10 ml of the collected samples were used, which were kept in a test tube with screw cap, in a water bath at 37 °C for 24 hours (HANSEN, 2017). In the first 6 hours, the pH was determined every 30 minutes and after this period, every 1 hour. The analyses were performed in duplicate using a digital potentiometer (AKSO instrumentos, model MP511) (AOAC, 2005).

For the industry's internal quality standard to be reached, the pH of the samples collected between two CIPs at points A and B1 must be reduced by one logarithmic scale in a minimum period of 12 hours; B2 samples should have their pH reduced by two scales in the same period. If this time is respected, it indicates that CIP cleaning process parameters (time, concentration, and temperature) are being efficient. As for the fermentation process of the fermented dairy products, the time predicted and established by the dairy industry was 4 to 5 h. Thus, it was guaranteed a final product with physical, microbiological, and sensory characteristics within legal limits and quality.

3 Results and Discussion

Samples (I to IV) from milk base pasteurizer (point A) and fermentation tank of the milk base before culture addition (point B1), showed a reduction of 1 pH scale in a period of 8h and 6h after the intermediate CIP, and 9 h and 8h after the complete CIP, respectively. Although the complete CIP showed better efficiency, the time determined by the internal control of the industry (12 hours) was not reached. Frequency, chemical dosages, and recommended time for CIP become key measures to prevent the presence of NSLAB (LATEC INGREDIENTES, 2020). The storage of cold milk for long periods benefits the growth of NSLAB, which can adhere to the surface of the equipment, hindering the recommended sanitization processes (HANSEN, 2017; FERREIRA, 2020).

In view of the results obtained, a change was proposed in the cleaning process at milk base pasteurizer (point A) (Protocol 2) maintaining only the complete CIP, which began to be performed every 10 hours and using alkaline cleaning solution in higher concentration (2.0 to 2.5%). The pumping speed of the solutions was 25 cubic meters/hour in 2-inch pipes.

The difference between the protocols (P1 and P2) was limited to the time between the cleaning processes and the concentration of the alkaline solution. The speed of the solutions circulation and the design of the pipes have not changed.

In points fermentation tank of the milk base before (B1) and after culture addition (B2), where cleaning was performed most frequently (between 4 and 6 h), the only change needed was a new training of employees to ensure the hygiene efficiency of these equipment, emphasizing the importance of correct performing the CIP, time, temperature, and concentration of the cleaning solutions used.

After the change of the cleaning protocol, new samples were collected, and pH monitoring was performed again as described in item 2.4.

The comparison of cleaning and sanitization efficiency between protocol 1 (P1) and protocol 2 (P2) was performed at milk base pasteurizer (point A) and fermentation tank of the milk base (before and after culture addition) (points B1 e B2), with sample collection immediately after the end of the complete CIP (sample 1) and after 12 hours of production (sample 2).

There was an increase of 1 hour in the reduction of pH in 1.0 scale, comparing samples 1 in milk base pasteurizer (point A), from 9 h (P1) to 10 h (P2) (Table 2). At this same point, the reduction of 1 pH scale was 8 h in samples 2, for both P1 and P2. This result showed an increase of 1h in the initial efficiency of P2, but without changing the final time of the efficiency of the cleaning and sanitization process.

Table 2 - Monitoring the pH of samples (1 and 2) collected in the pasteurizer (point A) between the cleaning processes (CIP) - Protocols 1 and 2**

Point A (Milk base pasteurizer)	**CIP Protocols****	**pH reduction time (h) on one logarithmic scale**
Sample 1*	1	9:00
	2	10:00
Sample 2*	1	8:00
	2	8:00

* Sample collection immediately after the end of the complete CIP (sample 1) and after 12 hours of production (sample 2). ** difference between P1 and P2: time between the cleaning processes and the concentration of the alkaline solution.
Source: resource data.

Comparing the cleaning efficiency of fermentation tank of the milk base before culture addition (point B1), using samples 1, the time for 1.0 scale pH reduction was 8 h in P1 and 9 h in P2. Observing samples 2, the reduction of 1.0 pH scale occurred in 6h and 9h in the P1 and P2 protocols, respectively. Thus, P2 had higher efficiency at point B1, showing a reduction of 1h in the initial efficiency of P2 and 3 h in the final time (Table 3).

Table 3 - Monitoring the pH of samples (1 and 2) collected in the fermentation tank before (B1) and after (B2) culture addition, between the cleaning processes (CIP) - Protocols 1 and 2**

Point B1 (Fermentation tank before culture addition)	**CIP Protocols**	**pH reduction time (h) on one logarithmic scale**
Sample 1*	1	8:00
	2	9:00
Sample 2*	1	6:00
	2	9:00

Point B2 (Fermentation tank after culture addition)	**CIP Protocols**	**pH reduction time (h) on two logarithmic scale**
Sample 1*	1	4:00
	2	7:00
Sample 2*	1	3:30
	2	5:30

* Sample collection immediately after the end of the complete CIP (sample 1) and after 12 hours of production (sample 2). ** difference between P1 and P2: time between the cleaning processes and the concentration of the alkaline solution.
Source: resource data.

At point B2 (fermentation tank of the milk base after culture addition), samples 1 showed a reduction of 2.0 pH scales in 4 h and 7h in P1 and P2, respectively. Samples 2, already considered fermented dairy products, showed 2.0 scale pH reduction in 3h30min in P1 and 5h30min in P2 (Table 3). The improvement of fermentation time can be associated with the reduction of NSLAB, since the growth kinetics of these microorganisms are opposite to those of starter dairy cultures. NSLAB can use alternative sources of nutrients and energy from lactic acid, citric acid, fatty acid, glycerol and peptides and amino acids derived from starter culture metabolism (MONTEL *et al.*, 2014; LUNARDI *et al.*, 2021)

4 Conclusion

After the changes in the cleaning and sanitization protocols, there was adequacy in the fermentation time of fermented dairy products, and the defects associated with the presence of NSLAB were no longer observed. Thus, it is possible to relate the equipment of the production line of fermented dairy products as a probable source of contamination by NSLAB.

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