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Effectiveness of enzymatic treatment for reducing dairy fouling at pilot-plant scale under real cleaning conditions

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ABSTRACT

Conventional cleaning with chemical products uses a substantial amount of water and energy to clean the fouling related to dairy production, resulting in significant economic costs. We evaluated an enzymatic treatment based on the use of protease and amylase to clean dairy fouling generated in an indirect plate heat exchanger and the spray dryer equipment of a pilot plant, representing real cleaning conditions in the dairy industry. The efficacy of the enzymatic treatment in removing fouling at 50 °C was comparable to that of the clean-in-place method, with alkaline–acid cleaning performed at a maximum temperature of 80 °C. Microbiological analysis showed that the cleaning treatments guaranteed adequate hygienic conditions of the dairy products manufactured. Monitoring fluorescence markers, such as tryptophan, riboflavin, Maillard compounds, and dityrosine could help improve the effectiveness of both alkaline and enzymatic cleaning. The enzymatic treatment fulfills dairy industry objectives, saving water and energy during washing by reducing chemical product use. Considering that enzymatic cleaning is biodegradable after use and that its economic cost is competitive compared to chemical cleaning, it represents a viable alternative to the chemical cleaning of dairy fouling.

1. Introduction

Every day, the dairy industry processes large quantities of milk for producing dairy products, increasing year on year (Eurostat, 2020). During pasteurization, milk is treated at high temperatures to preserve its quality and to keep it free of pathogens and most spoilage-causing microorganisms. Plate heat exchangers (PHEs) are a common thermal equipment used in the dairy industry for pasteurizing milk. The complex biological composition of milk means that when heated it forms considerable unwanted adhesion of deposits within the PHE, termed dairy fouling (De Jong, 1997; Wallhäußer, Hussein, & Becker, 2012a, 2012b). Other equipment, such as spray dryers, are also affected by fouling formation when hot air is used in the production of powdered milk. The presence of fouling in dairy plants is a major problem because of subsequent undesirable effects. The main effect of the formation of dairy fouling is the coating of the internal surfaces of pipelines, which increases their internal pressure and causes obstructions in the circulation of milk (Sadeghinezhad et al., 2015). This issue results in poor heat transmission due to the insulating properties of the dairy fouling, and it becomes necessary to apply more heat to the thermal process; thus, higher surface temperatures during heat treatment of milk result in more fouling (Bansal & Chen, 2006; Barish & Goddard, 2013; Hagsten et al., 2016). In addition to the operational effects, there are also health effects. When the cleaning procedures are not effective, the continuous presence of fouling can result in bacterial colonization and the formation of biofilms with high resistance to cleaning procedures (Brooks & Flint, 2008; González-Rivas, Ripolles-Avila, Fontecha-Umaña, Ríos-Castillo, & Rodríguez-Jerez, 2018). This implies a reduction in the quality of milk or other processed foods that may result in cross-contamination, affecting consumer health (Oliver, Jayarao, & Almeida, 2005).

Cleaning and disinfection involve the elimination of different types of microorganisms and the removal of fouling on food contact surfaces

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(Boyce, Piterina, & Walsh, 2010a, 2010b; Ríos-Castillo, González-Rivas, & Rodríguez-Jerez, 2017). In the dairy industry, cleaning-in-place (CIP) is a widely used cleaning and disinfection method for dairy product safety and for restoring PHE characteristics after production. A typical CIP cycle consists of a pre-rinse step with hot water, followed by an alkaline and acid step, and a final rinse step. The presence of fouling and its consequent cleaning presents immense economic loss because of the use of cleaners, increased energy consumption, and the periods of downtime. In addition, the cleaning time is usually longer than is strictly necessary due to the lack of reliable monitoring tools (Van Asselt, Van Houwelingen, & Te Giffel, 2002). All these economic expenses related to the cleaning of fouling are estimated to account for 80% of total production costs (Bansal & Chen, 2006). In addition, the chemical cleaner residues are corrosive, toxic, and non-biodegradable, and their prolonged use has an impact on the environment. Thus, the cleaning of equipment represents a challenge for dairy plants.

Enzymatic formulations with proteolytic activity are alternatives to chemical agents for removing fouling (Graβhoff, 2002; Lequette, Boels, Clarisse, & Faille, 2010a, 2010b). Among the benefits of using enzymes in cleaning are the fact that they are biodegradable, they operate with less energy and water consumption, and they generate less residues (Boyce et al., 2010a, 2010b; Guerrero-Navarro, Ríos-Castillo, Ripolles-Avila, Felipe, & Rodríguez-Jerez, 2020). Enzymes such as lipases and proteases have been studied as cleaning agents for fouling at laboratory scale, showing high efficiency (Allie, Jacobs, Maartens, & Swart, 2003). Moreover, the increased production of enzymes on a large scale has reduced the cost of enzyme products with cleaning properties to a competitive level (Petrus, Li, Chen, & Norazman, 2008). In contrast to laboratory-scale enzyme cleaning studies, the activity of enzymes under real conditions of use is not particularly accurate. The objective of the present study was to evaluate an enzymatic cleaning treatment for eliminating the dairy fouling formed in the production of pasteurized milk and milk powder, to be applied under real conditions of use in the dairy industry. The study was carried out in an experimental dairy production plant, where we monitored the cleaning process, performed the microbiological analysis, and compared the efficacy of the enzymatic and chemical treatments.

2. Materials and methods

2.1. Location

The study was performed at the pilot plant of the Institute of Agrifood Research and Technology (IRTA) experimental processing plant (Monells, Spain). A thermal system using an indirect PHE (HTST Pasteurizer. Inoxpa S.A.U., Banyoles, Spain) was used for the thermal heat treatment of milk. The PHE thermal treatment section had 18 plates and a total surface of 9 m^2. Raw liquid bovine whole milk refrigerated at 5 °C was used to test the cleaning of dairy fouling following milk pasteurization in the PHE and the production of powdered milk in the spray dryer. A fluidized spray dryer Niro FSD-6.3 (GEA, Alcobendas, Spain) was used to powder the milk. The spray dryer has a feed rate of 45 L/h (45% w/w total solid content of the product), inlet air temperature of 180 °C and outlet air temperature of 75 °C. A GEA-Niro pilot single effect falling-film evaporator composed of 25 evaporation tubes was used before the spray dryer to concentrate the milk. Dairy farms in the Baix Empordà region (Girona, Spain) supplied the whole milk (36.2 ± 0.98 g/L of fats, 33.5 ± 1.17 g/L of proteins, 55.31 ± 2.06 g/L of sugars and 124.8 ± 2.3 g/L of total solids). The IRTA plant has a 400 m^2 functional surface area for equipment and complies with food safety standards, according to European Union legislation.

2.2. Experimental conditions

The level of effectiveness to clean fouling using an enzymatic cleaning solution compared with CIP using chemical cleaners was evaluated. The dairy fouling cleaning also included microbiological analysis, detection of fluorescence markers, and the identification of components of fouling adhered in the PHE and the spray dryer once the cleaning had been completed. In addition, we determined the economic costs of the cleaning treatments. The experiments were performed using 4000 L milk, of which 3600 L was used in the PHE, and 400 L was used in the spray dryer. The raw milk pasteurization was performed to work at 110 °C for 15 min, at a rate of 600 L/h. For each cleaning treatment, dairy fouling derived from the spray drying was obtained after 40 min using 100 L of milk to produce milk powder.

Table 1
Chemical and enzymatic cleaning treatments performed in the plate heat exchanger and spray dryer.

	Chemical	Enzymatic
PHE	**Pre-rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Alkaline step:** 3% Brio Complex (NaOH and phosphonates) Duration: 20 min, 80 °C, 500 L/h. **Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Acid step:** 1% Acimix CIP (nitric acid) Duration: 20 min, 50 °C, 500 L/h. **Rinse** Duration: 20 min, 20–22 °C, 500 L tap water/h.	**Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Enzymatic cleaning:** 1% (3% Savinase + 3.3% Termamyl Ultra 300 L, diluted in 3% buffer solution). Duration: 20 min, 50 °C, 500 L/h. **Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h.
Spray dryer	**Pre-rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Alkaline step:** 3% Jet Foam (H_2O_2 and surfactants). Duration: 20 min, 80 °C, 500 L/h. **Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Acid step:** 1% Acid Jet (ortophosphoric acid, surfactants) Duration: 20 min, 80 °C, 500 L/h. **Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h.	**Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h. **Enzymatic cleaning:** 1% (3% Savinase + 3.3% Termamyl Ultra 300 L, diluted in 3% buffer solution) + alkaline foaming detergent. Duration: 20 min, 50 °C, 500 L/h. **Rinse:** Duration: 20 min, 20–22 °C, 500 L tap water/h.

2.3. Cleaning procedures

The chemical and enzymatic cleaning treatments were carried out once the processes in the PHE and the spray dryer had been completed. A schematic procedure of the two treatments is described in Table 1.

2.3.1. Chemical cleaning

The chemical treatment of the PHE, based on the previous description by Boyce et al. (2010a, 2010b) and Lyndgaard, Rasmussen, Engelsen, Thaysen, and van den Berg (2014), was carried out in two steps: a first-step treatment with an alkaline product (5–15% [v/v] sodium hydroxide, phosphonates, pH 13.0 ± 1.0 at 1%; 1.41 ± 0.02 g/cm^3 density at 20 °C) (Brio Complex; iTram Higiene, Vic, Spain). During this stage, the CIP process began with a pre-rinse using 200 L tap water for 20 min with a flow rate of 500 L/h at room temperature (20–22 °C). This rinse removes substances that are not highly adhered to surfaces. Similarly, any soluble substance derived from milk thermal processing is eliminated. Alkaline cleaning was then carried out using Brio Complex diluted to 3% v/v in 200 L tap water in recirculation (500 L/h) for 20 min at 80 °C. Once the alkaline cleaning process had been completed, a water rinse (500 L/h) was performed to remove solubilized residues

resulting from this step. Next, the acid stage consisted of cleaning using a 1% v/v acid product (42% nitric acid, pH 1.1 ± 0.5 at 100%, 1.3 ± 0.02 g/cm^3 density at 20 °C) (Acimix CIP; iTram Higiene, Spain) in 200 L recirculated water at 50 °C for 20 min. Last, after this step, a water rinse was performed.

The chemical cleaning of the spray dryer was performed with a pre-rinse with tap water at room temperature, followed by the use of 3% v/v alkaline product (3–5% hydrogen peroxide and ethoxylated alcohols, < 5% phosphonates, < 5% anionic surfactants, < 5% nonionic surfactants; pH 13.5 ± 1.0; 1.075 ± 0.025 g/cm^3 density at 20 °C) (Jet Foam; iTram Higiene, Spain) performed at 500 L/h. The treatment was carried out at 80 °C for 20 min. Subsequently, a second 20-min rinse was performed, and a 1% v/v acid product (34% orthophosphoric acid, < 5% nonionic surfactants; pH 2.25 ± 0.5 at 1%; 1.2 ± 0.025 g/cm^3 density at 20 °C) (Acid Jet; iTram Higiene, Spain) was used, using tap water for 20 min at 50 °C. Last, a 20-min rinse was performed.

2.3.2. Enzymatic cleaning

The enzymatic cleaning in the PHE was carried out with a formulation patented by Rodríguez-Jerez, Ríos-Castillo, and Guerrero-Navarro (2020), which consists of protease (3.0% [v/v] Savinase® 16 L [16 KNPU–S/g]; Novozymes A/Z, Bagsværd, Denmark) and alpha-amylase (3.2% [v/v] Termamyl® Ultra 300 L [300 KNU/g]; Novozymes A/Z), diluted in 3% (v/v) buffer solution (nonylphenoxy poly[ethyleneoxy)] ethanol 15EO [10.0% v/v]). The pre-rinse was performed with a flow rate of 500 L/h using tap water at room temperature for 20 min to remove the soluble milk components. Subsequently, a 1% (v/v) enzymatic formulation in 200 L of tap water in recirculation at 500 L/h and 50 °C for 20 min was used. Last, a 20-min rinse with tap water was performed at room temperature. The enzymatic cleaning of the spray dryer was preceded by a first 20-min rinse step with tap water at room temperature. Next, a 1% (v/v) enzymatic formula was applied, adding an alkaline foaming detergent (1% v/v) to produce foam and increase the contact time of the enzymes with the surfaces. The enzymatic treatment was applied for 20 min at 50 °C. Last, a 20-min rinse with tap water was performed at room temperature.

2.3.3. Pre-cleaning of plates before thermal treatment and cleaning

Before each cleaning treatment, the PHE unit was opened, separating all the plates, and removing all the incrustations formed. We used a Kärcher unit (HD 10/15-4 Cage Food) (Kärcher España, Granollers, Barcelona, Spain) with microfiber tubular browsers (Karcher tubular browser 500) (Kärcher España, Granollers, Barcelona, Spain). The cleaning temperature of the water was 60 °C. Consequently, before each heat treatment, the PHE was thoroughly cleaned, and any organic residue present on the inner surface of each plate was removed. The level of organic matter in the internal surface of each plate was the same before each treatment.

2.4. Cleaning analysis

2.4.1. Dry residues

Once the chemical and enzymatic cleaning had been completed, 100 mL of each cleaning solution were collected in duplicate from the PHE system and spray dryer, and dried. Drying was performed using 20 mL of each sample in an IDL–FI–80 forced air oven (Labolan, SL, Esparzar de Galar, Spain) at 90 °C for 1–1.5 h. Next, the dried residues from each treatment were weighed on a Mettler AE100 analytical balance (Mettler-Toledo S.A.E., Hospitalet del Llobregat, Spain). The average density of the cleaning products was subsequently discarded, obtaining the amount of milk fouling eliminated during the cleaning. The results were expressed in g/L.

2.4.2. Fouling components

The samples to analyze the milk fouling components were obtained from the PHE, once the cleaning stages were completed. The composition of proteins, lipids, and sugars was identified using a direct epifluorescence microscope (DEM) with three selective fluorescent dyes (fluorescein isothiocyanate [FITC], Nile red [NR], and concanavalin A-Alexa Fluor 350 [ConA-350]). This mixture of dyes was used because it has previously been used for dairy fouling samples (Guerrero-Navarro et al., 2020). Before the analysis, all dyes were mixed homogeneously in 60 μL 0.1 M sodium bicarbonate (Panreac, Castellar del Vallès, Spain) to avoid dimerization of the ConA-350 dye. In addition, the fouling residue samples were analyzed with near-infrared spectrometry (NIRS; NIR 5000, FOSS-NIR Systems Inc., Silver Springs, MD, USA) at 1100–2500 nm wavelength. Additionally, the samples were carbonized in an oven at 550 °C for 5 h to determine the mineral fraction.

2.4.3. Microbiological analysis

Microbiological analysis of the cleaning was performed after each cleaning treatment using the water used for the cleaning rinses and the whole milk used for the tests. For this step, 10 mL of each sample was transferred to 90 mL buffered peptone water (bioMérieux, Marcy-l'Étoile, France). The samples were then homogenized on a shaker for 30 s, and serial dilutions were made using buffered peptone water (bioMérieux, France). From each dilution, Petri dishes were cultured using five types of culture media: (1) Total viable count (TVC), using the automated TEMPO® system (bioMérieux, France), incubated at 30 °C for 48 h (2) Lactic acid bacteria count (LAB) using de Man-Rogosa-Sharpe agar (MRS; Oxoid, England), incubated at 30 °C for 48 h (3) *Staphylococcus aureus* count using Baird-Parker agar with rabbit plasma fibrinogen supplement (BP-RPF; Oxoid, England), incubated at 37 °C for 24 h (4) Enterobacteria count (EC) using violet red bile glucose agar (VRBGA; Oxoid, England), incubated at 37 °C for 24 h (5) Yeast and molds (Y&M), cultured in Sabouraud (SAB) medium with dextrose and chloramphenicol (bioMérieux, France), incubated at 20 °C for 120 h.

2.4.4. Cleaning monitoring

The monitoring of the fluorescent markers during the cleaning of the spray dryer was carried out by taking samples of the water used, the cleaning solutions, and the rinse waters (Guerrero-Navarro et al., 2020). The fluorescent analysis was performed with a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Madrid, Spain) coupled with a 15 W xenon lamp and a front-face geometry accessory (Agilent Technologies). The analysis was performed at angles of incidence of 90°, and at 35° to analyze thinly diluted samples, using Suprasil® quartz cuvettes (UV fluorescence cell, Agilent Technologies). Quantification of four markers whose fluorescence response depends on the heat treatment of milk (Ayala, Zamora, González, Saldo, & Castillo, 2017a, 2017b) was performed with standard curves and front-face fluorescence of: (1) Dityrosine (Dt), detected at 315 nm excitation wavelength and an emission wavelength of 350–500 nm. (2) Maillard compounds (MC), captured with an excitation wavelength of 330 nm and an emission wavelength of 350–500 nm. (3) Tryptophan fluorescence (L-Trp), obtained with an excitation wavelength of 290 nm and an emission wavelength of 300–450 nm. (4) Riboflavin (Rbf), detected with an excitation wavelength of 450 nm and an emission wavelength of 470–570 nm.

2.5. Cost of cleaning treatments

The average economic costs each cleaning treatment in the year 2020 were calculated in euros (€) based on the manufacturing prices provided by the manufacturer.

2.6. Statistical analysis

The chemical and enzymatic cleaning of the PHE and spray dryer were carried out twice, on separate days. The sampling of each type of cleaning was performed by taking six aliquot samples of cleaning solutions and rinse waters. All the data collected from the cleaning

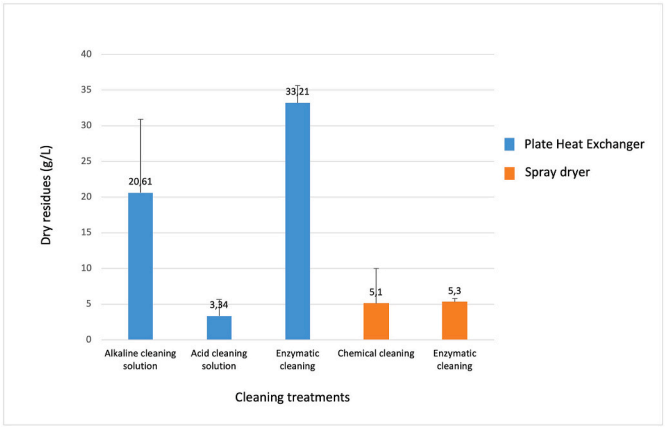

Fig. 1. Amount of dairy fouling (g/L) removed in each treatment.

treatments were processed using R free software (R Development Core Team). The comparison between the cleaning treatments was performed with the unpaired T-test. A p-value ≤ 0.05 was considered statistically significant.

3. Results and discussion

3.1. Fouling cleaning residues

The results of the dry residues during the cleaning of the dairy fouling are shown in Fig. 1. The dry residue value of the tap water used during cleaning was 0.59 g/L. The dry residues from the cleaning solutions analyzed for the PHE were calculated as 23.95 g/L for the chemical treatment, of which 20.61 g/L was obtained during the alkaline cleaning step and 3.3 g/L during the acid step. By contrast, the efficacy of the enzymatic solution for cleaning the dairy fouling showed an increase of up to 33.21 g/L, which was statistically significant ($p < 0.05$) compared to the chemical treatment. However, the results for the dry residues from the spray dryer did not show statistical differences ($p > 0.05$) between the enzymatic treatment (5.30 g/L) and the chemical treatment (5.10 g/L).

Chemical cleaning has high efficiency because the alkaline agents saponifies fats and degrades fouling proteins, dissolving the residues adhered to the surfaces (Ogunbiyi, Miles, & Hilal, 2008). In the present study, sodium hydroxide was used in the alkaline product because of its high pH. Besides, the acid step performs a descaling function, especially on the mineral elements adhered to the surfaces (Graβhoff, 2002). Here, we found that the enzymatic solution cleaned a greater amount of milk fouling than the two-stage chemical method. This may be because the protease and amylase in the enzymatic solution act together to dissolve and degrade the milk organic components. Proteases and amylases are the first and second most used enzymes in the formulation of detergents with enzymatic properties (Mitidieri, Souza Martinelli, Schrank, & Vainstein, 2006). The use of proteases has demonstrated cleaning benefits for the detergent industry. Boyce and Walsh (2012) evaluated fungal-origin proteases on a laboratory scale to determine their potential suitability for cleaning in the dairy industry. Using these proteases achieved adequate industrial-like milk fouling cleaning of stainless-steel surfaces at 40 °C, replacing caustic-based cleaning solutions that act at 70–80 °C. Amylase has been reported in the literature to improve cleaning procedures. In the case of our study, Termamyl® 300 L is a commercial heat-stable alpha-amylase produced by *Bacillus licheniformis* used to hydrolyze glycosidic linkages, which cleaves starch at 96 °C (Allala et al., 2019; Marasca, Boulos, & Nyström, 2020). In a laboratory-scale study, Guerrero-Navarro, Ríos-Castillo, Ripolles-Avila, Hascoët, Felipe, and Rodríguez-Jerez (2019) observed that a formulation based on proteases and alpha-amylase (Termamyl®) acting at 55 °C had 75% efficiency in cleaning milk fouling. Likewise, the authors observed that enzymatic cleaning has comparable efficiency to chemical cleaning for eliminating fouling. Besides, Ripolles-Avila, Ríos-Castillo, Fontecha-Umaña, and Rodríguez-Jerez (2020) studied the efficacy of an enzymatic formulation based on protease (5.0%), lipase (0.5%), and amylase (2.5%) as a cleaning treatment to prevent the presence of biofilms, reporting 34.48–37.5% biofilm detachment when the formula was tested on stainless steel surfaces.

In dairy plants, the principle of the spray drying process performed in a drying chamber is the atomization of dairy concentrate in a stream of heated air, followed by dehydration, yielding milk powder with a low water content (Moejes & van Boxtel, 2017). Although the cleaning of the spray dryer atomizer can be more efficient because its ease of access during maintenance allows a deeper clean than for the PHE system, it has been observed that the accumulation of fouling in this equipment could mean a decrease in the quality of the processed foods (Goula & Adamopoulos, 2004). In the case of powdered milk production, cleaning of the dryers by CIP represents 10–26% of the energy costs during this stage (Ramírez, Patel, & Blok, 2006). Our results show that the enzymatic formulation could be an alternative to chemical products under real conditions of use for CIP of thermal equipment for pasteurizing milk and producing powdered milk. Graβhoff (2002) demonstrated the efficacy of Savinase® for cleaning a milk pasteurizer, with an optimal use temperature of 50 °C, followed by a 15-min acid wash. Our results show efficacy at 50 °C under real conditions to reduce dairy fouling. These results are comparable with those observed by Guerrero-Navarro, Ríos-Castillo, Ripolles-Avila, et al. (2019), who tested the effectiveness of an

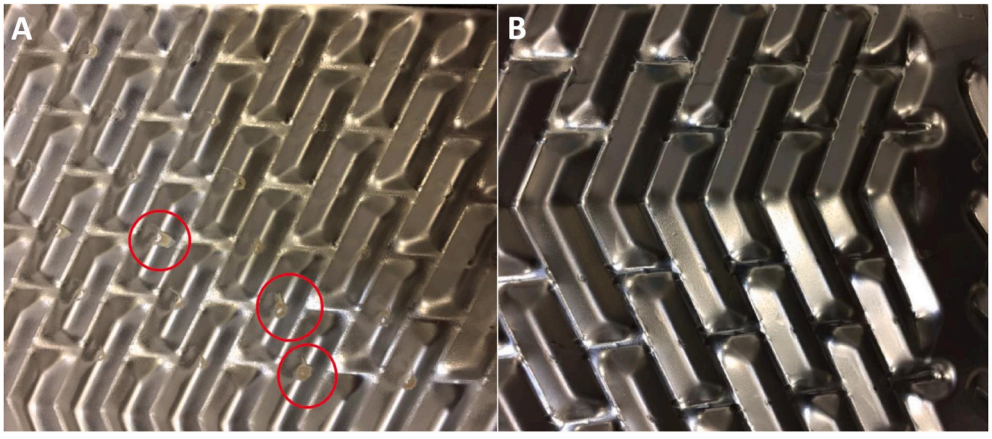

Fig. 2. Photographs of PHE after enzymatic cleaning. Red circles indicate fouling residues adhered to stainless-steel surfaces (A) and cleaned surfaces (B). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

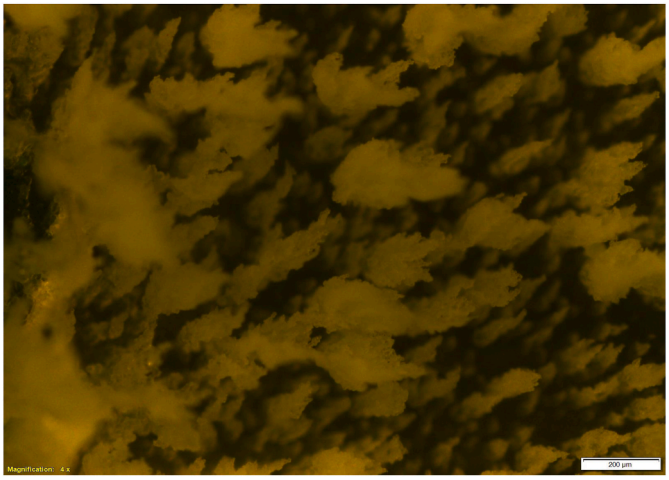

Fig. 3. Dairy fouling obtained after enzymatic treatment from PHE: (A) dried residues with a soft, white appearance. Scale bar: 10 cm. (B) Direct epifluorescence microscopy image of fouling stained with fluorescein isothiocyanate [FITC], Nile red [NR], and concanavalin A-Alexa Fluor 350 [ConA-350]), visualized in the green channel. Protein components in yellow-greenish coloration. Scale bar: 200 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Composition of the residual fouling layer from PHE after enzymatic cleaning obtained from NIRS and mineral fraction analysis.

Component	Content (% w/w) ± standard deviation
Mineral	56.6 ± 3.7
Protein	23.1 ± 1.6
Fats	15.4 ± 1.9
Water	5.0 ± 0.5
Total	100.0

enzymatic solution at the same temperature to clean milk fouling developed under laboratory conditions. In addition, due to the reduction in rinses and washing times when using the enzymatic cleaning, water consumption and washing temperature are also decreased, consequently saving energy compared to chemical cleaning.

3.2. Fouling analysis

After the cleaning treatments, the PHEs were opened, the plates through which the heat-treated milk flowed separated, and the milk fouling removal determined. The enzymatic cleaning eliminated 85–95% of fouling. The presence of fouling was particularly evident in the areas of the plates where the temperature increased the most during the thermal pasteurization (Fig. 2A) and was soft and whitish in color (Fig. 3A). The other areas did not present adhered fouling (Fig. 2B). The remaining fouling was extracted, and NIRS (organic fraction) and carbonization (mineral fraction) evaluation of its composition showed a high percentage of mineral and protein contents (Table 2). Microscopy also revealed a high protein content (Fig. 3B). Our results agree with those of Visser and Jeurnink (1997), who indicated that proteins and minerals are the main components of the fouling produced during the heat treatment of milk. Guerrero-Navarro et al. (2020) showed that, under laboratory conditions, the proportion of protein analyzed by confocal laser scanning microscopy (CLSM) was 39.4% prior to a cleaning treatment. Although in our study we did not initially analyze the protein content of the fouling, once the enzymatic cleaning was completed under real conditions, we found a low protein content (23.1%). By contrast, a higher mineral content was found (56.6%). The enzymatic cleaning of proteins can be attributed to the proteolytic

Table 3
Microbiological analysis of milk and rinse waters from chemical and enzymatic treatments performed in the PHE and spray dryer processes (CFU/mL[a]).

Process	Sample	Total viable count	Lactic acid bacteria	Enterobacteria	Yeast and molds
PHE	Raw milk	3.2×10^5	1.9×10^3	4.0×10^3	2.6×10^3
	Pasteurized milk	2.4×10^4	0	0	1.6×10^3
	Post-alkaline rinse water	1.0×10^0	0	0	0
	Post-acid rinse water	3.4×10^1	0	0	0
	Post-enzymatic rinse water	1.0×10^0	0	0	1.0×10^1
Spray dryer	Powder milk	2.9×10^2	0	0	0
	Powder milk fouling	1.0×10^2	0	0	0
	Post-alkaline rinse water	4.0×10^0	0	0	0
	Post-acid rinse water	2.0×10^0	0	0	0
	Post-enzymatic rinse water	0	0	0	1.0×10^1

[a] Mean values expressed as CFU/mL; n = 4 for every item of data.

action of savinase®, which is an alkaline protease that has an adequate proteolytic activity at 50 °C and under most washing conditions (Haggag, El-Sayed, & Allam, 2007; Nazari, Montazer, Afzali, & Sheibani, 2014). Although the enzymatic activity does not have a direct effect on the mineral composition, the mineral cleaning may be favored when removed together with proteins such as casein during the enzymatic cleaning without increasing the cleaning temperature (50 °C). Otherwise, with a heat treatment at a higher temperature, the adherence of minerals to the equipment would have been higher, resulting in a greater energy consumption to remove it (Hagsten et al., 2016; Visser & Jeurnink, 1997). Therefore, the protein composition of milk can also be used to eliminate compounds such as minerals. In this sense, it should be noted that a high content of mineral and macromineral elements, such as calcium phosphate, calcium phosphocaseinate, and magnesium, are associated with milk proteins (Gaucheron, 2005; Regnault, Dumay, & Cheftel, 2006; Sola-Larrañaga & Navarro-Blasco, 2009). Considering the effectiveness of enzymatic cleaning, it could either be used as a sole cleaning treatment or it could be complemented with acid CIP treatments to guarantee the total elimination of possible mineral residues. Nevertheless, the different characteristics and operating conditions of the pasteurization and spray drying equipment must be considered.

3.3. Microbiological analysis

As expected, the microbiological analysis of the pasteurized milk showed that the highest microbial load was obtained from raw milk (Table 3). By contrast, *S. aureus* was not detected in raw milk or in any cleaning solutions and rinse waters (data not shown). After pasteurization, LAB and EC were not detected, the presence of TVC was reduced, and the Y&M count remained at 10^1 CFU/mL. During the alkaline and acid cleaning steps, no microbiological load was detected, possibly due to the effect of the cleaning products during washing, although the post-treatment rinse showed TVC counts that were higher with the acid step compared to the alkaline one. This observation may have been due to the presence of microorganisms adhered to the surfaces prior to cleaning or from milk in a state which, due to the effect of chemical treatments, was viable but non-culturable (VBNC) (Ayrapetyan & Oliver, 2016), and were only detected from rinses after chemical treatments. In this regard, an adequate post-cleaning rinse is essential to eliminate the injured

Table 4
Concentrations of fluorescence markers in the different stages of spray dryer cleaning.

Sample	Concentration[a] ± standard deviation			
	L-Trp (mg/L)	Rbf (mg/L)	MC (mL/L)	Dt (mL/L)
Tap water	0.05 ± 0.04	0.00	0.25 ± 0.05	0.23 ± 0.06
Chemical cleaning				
Pre-rinse	0.06 ± 0.04	1.02 ± 0.50	0.30 ± 0.10	0.30 ± 0.10
Alkaline solution	0.22 ± 0.10	2.36 ± 1.00	0.76 ± 0.30	0.97 ± 0.40
Rinse after alkaline treatment	0.05 ± 0.04	0.37 ± 0.04	0.28 ±	0.27 ± 0.10
Acid solution	0.05 ± 0.04	0.85 ± 0.45	0.31 ±	0.28 ± 0.10
Rinse after acid treatment	0.06 ± 0.04	0.95 ± 0.40	0.29 ±	0.31 ± 0.12
Enzymatic cleaning				
Enzymatic solution	0.23 ± 0.10	o.d.[b]	2.41 ±	2.73 ±
Rinse after enzymatic treatment	0.05 ± 0.04	0.98 ± 0.45	0.28 ± 0.12	0.27 ± 0.10

Tryptophan (L-Trp), Riboflavin (Rbf), Maillard compounds (MC), Dityrosine (Dt).
[a] Mean values expressed as mg/L or mL/L; n = 4 for every item of data.
[b] Out of detection range.

microorganisms that can be reactivated after cleaning treatments. In the dairy industry, chemical cleaning with alkaline products such as sodium hydroxide reduces the presence of bacterial microorganisms and improves the removal of dairy biofilms and fouling (Bremer, Fillery, & McQuillan, 2006). In our work, the combination of NaOH and phosphonates reduced the presence of total aerobic bacteria, observed as a reduction in their presence in the alkaline post-cleaning rinse. Although acid cleaning reduces bacterial load to a lesser extent than alkaline cleaning, its activity also has an effect on bacterial growth. Unlike the chemical cleaning, the presence of TVC was observed during the enzymatic cleaning. This can be explained by the fact that enzymes do not have a direct antimicrobial effect on cells, but have activity to descale them when they are attached to surfaces (Lequette et al., 2010a, 2010b; Ripolles-Avila et al., 2020). The effect of detaching microorganisms by the enzymatic cleaning solution was evidenced in the reduction of TVC on analyzing the rinse after the enzymatic treatment. These results indicate an effective microbiological cleaning of the installation, and thus removal of fouling deposit, using the chemical and enzymatic solutions.

Microbiological values of the powdered milk fouling obtained during the cleaning of the spray dryer were low. A TVC count of no more than 10^2 CFU/mL was found, and the presence of yeasts and molds was only evidenced in the rinse water after the enzymatic cleaning. The low microorganism count after the spray dryer cleaning can also be related to the ease of cleaning the fouling compared to the PHE. However, the formation of fouling facilitates the development of biofilms; therefore, proper cleaning should ensure complete elimination of fouling in dairy plants with spray drying systems for producing milk powder (Walmsley, Walmsley, Atkins, Neale, & Tarighaleslami, 2015). One of the bacterial microorganisms isolated most often from the spray dryer was *Bacillus amyloliquefaciens*, which was biochemically identified with the API 50 CBH system (bioMérieux, France). Although *B. amyloliquefaciens* has not been implicated in food poisoning cases, it has the ability to produce highly thermoresistant spores (surviving 125 °C, 30 min), and has proteolytic activity that affects the quality of dairy products (Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz, 2013). In the case of yeast and molds, there were low counts of these microorganisms, with *Rhodotorula* spp. one of the more frequently detected microorganisms in the rinse water after milk pasteurization. *Rhodotorula* is a common environmental yeast recognized as an emerging pathogen and, because this genus produces extracellular proteases and lipases, it is likely to contribute to the spoilage of dairy products (Wirth & Goldani, 2012).

3.4. Fluorescence markers

The results of the front-face analysis of the markers of the fouling obtained from the spray dryer are shown in Table 4. For chemical cleaning, it was observed that the L-Trp, MC, and Dt values were similar between tap water and pre-rinse, with the exception of Rbf (0.0 mg/L in tap water and 1.02 mg/L in pre-rinse). The alkaline solution showed the highest values of the markers (LTrp, Rbf, MC, and Dt), which were reduced with the rinse after alkaline treatment. In this sense, an increased reduction was observed with the Rbf marker (2.36 mg/L to 0.37 mg/L). In the case of the acid step of the chemical cleaning, these values were found to be similar when comparing the acid solution and the rinse after acid treatment in all the markers tested. During the enzymatic cleaning, we observed a reduction in the presence of L-Trp (0.05 mg/L), MC (0.28 mL/L), and Dt (0.27 mL/L) in the samples obtained from the rinse after enzymatic treatment compared with the enzymatic solution. In the case of the Rbf results, the changes could not be compared because although the result of the rinse after the enzymatic treatment was 0.98 mg/L, the results for this marker were out of the detection range in the enzymatic solution.

Changes in fluorescence intensity, particularly during the alkaline step of the chemical cleaning and the enzymatic cleaning, may be due to the use of Jet Foam alkaline cleaning agent based on hydrogen peroxide and enzymes, which contain fluorescent compounds. In addition, the presence of compounds released from the milk fouling due to the use of cleaning agents must also have influenced the changes in the fluorescent marker intensities. Because there were changes in the intensity of the markers, front-face measurement of the concentration of fluorophores could be used as a method for evaluating the cleaning efficacy of alkaline and enzymatic solutions for dairy fouling. Given that acid treatment mostly descales inorganic matter, analysis with these markers is not highly recommended. Previous authors have traced the cleaning process using fluorescent measurements. Berg, Ottosen, van den Berg, and Ipsen (2017) observed that inline ultraviolet–visible (UV–Vis) spectroscopy could be used to optimize the processing time, energy, and chemicals required for cleaning in place of membrane filtration plants. Lyndgaard et al. (2014) demonstrated that UV spectroscopy in combination with an explorative multivariate classification method such as principal component analysis can be used as a method for monitoring the performance of the cleaning procedures. In addition, previous studies have demonstrated the efficacy of measuring fluorophore compounds for determining the compounds in milk subjected to heat treatments. Ayala, Zamora, Rinnan, Saldo, and Castillo (2020) found that tryptophan fluorescence allowed sample classification by PCA analysis according to heat load, i.e., temperature and time. In another study, the same authors evaluated the correlation of lactulose concentration with fluorescence markers such as tryptophan, dityrosine, Maillard intermediate compounds, and riboflavin to obtain prediction models of lactulose concentration in heat-treated milk based on front-face fluorescence (Ayala et al., 2017a, 2017b). Liu, Zamora, Castillo, and Saldo (2018) and Alvarado, Zamora, Liu, Saldo, and Castillo (2019) studied the potential of predicting retinol loss and riboflavin, respectively, in milk during thermal processing using front-face fluorescence spectroscopy, concluding that this technique has the potential to replace existing conventional analytical techniques for heat-treated milk.

3.5. Costs

The analysis of the costs derived from the cleaning in the pilot plant shows that the alkaline treatment costs 9.36 €/200 L, whereas the combined alkaline–acid treatment costs 12.20 €. The enzymatic treatment (9.00 €) together with the expense of the buffer for the treatment

Table 5
Product costs (€) of each cleaning treatment.

Product	Main component	Concentration (% v/v)	Kg/ 200 L	€/Kg	Total cost
Alkaline cleaner	NaOH	3%	6.00	1.56	9.40
Acid cleaner	HNO_3	1%	2.00	1.42	2.80
Enzymes	Enzymes and surfactants	1%	2.00	4.50	9.00
Enzymatic buffer	Buffer	3%	6.00	1.50	9.00

(9.00 €) costs 18.00 € (Table 5). The higher cost of the enzymatic treatment than the chemical treatment is due to the high cost of the buffer used. However, the buffer can be diluted up to 1/10 v/v as a savings strategy for commercial use. This concentration reduction in buffer usage could represent an enzyme treatment cost of 9.90 €, which would make it more competitive against current cleaning treatments, with a 25% cost reduction. In addition, the advantages of enzymatic cleaning compared to chemical cleaning go beyond the immediate costs, as it does not corrode the processing equipment and is biodegradable in the environment after use. Other works have also reported methods aimed at reducing the costs of cleaning fouling, including the application of ultrasound and nanofiltration procedures to recover and reuse cleaning products (Fernández, Riera, Álvarez, & Álvarez, 2010; Luján-Facundo, Mendoza-Roca, Cuartas-Uribe, & Álvarez-Blanco, 2016).

4. Conclusions

The enzymatic cleaning based on the use of protease and amylase demonstrated comparable efficacy to the alkaline–acid chemical CIP for cleaning the milk fouling generated in a pilot-scale plate heat exchanger and spray dryer operating under real cleaning conditions in the dairy industry. The enzymatic formulation may ensure an efficient cleaning by removing microorganisms present in the equipment, thus meeting the hygienic conditions necessary for producing dairy products. Monitoring fluorescence markers during washes can be a method for improving the efficiency of alkaline and enzymatic cleaning procedures. The enzymatic formulation tested fulfills the objectives of the dairy industry, including saving water and energy consumption, by reducing the use of chemical products. Considering that the enzymatic cleaning treatment is biodegradable and non-corrosive and is competitively priced compared to traditional chemical cleaning, it could be used in a complementary manner or may be an alternative to the use of chemical products for the cleaning of milk fouling.

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CRediT authorship contribution statement

Alfons Eduard Guerrero-Navarro: Methodology, Formal analysis, Investigation, Writing – original draft. **Abel Guillermo Ríos-Castillo:** Conceptualization, Methodology, Software, Validation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Carolina Ripolles-Avila:** Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Anna Zamora:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing. **Anne-Sophie Hascoët:** Formal analysis, Investigation, Resources, Writing – original draft, Writing. **Xavier Felipe:** Conceptualization, Validation, Investigation, Resources, Writing – review & editing, Visualization, Funding acquisition. **Manuel Castillo:** Conceptualization, Validation, Writing – review & editing, Visualization. **José Juan Rodríguez-Jerez:** Conceptualization, Validation, Investigation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Boyce, A., Piterina, A. V., & Walsh, G. (2010b). Assessment of the potential suitability of selected commercially available enzymes for cleaning-in-place (CIP) in the dairy industry. *Biofouling, 26*(7), 837–850. https://doi.org/10.1080/08927014.2010.522705This study reported the efficacy of lipase and protease for CIP application on laboratory scales that we used to determine the efficacy of enzymatic cleaning performed on real scales at a dairy pilot plant.

Guerrero-Navarro, A. E., Ríos-Castillo, A. G., Ripolles-Avila, C. R., Hascoët, A. S., Felipe, X., & Rodriguez Jerez, J. J. (2019). Development of a dairy fouling model to assess the efficacy of cleaning procedures using alkaline and enzymatic products. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 106*, 44–49. https://doi.org/10.1016/j.lwt.2019.02.057We used this study, performed under laboratory conditions, as a base for our study to evaluate dairy fouling formed under real conditions during the milk pasteurization and spray drying processes.

Lequette, Y., Boels, G., Clarisse, M., & Faille, C. (2010b). Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling, 26*(4), 421–431. https://doi.org/10.1080/08927011003699535We used this study as a base for our work selecting cleaning agents that are active against microorganisms because it reported enzymatic treatments that are capable of removing bacterial biofilms from samples in the food industry.

Wallhäußer, E., Hussein, M. A., & Becker, T. (2012). Detection methods of fouling in heat exchangers in the food industry. *Food Control, 27*, 1–10. https://doi.org/10.1016/j.foodcont.2012.02.033This review shows that fouling formation on heat transfer surfaces is a major problem in the food and dairy industry, limiting processing times and increasing plant downtime and economic costs.