

Heat treatment design for the valorization of sheep cheese whey in artisanal production

Desenho de processo térmico de soro de queijo de ovelha visando sua valorização em produções artesanais

Diseño del proceso térmico de suero de queso de oveja para su valorización en producciones artesanales

Recebido: 15/06/2022 | Revisado: 26/06/2022 | Aceito: 28/06/2022 | Publicado: 07/07/2022

Alline Artigiani Lima Tribst

ORCID: <https://orcid.org/0000-0003-1946-9354>

Universidade Estadual de Campinas, Brazil

E-mail: tribst@unucamp.br

Bruno Ricardo de Castro Leite Junior

ORCID: <https://orcid.org/0000-0001-9030-2819>

Universidade Federal de Viçosa, Brazil

E-mail: bruno.leite@ufv.br

Abstract

We evaluated the thermal death (62-85 °C) of the native microbial load in sheep cheese whey (SCW), aiming to allow its use as an ingredient in smallholdings. The results showed a microbiota with mixed thermal resistance and its inactivation kinetic was fitted to a biphasic first-order kinetic model. D-values were 60.8-1.2 min at 62-70 °C (sensitive population) and 35.4-14.1 min at 70-75 °C (resistant population). In addition, a thermoduric population (~1 log CFU/mL) was not inactivated up to 85 °C/ 30 min. We selected three binomials (68, 75, and 80 °C for 20, 6, and 1 min, respectively) to study the microbial growth and the physical and physicochemical changes of SCW during storage (7 °C/ 21 days). The results demonstrated that a binomial temperature of 75 °C/ 6 min was the best option for SCW stabilization, guaranteeing acceptable microbial counts up to 14 days of storage and preserving its physical and physicochemical characteristics. These results are fundamental to guide the processing conditions of this by-product in smallholdings and can be directly applied by researchers and sheep cheese producers.

Keywords: Sheep cheese whey; Holder pasteurization; Microbial inactivation; Kinetic parameters; Shelf life; Stability.

Resumo

Nós avaliamos a morte térmica (62-85 °C) da carga microbiana nativa presente em soro de queijo de ovelha (SQO) com o objetivo de estabilizar o soro para posterior uso como ingrediente em produções artesanais. Os resultados mostraram uma microbiota com resistência térmica mista, cuja cinética de inativação foi descrita por um modelo bifásico de primeira ordem. Os valores D foram 60,8-1,2 min a 62-70 °C (população sensível) e 35,4-14,1 min a 70-75 °C (população resistente). Além disso, foi observada uma população termodúrica (~1 log UFC/mL), resistente a tratamentos térmicos de até 85 °C/ 30 min. Selecionamos três binômios (68, 75 e 80 °C por 20, 6 e 1 min, respectivamente) para estudar o crescimento microbiano e as alterações físicas e físico-químicas dos SQO durante o armazenamento (7 °C/ 21 dias). Os resultados demonstraram que o binômio de 75 °C/ 6 min foi a melhor opção para estabilização do SQO, garantindo contagens microbianas aceitáveis por até 14 dias de armazenamento e, ao mesmo tempo, preservando suas características físicas e físico-químicas. Esses resultados são fundamentais para orientar as condições de processamento desse subproduto em pequenas propriedades ligadas à agricultura familiar e podem ser aplicados diretamente por pesquisadores e produtores de queijo de ovelha.

Palavras-chave: Soro de queijo de ovelha; Pasteurização lenta; Inativação microbiana; Parâmetros cinéticos; Vida de prateleira; Estabilidade.

Resumen

Evaluamos la muerte térmica (62-85 °C) de la carga microbiana nativa presente en el suero de queso de oveja (SQO) con el fin de estabilizar el suero para su posterior uso como ingrediente en producciones artesanales. Los resultados mostraron una microbiota con resistencia térmica mixta, cuya cinética de inactivación fue descrita por un modelo bifásico de primer orden. Los valores de D fueron 60,8-1,2 minutos a 62-70°C (población sensible) y 35,4-14,1 minutos a 70-75°C (población resistente). Además, se observó una población termodúrica (~1 log UFC/mL), resistente a tratamientos térmicos de hasta 85 °C/30 min. Seleccionamos tres binomios (68, 75 y 80 °C durante 20, 6 y 1 min, respectivamente) para estudiar el crecimiento microbiano y los cambios físicos y fisicoquímicos de la SQO durante el

almacenamiento (7 °C/ 21 días). Los resultados mostraron que el binomio de 75 °C/6 min fue la mejor opción para estabilizar la SQO, asegurando conteos microbianos aceptables hasta por 14 días de almacenamiento y, al mismo tiempo, preservando sus características físicas y fisicoquímicas. Estos resultados son fundamentales para orientar las condiciones de procesamiento de este subproducto en pequeñas propiedades vinculadas a la agricultura familiar y pueden ser aplicados directamente por investigadores y productores de queso de oveja.

Palabras clave: Suero de queso de oveja; Pasteurización lenta; Inactivación microbiana; Parámetros cinéticos; Estabilidad.

1. Introduction

Cheese whey is a by-product obtained from cheese (Macedo et al., 2021) that can be used as a food ingredient after stabilization, commonly reached through spray-drying processing (da Silva et al., 2018) or membrane concentration (Johnson, 2017; Macedo et al., 2021). Currently, cow cheese whey is widely used to improve the functionality and nutritional value of processed foods and to replace milk in some processed products due to its lower cost (Johnson, 2017); nevertheless, the processing of small ruminant cheese whey is unusual due to its production scale and characteristics (Johnson, 2017; Tribst et al., 2020).

Sheep milk is mainly used to produce artisanal cheese in most countries (Tribst et al., 2019; Macedo et al., 2021). Production commonly occurs on small family farms that are responsible for the entire production chain, ranging from flock management to the sale of cheeses. In many regions, this is explained by the geographical distance between sheep milk producers (Tribst et al., 2019), which limits processing in larger-volume cooperatives. Consequently, the costs of purchasing a spray-dryer and/or filtration membrane or even those involved in transporting the whey to a processing unit have become prohibitive due to the small volume of whey produced daily (Tribst et al., 2020; Macedo et al., 2021). Furthermore, the lack of technical knowledge of artisanal producers on how to stabilize and utilize the whey produced restricts other possibilities for whey valorization, including the development of whey drinks and vinegar (Zotta et al., 2020).

In this context, part of sheep cheese whey is used to manufacture whey cheese (such as Ricotta fresca – Pala et al., 2016), and a large volume is discarded or used for animal feed, reducing the monetary gains of smallholdings (Macedo et al., 2021). Moreover, sometimes this effluent is incorrectly disposed, being an environmental concern, even in a small proportion.

Thus, the study of alternatives compatible with artisanal production to use whey produced from sheep cheese is essential. The first step to fill this gap is to study the heat treatment binomials able to ensure microbiological stabilization of whey with minimal adverse consequences to its physical stability and physicochemical parameters.

The high pH and nutrient availability make whey very perishable (Zotta et al., 2020). The native whey microbiota has microorganisms with different heat resistance, such as starter cultures added to cheese (Zotta et al., 2020), thermophilic spores and non-spore-forming microorganisms from milk that survived to pasteurization, such as *Bacillus*, *Brachybacterium*, *Paenibacillus*, *Staphylococcus*, and *Micrococcus*, as described by Ribeiro-Júnior et al. (2018), and environmental contaminants from cheese factories (Pala et al., 2016).

In addition to microbial inactivation, the whey heat treatment design must also consider the sensitivity of proteins to thermal destabilization. β -Lactoglobulins, which are found in abundance in sheep cheese whey, are denatured at 80 °C/1 min (Dumitraşcu et al., 2013). This denaturation leads to protein dimer dissociation, unfolding, and aggregation with a consequent reduction in protein physical stability, resulting in phase separation and sedimentation (Dumitraşcu et al., 2013).

Considering these issues, this research aimed to evaluate the impact of different binomials of holder pasteurization in sheep cheese whey, elucidating the kinetics of inactivation and microorganism growth rate after processing and the impact of different processes on the stability and physicochemical characteristics of whey.

2. Material and Methods

2.1 Sheep cheese whey

Sweet whey from sheep milk cheese was obtained from the process of a semihard cheese produced with recombinant chymosin and a starter culture containing lactic acid bacteria (Rima Artisanal cheese factory, Porto Feliz, Brazil). The whey was filtered through food-grade cheesecloth to retain any cheese curds and stored at 1°C until processed (48 h).

2.2 Thermal processing of whey and kinetic parameters of thermal death curves

Thermal inactivation of native whey microbiota was performed simulating the heating rate of traditional devices for batch pasteurization, which is mandatory in artisanal cheese factories due to the low volume processed. A volume of 800 mL of whey was added to a 1 L glass beaker. A T-type thermocouple was inserted in the center of the beaker and the sample was heated in a boiling water bath under stirring until reaching the process temperature. Then, the beaker was transferred to another water bath set at the fixed process temperature, and the time counting was started. Aliquots of 10 mL were taken at predetermined time intervals and immediately transferred to test tubes immersed in cold water. Temperatures were recorded at 5 s intervals during all experiments.

The processes were carried out at 62 °C for up to 60 min, 65 °C for up to 45 min, and 68, 70, 75, 80, and 85 °C for up to 30 minutes. Up to 68 °C, aliquots were taken at 5-min intervals, and for samples processed at 70-85 °C, aliquots were collected at 1, 3, 6, and 10 min and then at 5-min intervals for up to 30 minutes. Surviving mesophilic microorganisms were counted in plate count agar incubated at 35 °C/ 48 h (Tribst et al., 2019). Processes were carried out in triplicate and enumerations in duplicate.

Microbial inactivation showed mixed population behavior, with biphasic first-order kinetics. Therefore, the decimal reduction time (D) values were obtained from the slope of each segment in the thermal inactivation curves (Augusto et al., 2011), following Equation 1. The thermal coefficient (z) value was obtained from the slope of the thermal death time curve (log[D] versus temperature), following Equation 2 (Pflug 1988).

$$\frac{N_t}{N_0} = 10^{-\frac{t}{D}} \therefore D = \frac{t}{(\text{Log } N_0 - N_t)} \quad (\text{Equation 1})$$

where:

N_t is the number of microbial cells at time t (min), N_0 is the initial number of microbial cells, t is the processing time (min) at fixed temperature (T), and D is the decimal reduction time (min) at fixed temperature (T).

$$D_2 = D_1 \cdot 10^{\left(\frac{T_1 - T_2}{z}\right)} \therefore z = \frac{T_1 - T_2}{\log D_2 - \log D_1} \quad (\text{Equation 2})$$

where:

D_1 and D_2 are the decimal reduction time (min) at fixed temperatures, T_1 and T_2 (°C), respectively, and z is the number of degrees Celsius required to reduce D by a factor of 10.

2.3 Impact of heat treatment on whey shelf life

For this study, three binomials with residual counts close to 3 (68 °C/ 20 min), 2 (75 °C/ 6 min), and 1 log CFU/mL (80 °C/1 min) were selected. The samples were sweetened (3 or 6% sugar) or not and processed following the procedure described above. After the residence time, the beaker containing the entire volume (800 mL) was transferred to a cold water bath for rapid temperature reduction. Then, the samples were divided into 4 glass flasks (120 mL) and 4 tubes (8 mL) previously sterilized. To evaluate the residual activity of the rennet, an aliquot of 10 mL was used. Each process was carried out in triplicate. Samples were stored at 7 °C and evaluated after 0, 7, 14, and 21 days.

2.4 Microbiological and physicochemical characterization

Samples were serially diluted in saline solution (0.85% NaCl) and plated in plate count agar (PCA) for total bacteria count (TBC) and total psychrotrophic count (TPC). TBC was determined after incubation at 35 °C/ 48h and TPC was determined after incubation at 7 °C/ 10 days (Tribst et al., 2019). Counts of each sample were performed in duplicate.

Titrate acidity, pH (AOAC International, 1999), and stability to ethanol (Tribst et al., 2019) were determined in triplicate. The percentage of sedimentation in each sample was measured after 21 days of storage, measuring the volume of sediment formed in the base of the tubes in relation to the total volume (8 mL) of the sample (Kubo et al., 2013). Furthermore, at day 0, the residual activity of rennet was measured in triplicate, following the method described by Leite Júnior et al. (2014), replacing the enzyme solution with whey and carrying the reaction by 120 min.

2.5 Statistical analysis

The differences in kinetic parameters of thermal death curves and in microbiological and physicochemical parameters of the whey were evaluated by using the analysis of variance (ANOVA) and the Tukey test at a 95% confidence level (XLSTAT software, version 2015.2.02, Microsoft, Inc., USA). The results are expressed as the mean \pm standard deviation.

3. Results and Discussion

3.1 Kinetic parameters of thermal death curves

The TBC and pH in raw whey were 6.96 ± 0.01 log CFU/mL and 6.72 ± 0.02 , respectively. This high bacterial count was expected, considering that the whey was obtained from cheese with a starter culture. On the other hand, the pH suggests that these cultures did not have enough time to acidify the cheese mass before whey separation. According to Lo et al. (2016), this type of whey is commonly dominated by starter bacteria, including the *Lactobacillus* and *Streptococcus* genera.

The kinetic parameters of the thermal death curves of the whey native microbial load were compiled (Table 1). The results showed that the come-up time increased with increasing temperature, reaching ~10 minutes for processing at 85 °C. Although long, this come-up time is usual in batch processes carried out in jacketed tanks heated by steam or hot water, depending on parameters such as tank design, type/ratio of heating media, and stirring.

Table 1. Kinetic parameters of thermal death curve of microbial load in sheep cheese whey.

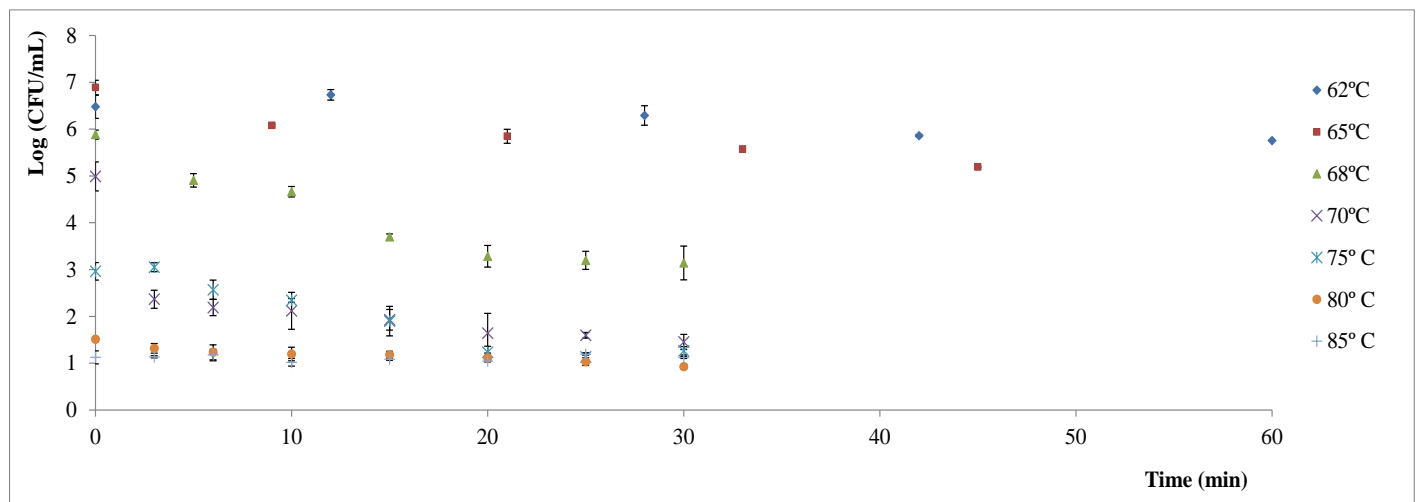
Set temp (°C)	Real Temp (°C)	Come-up time		D-values				Impact at the end of processing	
		Come-up time (s)	Inactivation log(CFU/mL)	Sensitive Fraction		Resistant fraction		Max. residence time (min)	Residual count (at max. residence time) log(CFU/mL)
				D	R ²	D	R ²		
62	61.5 ± 0.6	294 ± 8.5	0.41 ± 0.22 ^f	60.8 ± 9.5 ^a	0.70 - 0.89	§n.i.	---	60	5.74 ± 0.02 ^a
65	65.3 ± 1.8	380 ± 10.6	0.07 ± 0.11 ^f	29.3 ± 1.7 ^b	0.90 - 0.97	n.i	---	45	5.19 ± 0.01 ^b
68	67.9 ± 0.8	414 ± 8.5	1.08 ± 0.12 ^e	10.2 ± 0.6 ^c	0.88 - 0.96	n.i	---	30	2.97 ± 0.25 ^c
70	70.3 ± 1.4	455 ± 6.4	1.97 ± 0.30 ^d	¥1.2 ± 0.2 ^d	1.00	35.4 ± 4.7 ^a	0.76 - 0.79	30	1.48 ± 0.23 ^d
75	74.8 ± 1.5	447 ± 4.2	4.00 ± 0.18 ^c	#i-c.u.t	---	14.1 ± 0.6 ^b	0.87 - 0.99	30	1.26 ± 0.09 ^{de}
80	79.9 ± 1.4	501 ± 44.5	5.45 ± 0.07 ^b	i-c.u.t	---	i-c.u.t	---	30	0.93 ± 0.06 ^f
85	84.8 ± 0.9	586 ± 2.2	5.84 ± 0.14 ^a	i-c.u.t	---	i-c.u.t	---	30	1.12 ± 0.09 ^{ef}

¥ D-value estimated using 2-points linear regression, #i-c.u.t means inactivated at come up time, §n.i. means not inactivated. ^{a-f} Different superscript letters mean significant differences among processes (p<0.05). Source: Authors.

At the come-up time, inactivation became significant (> 0.5 log CFU/mL) for samples processed at temperatures ≥ 68°C, with intense inactivation for processes carried out at ≥75 °C. This corroborates the statement that the come-up time plays an important role in microbial inactivation and needs to be considered in the evaluation of process lethality in holder pasteurization (Bhalerao, Chakraborty 2021). Furthermore, the comparison between real and set temperatures showed a satisfactory variation (≤ 0.5 °C in the average temperature, with a standard deviation ≤ 1.8 °C; which is acceptable in artisanal processing due to the usual manual control of the heating equipment).

As expected for the native microbial load, the inactivation curve (Figure 1) clearly showed the existence of a microbial population with a diverse thermal resistance (da Silva Duarte et al., 2020), and this behavior was also observed in milk samples with a pool of strains of microorganisms of the same species (Gabriel et al., 2020). In addition to microbial diversity, the occurrence of cell clumping in samples with a high density of microorganisms can also favor a non-linear inactivation behavior (Mullan 2019) since inactivated cells on the surface of the clump act as a physical barrier to heat transfer, slowing the inactivation rate (Gabriel et al., 2020).

Figure 1. Total bacterial counts in sheep cheese whey during different thermal processes.



Source: Authors.

Inactivation curves had a biphasic first-order behavior, with each segment described by first-order kinetics (Equation 1) with an acceptable fit (R² higher than 0.9 for most replicates, except for the curve at 62 °C due to the almost negligible inactivation). The first segment, named the “sensitive fraction”, had almost 3 log cycles and D-values from 60.8 to 1.2 min between 62 and 72 °C, with a z-value, calculated using Equation 2, of 5.72 °C (R² = 0.93). This fraction was completely

inactivated by processes at 68 °C/ 20 min or 70 °C/ 1 min. Furthermore, for temperatures ≥ 75 °C, its complete inactivation occurred at the come-up time. Conversely, the second segment (~ 2 log CFU/mL), called the “resistant fraction”, had marginal inactivation up to 70 °C and total inactivation at 75 °C/ 20 min or during the come-up time for processes at temperatures ≥ 80 °C. For this fraction, it was not possible to determine the z-value due to insufficient temperatures with measurable linear inactivation. Finally, 1 log CFU/mL of thermoduric microorganisms (resistant to 85 °C/ 30 min) was observed.

The evaluation of the inactivation profile suggests that the sensitive fraction of microorganisms may include those from contamination during cheese manufacturing (Pala et al., 2016), such as coliforms (Johnson, 2017), and some culture starters, especially lactobacilli that have a low thermal resistance (Lo et al., 2016). On the other hand, the resistant fraction can be explained by some species of vegetative heat-resistant bacteria, such as *Streptococcus* (Lo et al., 2016; Johnson, 2017), *Staphylococcus* and *Micrococcus* genera (Ribeiro-Júnior et al., 2018). Finally, the most heat-resistant fraction probably comprises spore-forming microorganisms, such as *Bacillus*, representing less than 0.1% of the total population, as observed in previous studies (da Silva Duarte et al., 2020).

Regarding whey stability, it was observed that processes at 85 °C caused visible and instantaneous sedimentation (data not shown), explained by whey protein denaturation, especially β -lactoglobulin, which is the major protein constituent in sheep cheese whey (Dumitraşcu et al., 2013). Thus, temperatures above 85 °C were not tested, even considering the remaining count of microorganisms.

The overall evaluation of the results highlights that it is not possible to use the binomials traditionally applied in milk pasteurization (63 – 65 °C/ 30 – 32 min or 72 – 75 °C/ 15 – 30 s) for processing sheep cheese whey. This can be explained by considering the lower counts expected for sheep milk obtained following good manufacturing practices and the differences in the microorganism diversity found in raw milk and whey. Raw milk has a higher percentage of microorganisms with low heat resistance, such as coliforms, non-thermoduric psychrotrophics, and pathogens (da Silva Duarte et al., 2020), while *Lactobacillus* and/or *Streptococcus* from starter cultures are the main genera found in this kind of whey (Lo et al., 2016; Nalepa, et al., 2020), commonly at counts of $\sim 10^7$ CFU/mL (Nalepa et al., 2020).

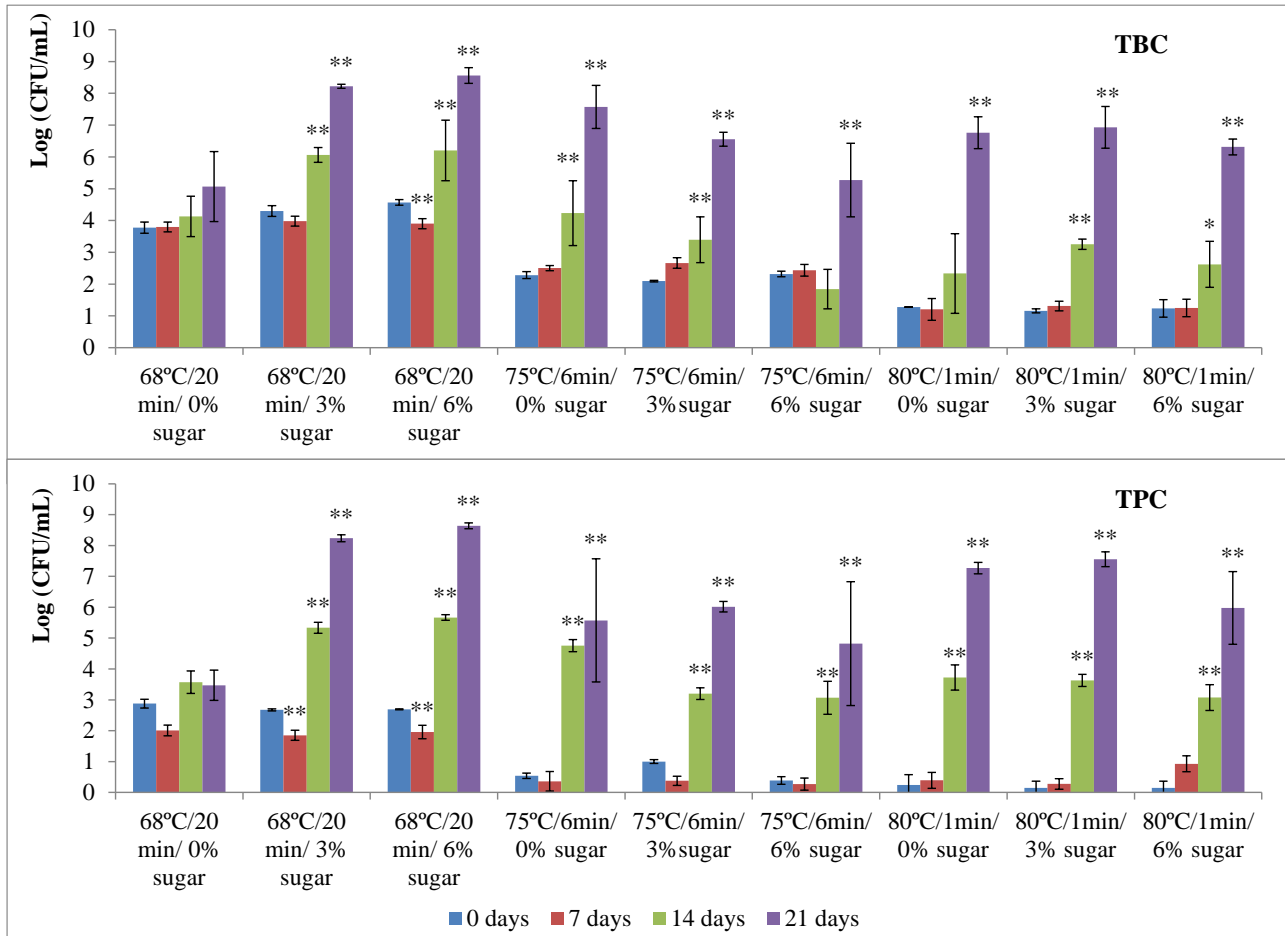
Based on these results, we chose binomials capable of inactivating different microbial loads, aiming to evaluate the impact of each residual count on the whey shelf life. Thus, binomials chosen were 68 °C/ 20 min (inactivation of the sensitive fraction), 75 °C/ 6 min (part of the resistant fraction also inactivated), and 80 °C/ 1 min (total inactivation of the sensitive and resistant fractions, remaining only the thermoduric microorganisms).

For the binomial 68 °C/ 20 min, the residual activity of the rennet was measured and the results showed a low activity ($\leq 1.6\%$), demonstrating that this binomial and, consequently, all those more intense than it, are sufficient to paralyze the proteolysis caused by rennet in whey during refrigerated storage. These results corroborate previous data that estimated the inactivation of recombinant chymosin at temperatures ranging from 50 to 60 °C, depending on the source of the enzyme (Belenkaya et al., 2018)

3.2 Impact of heat treatment on whey shelf life

The microbial growth in the samples was accompanied by weekly enumeration of total and psychrotrophic bacteria, and the results showed that the growth was affected by the addition of sugar (samples processed at 68 °C/20 min) and by the applied binomials (Figure 2). For the unsweetened sample processed at 68 °C/ 20 min, counts remained stable (~ 4 log CFU/mL) throughout storage ($p > 0.05$). However, for samples with sugar (3 or 6%), an increase in TBC and TPC was observed after 14 days of storage (growth of 2 log CFU/mL, $p < 0.05$), with a consequent increase in acidity and a reduction in pH and ethanol stability ($p < 0.05$) of whey (Figure 3). According to these results, the adequate shelf life of sweetened whey processed at 68 °C/20 min is limited to 7 days.

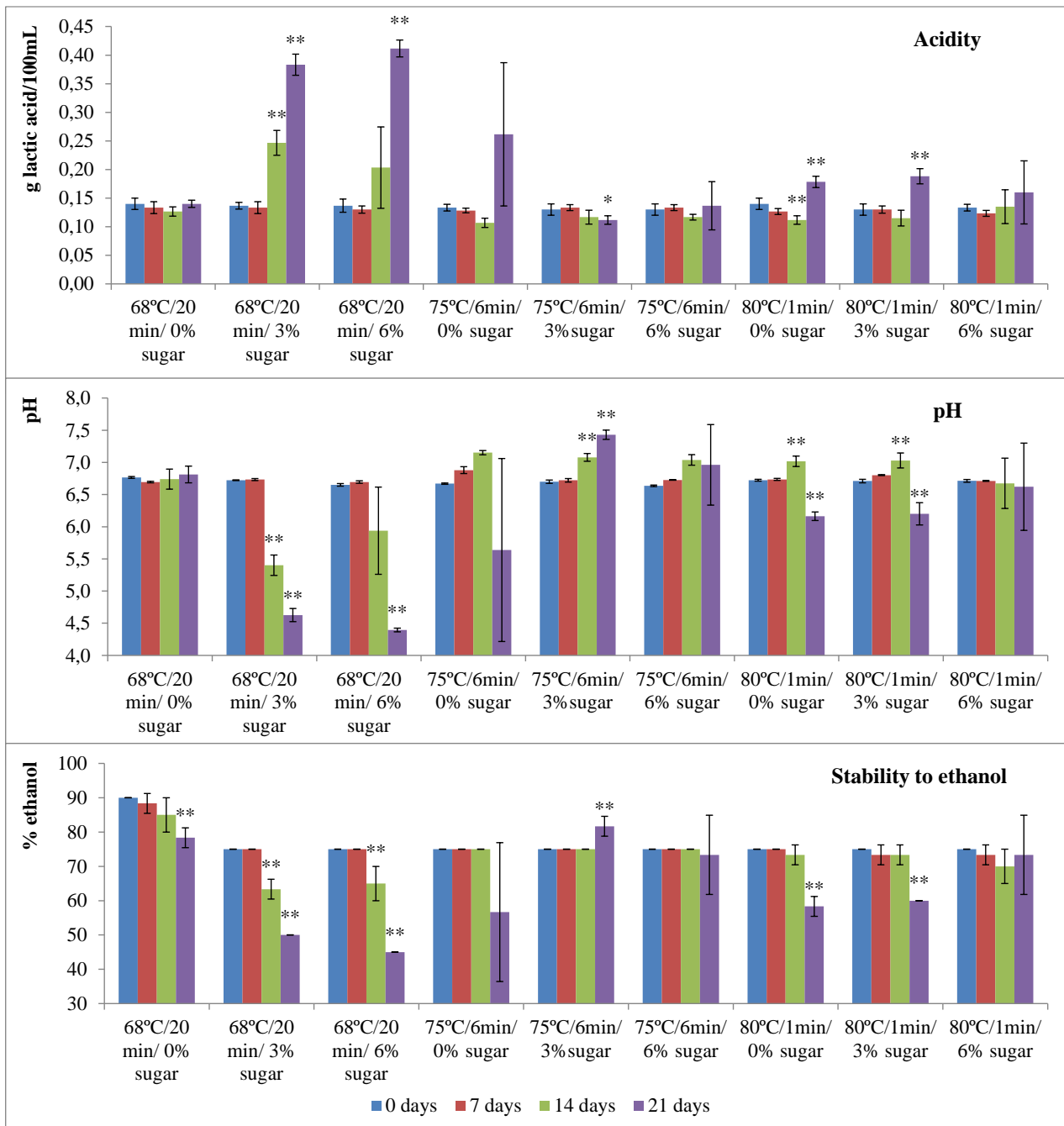
Figure 2. Impact of heat treatment on the sheep cheese whey shelf life: enumeration of total (TBC) and psychrotrophic (TPC) bacteria.



* means difference at $p < 0.05$ and ** means difference $p < 0.01$ compared to sample at day 0. Source: Authors.

Comparing the bacterial counts immediately after processing in samples with or without sugar, we observed that the number of survivors was similar (less than 1 log CFU/mL difference), which suggests that sugar contaminants resistant to this binomial had an acidifying profile and good ability to grow at low temperatures. As described by Thompson (2009), refined sugar is a stable product due to its low a_w but is commonly contaminated by spore-forming microorganisms (resistant to obtaining and refining sugar) and vegetative microorganisms due to the usual cross-contamination that occurs in this kind of processing. Thus, the addition of sugar in a high-moisture product may be a concern regarding the effectiveness of thermal processing and the ability of the residual population to grow (Thompson, 2009).

Figure 3. Physicochemical parameters of heat-processed sheep cheese whey during shelf life.



* means difference at $p < 0.05$ and ** means difference < 0.01 compared to sample at day 0. Source: Authors.

For samples processed at 75 °C/ 6 min and 80 °C / 1 min, psychrotrophic and mesophilic bacterial growth was statistically detectable in most of the samples on the 14th day of storage ($p < 0.05$), reaching counts close to the initial count of the processed whey at 68 °C/20 min. This relatively low count explains why the physicochemical parameters studied (Figure 3) were not changed after this period for almost all these samples. On the other hand, an intensive increase in TBC and TPC was observed after 21 days of these samples' storage ($p < 0.05$), reaching counts ≥ 6 log CFU/mL for mesophilic and psychrotrophic.

Despite this growth, the impact on pH, acidity, and ethanol stability was inconsistent, being statistically measurable ($p < 0.05$) only for samples with added 3% sugar and processed at 75 °C/6 min and/or those heat treated at 80 °C/1 min, without sugar or with added 3%. This can be explained by the high standard deviation of the measurements performed on the 21st day

of storage, which is attributed to the differences in the growth profile of each repetition. Some replicates showed a typical predominance of acidifying microorganisms (with increased acidity and reduced pH and ethanol stability), whereas others showed an opposite behavior, suggesting the growth of low- or non-acidifying and proteolytic microorganisms, which are characteristically found among psychrotrophic microorganisms (Juven et al., 1981).

Important differences were observed in the microbial growth in the whey processed at 68 °C/ 20 min and other samples. First, sugar contaminants were apparently only relevant for samples processed at low temperature, suggesting an intermediate heat resistance between the sensitive and resistant fractions found in whey. Second, the absence of growth in the unsweetened sample processed at 68°C/20 min showed that its survivors (~4 log CFU/mL) were unable to grow at 7°C. This result suggests that these microorganisms were mostly non-psychrotrophic and the small fraction of thermoduric microorganisms found in the samples processed at the highest binomials (1-2 log CFU/mL, i.e., <1% of the total survivor load at 68 °C/ 20 min) were: (i) inhibited by the other microorganisms (Chapman et al., 2012) or (ii) were endospores, and heat treatment was not sufficient to induce their activation and germination (Kakagianni et al., 2020), affecting their ability to grow. Nevertheless, choosing 68 °C/ 20 min as a processing condition may not be safe due to the high residual load and the possibility of reaching unacceptable counts quickly if part of this load exhibits psychrotrophic behavior.

On the other hand, although pasteurization at high binomials (such as 75 °C/ 6 min or 80 °C/ 1 min) was effective in reducing more than 5 log CFU/mL of the microbial population in sheep cheese whey, the low residual bacterial count grew during refrigerated storage (Masiello et al., 2017), limiting the shelf life of the heat-stabilized whey. This suggests that at least some of these thermoduric microorganisms were also psychrotrophic (Masiello et al., 2017). Likewise, previous results obtained for pasteurized milk (80 °C/ 12 min) showed the growth of low counts of psychrotrophic thermoduric microorganisms, predominantly *Bacillus* and *Paenibacillus* spp., reaching $\geq 20,000$ CFU/mL after 21 days of storage at 6 °C (Masiello et al., 2017).

Whereas thermoduric microorganisms are regularly isolated from environmental samples taken from dairy farms, adjustment of cleaning practices in bulk milk and milking areas and equipment (Elmoslemany et al., 2010), efficient scraping of the flock house area, and segregation of problem animals during milking can help to keep counts of these microorganisms at very low levels (Masiello et al., 2017).

However, depending on the production and environmental characteristics, it is possible that the presence of psychrotrophic thermoduric microorganisms remains a problem even after these adjustments. In this case, it is possible to try other control alternatives, such as the addition of competitive cultures for bioprotection (Canon et al., 2020), the use of antimicrobials, such as nisin (Castellano et al., 2018), or whey acidified by fermentation (Pereira et al., 2015) or direct acid addition, to guarantee an extended shelf-life for processed sheep cheese whey.

Finally, the evaluation of sedimentation in the samples processed in different binomials showed that whey subjected at 68 °C/ 20 min remained stable, and a slight sedimentation (<0.8%, $p < 0.05$) was observed for those processed at 75 °C/ 6 min, regardless of sugar addition ($p > 0.05$). On the other hand, for samples processed at 80 °C/ 1 min, sedimentations were expressive and dependent on the sugar concentration: $7.3 \pm 1.5\%$ (without sugar), $6.9 \pm 1.4\%$ (3% sugar) and $3.9 \pm 2.1\%$ (6% sugar), showing that the higher sugar concentration reduced the impact of heating on protein destabilization ($p < 0.05$). These results were expected due to the abundance of β -lactoglobulins in sheep cheese whey, which is denatured under this condition (Dumitraşcu et al., 2013).

4. Conclusions

Studies on microbial stabilization are a crucial step for product development and, for immediate application in food processing, it should consider the native microbiota of the raw material. The microbial reduction profile under different temperatures suggested a mixed microbial population in sheep cheese whey and the existence of a thermoduric fraction, able to survive after 85°C/ 30 min. Even in low concentration (~1 log CFU/mL), this fraction grew and spoiled sheep cheese whey after 21 days under refrigeration. Furthermore, the results suggest that the growth rate was dependent on microbial competition.

Among the studied processes, the binomial 75 °C/ 6 min was the best option for processing sheep cheese whey, guaranteeing a shelf life of 14 days. If extended shelf life is desirable, smallholdings need to consider improvements to the raw material to minimize the occurrence of thermoduric microorganisms and/or the association of other barriers to control the growth of this population.

Therefore, future research for whey stabilization and valorization can focus on whey stabilization using pH reduction, antimicrobials, and bioprotective cultures as a hurdle associated with thermal treatment to achieve adequate shelf life, ensuring sufficient time for processing, distribution, and consumption. In addition, it is also necessary to develop strategies to improve the physical stability and sensory profile of the sheep cheese whey.

Acknowledgments

The authors would like to thank the Rima smallholding for the milk donation. This work was supported by the São Paulo Research Foundation (FAPESP, project no. 2020/10930-9) and by the National Council for Scientific and Technological Development with the productivity grants of B.R.C. Leite Júnior (306514/2020-6) and A.A.L. Tribst (305050/2020-6).

References

- AOAC International. (1999). *Official Methods of Analysis*. (16th ed.), AOAC Int.
- Augusto, P. E. D., Tribst, A. A. L., & Cristianini, M. (2011). Thermal inactivation of *Lactobacillus plantarum* in a model liquid food. *Journal of Food Process Engineering*, 34(4), 1013-1027.
- Belenkaya, S. V., Rudometov, A. P., Shcherbakov, D. N., Balabova, D. V., Kriger, a. V., Belov, A. N., Koval, A. D., & Elchaninov, V. V. (2018). Biochemical Properties of Recombinant Chymosin in Alpaca (*Vicugna pacos L.*). *Applied Biochemistry and Microbiology*, 54, 569–576.
- Bhalerao, P. P. & Chakraborty, S. (2021). Integrated calculation of pasteurization time: A case study for thermal inactivation kinetics of a mixed fruit beverage. *Journal of Food Process Engineering*, 44 (8), e13761.
- Canon, F., Nidelet, T., Guédon, E., Thierry, A., & Gagnaire, V. (2020). Understanding the Mechanisms of Positive Microbial Interactions That Benefit Lactic Acid Bacteria Co-cultures. *Frontiers in Microbiology*, 11, 2088.
- Chapman, C. M., Gibson, G. R., & Rowland, I. (2012). In vitro evaluation of single- and multi-strain probiotics: inter-species inhibition between probiotic strains, and inhibition of pathogens. *Anaerobe*, 18, 405-413.
- Castellano, P., Peña, N., Ibarreche, M. P., Carduza, F., Soteras, T., & Vignolo, G. (2018). Antilisterial efficacy of *Lactobacillus* bacteriocins and organic acids on frankfurters. Impact on sensory characteristics. *Journal of Food Science and Technology*, 55, 689–697.
- da Silva Duarte, V., Carlot, M., Pakroo, S., Tarrach, A., Lombardi, A., Santiago, H., Corich, V., & Giacomini, A. (2020). Comparative evaluation of cheese whey microbial composition from four Italian cheese factories by viable counts and 16S rRNA gene amplicon sequencing. *International Dairy Journal*, 104, 104656.
- da Silva, D. F., Ahmé, L., Larsen, F. H., Hougaard, A. B., & Ipsen, R. (2018). Physical and functional properties of cheese powders affected by sweet whey powder addition before or after spray drying. *Powder Technology*, 323, 139-148.
- Dumitrașcu, L., Moschopoulou, E., Aprodu, I., Stanciu, S., Râpeanu, G., & Stănciuc, N. (2013). Assessing the heat induced changes in major cow and non-cow whey proteins conformation on kinetic and thermodynamic basis. *Small Ruminant Research*, 111(1-3), 129-138.
- Elmoslemany, A. M., Keefe, G. P., Dohoo, I. R., Witchel, J. J., Stryhn, H., & Dingwell, R. T. (2010). The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine*, 95, 32-40.
- Gabriel, A. A., Bayaga, C. L. T., Magallanes, E. A., Aba, R. P. M., & Tanguilig, K. M. N. (2020). Fates of pathogenic bacteria in time-temperature-abused and Holder-pasteurized human donor-, infant formula-, and full cream cow's milk. *Food Microbiology*, 89, 103450.

- Johnson, M. E. (2017). A 100-year review: cheese production and quality. *Journal of Dairy Science*, *100* (12), 9952–9965.
- Juven, B., Gordin, S., Rosenthal, I., & Laufer, A. (1981). Changes in refrigerated milk caused by Enterobacteriaceae. *Journal of Dairy Science*, *64*, 1781-1784.
- Kakagianni, M., Chatzitzika, C., Koutsoumanis, k. P., & Valdramidis, V. P. (2020). The impact of high power ultrasound for controlling spoilage by *Alicyclobacillus acidoterrestris*: A population and a single spore assessment. *Innovative Food Science & Emerging Technologies*, *64*, 102405.
- Kubo, M. T. K., Augusto, P. E. D., & Cristianini, M. (2013). Effect of High Pressure Homogenization (HPH) on the Physical Stability of Tomato Juice. *Food Research International*, *51*, 170-179.
- Leite Júnior, B. R. C., Tribst, A. A. L., & Cristianini, M. (2014). Proteolytic and milk-clotting activities of calf rennet processed by high pressure homogenization and the influence on the rheological behavior of the milk coagulation process. *Innovative Food Science and Emerging Technology*, *21*, 44-49.
- Lo, R., Xue, T., Weeks, M., Turner, M. S., & Bansal, N. (2016). Inhibition of bacterial growth in sweet cheese whey by carbon dioxide as determined by culture-independent community profiling. *International Journal of Food Microbiology*, *217*, 20-28.
- Macedo, A., Bilau, J., Camboias, E., & Duarte, E. C. N. F. D. (2021). Integration of Membrane Processes for By-Product Valorization to Improve the Eco-Efficiency of Small/Medium Size Cheese Dairy Plants. *Foods*, *10* (8), 1740.
- Masiello, S. N., Kent, D., Martin, N. H., Schukken, Y. H., Wiedmann, M., & Boor, K. J. (2017). Longitudinal assessment of dairy farm management practices associated with the presence of psychrotolerant Bacillales spores in bulk tank milk on 10 New York State dairy farms. *Journal of Dairy Science*, *100*, 8783-8795.
- Mullan, W. M. A. (2019). Are we closer to understanding why viable cells of *Mycobacterium avium subsp. paratuberculosis* are still being reported in pasteurized milk? *International Journal of Dairy Technology*, *72*, 332-344.
- Nalepa, B., Ciesielski, S., Aljewicz, M. (2020). The microbiota of edam cheeses determined by cultivation and high-throughput sequencing of the 16S rRNA amplicon. *Applied Science*, *10*(12), 4063.
- Pala, C., Scarano, C., Venusti, M., Sardo, D., Casti, D., Cossu, F., Lamon, S., Spanu, V., Ibba, M., Marras, M., Paba, A., Spanu, C., De Santis, E. P. L. (2016). Shelf Life Evaluation of Ricotta Fresca Sheep Cheese in Modified Atmosphere Packaging. *Italian Journal of Food Safety*, *5* (3), 5502.
- Pereira, C. D., Henriques, M., Gomes, D., Gouveia, R., Gomez-Zavaglia, A., de Antoni, G. (2015). Fermented dairy products based on ovine cheese whey. *Journal of Food Science and Technology*, *52*, 7401–7408.
- Pflug, I. J. Selected papers on the microbiology and engineering of sterilization processes. Minneapolis: Environmental Sterilization Laboratory, 5th ed., 1988.
- Ribeiro-Júnior, J. C., Tamanini, R., Oliveira, A. L. M., Alfieri, A. A., & Beloti, V. (2018). Genetic diversity of thermophilic spoilage microorganisms of milk from Brazilian dairy farms. *Journal of Dairy Science*, *101* (8), 6927-6936.
- Thompson S. (2009) Microbiological Spoilage of High-Sugar Products. In: Sperber W. & Doyle M. Compendium of the Microbiological Spoilage of Foods and Beverages. Food Microbiology and Food Safety. Springer, New York, NY. 10.1007/978-1-4419-0826-1_11
- Tribst, A. A. L., Falcade, L. T. P., Carvalho, N. S., Leite Júnior, B. R. D. C., & Oliveira, M. M. (2020). Manufacture of a fermented dairy product using whey from sheep's milk cheese: An alternative to using the main by-product of sheep's milk cheese production in small farms. *International Dairy Journal*, *111*, 104833.
- Tribst, A. A. L., Falcade, L. T. P., & Oliveira, M. M. D. (2019). Strategies for raw sheep milk storage in smallholdings: Effect of freezing or long-term refrigerated storage on microbial growth. *Journal of Dairy Science*, *102*(6), 4960-4971.
- Zotta, T., Solieri, L., Iacumin, L., Picozzi, C., & Gullo, M. (2020). Valorization of cheese whey using microbial fermentations. *Applied Microbiology and Biotechnology*, *104* (7), 2749-2764.