Development of a new Brazilian semi-hard (Coalho) Buffalo cheese made with the inclusion of cow milk and functional potential

Desenvolvimento de um novo queijo semiduro brasileiro (Coalho) de búfalo feito com a inclusão de leite de vaca e potencial funcional

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ABSTRACT
This study aimed to produce a new Brazilian Coalho buffalo cheese with 45% cow milk and potential functional through inulin and Lactobacillus plantarum addition. The Four treatments were made: the control (CTL) without functional ingredients, prebiotic (PRE) with Inulin, probiotic (PRO) with L. plantarum, and symbiotic (SYM) with both Inulin and L. plantarum. The starter culture (Lactococcus spp.) and L. plantarum viability and pH were analyzed during cheese ripening (0, 4, 8, and 10 days). While moisture, fat, and fat in the dry matter parameters were evaluated after ten days of storage ripening. During the ripening, the Lactococcus spp. growth was significant in the first eight days. The starter culture stabilized in counts above 7.1 log UFC.g^{-1}. In the same period, L. plantarum was no growth (< 10) neither in PRO nor SYM treatments. The higher Lactococcus spp. counts (up to 10.6 Log UFC.g^{-1}) in PRE and PRO cheeses leads to the most intense pH decrease (5.4) during the ripening. All treatments pointed to a moisture content between 38.09% to 40.49% and classified as Brazilian Coalho cheeses. The PRE had a higher FDM content (42.66%), while the lowest values were from SYM (35.53%). Thus, inulin addition does not negatively affect parameters tested, indicating a good fiber source for prebiotic semi-cooked and semi-hard cheeses. The L. plantarum viability was compromised during the process. Future studies about temperature adaptation, previous activation of strains or even encapsulation techniques associated with starter function should be evaluated to draw the best inoculation strategies for this probiotic for Coalho buffalo cheese.

Key-word: Dairy product, Inulin, Lactobacillus plantarum, Prebiotic, Probiotic

RESUMO
Este estudo visava produzir um novo queijo de búfalo Coalho brasileiro com 45% de leite de vaca e potencial funcional através da adição de inulina e Lactobacillus plantarum. Os Quatro tratamentos foram feitos: o controlo (CTL) sem ingredientes funcionais, o prebiótico (PRE) com Inulina, o probiótico (PRO) com L. plantarum, e o simbiótico (SYM) com Inulina e L. plantarum. A cultura inicial (Lactococcus spp.) e L. plantarum viabilidade e pH foram analisados durante a maturação do queijo (0, 4, 8, e 10 dias). Enquanto a humidade, gordura e gordura nos parâmetros de matéria seca foram avaliadas após dez dias de maturação de armazenamento. Durante a maturação, o crescimento de Lactococcus spp. foi significativo nos primeiros oito dias. A cultura inicial estabilizou em contagens superiores a 7,1 log UFC.g-1. No mesmo período, L. plantarum não teve crescimento (< 10) nem em tratamentos PRO nem SYM. As contagens mais elevadas de Lactococcus spp. (até 10,6 log UFC.g-1) em queijos PRE e PRO levam à diminuição mais intensa do pH (5,4) durante a maturação. Todos os tratamentos apontam para um teor de humidade entre 38,09% a 40,49% e classificados como queijos Coalho brasileiros. O PRE tinha um maior teor de FDM (42,66%), enquanto que os valores mais baixos eram de SYM (35,53%). Assim, a adição de inulina não afecta negativamente os parâmetros testados, indicando uma boa fonte de fibras para queijos pré-bióticos semi-cozidos e semi-duros. A viabilidade da L. plantarum foi comprometida durante o processo. Estudos futuros sobre adaptação à temperatura, activação prévia de estirpes ou mesmo técnicas de encapsulação associadas à função de arranque devem ser avaliados para desenhar as melhores estratégias de inoculação para este probiótico para o queijo de búfalo Coalho.

Palavra-chave: Produto lácteo, Inulina, Lactobacillus plantarum, Prebiótico, Probiótico.

1 INTRODUCTION

The coalho cheese is a semi-hard variety produced mainly from cow milk fermented by starter cultures, with a ripening time of 10 days, resulting in a widely consumed dairy product in Brazil (Costa Lima et al., 2018). However, the buffalo milk should also be considered a viable alternative of raw material since it has a high content of total solids, leading to a higher manufacturing yield, associated with nutritional benefits due to a rich composition of calcium, vitamin A, and fatty acids, such as conjugated linoleic acids (Khan et al., 2017; Nayak, Ramachandra, and Kumar, 2020). In this context, cheeses are considered probiotic vehicles because the cheese matrix creates a protective environment that improves the probiotic ability to survive the passage through the gastric tract (Rolim et al., 2020). Due to the great acceptance of Coalho cheese on the market (Soares et al., 2019) and because it is known to promote the viability of the starter and different probiotic bacteria during both the storage and the gastrointestinal passage (Oliveira et al., 2014), the Brazilian Coalho cheese should be considered a powerful probiotic carrier.

Several studies confirm the probiotic characteristics of Lactobacillus plantarum, which can be found in several types of cheese (Amira et al., 2019). They have been well evaluated for the better development of the sensory profile of cheeses by forming desirable volatile compounds (Belkheir et al., 2020). Among the main benefits linked to the presence of L. plantarum, it can be mentioned the production of exopolysaccharides with antioxidant activity plays a role in reducing cholesterol levels and inducing the activity of the immune system (Yılmaz and Şimşek, 2020).

Inulin is a colorless and odorless fructose polymer that possesses a slightly sweet taste used to fortify foods. This prebiotic ingredient is considered an "invisible" and ideal fiber source since its addition does not compromise the elaborated products' sensory characteristics (Karimi et al., 2015). Apart from that, the inclusion of inulin in food products favors their stability as inulin is considered a fat substitute when developing products with the desired texture (Schädle et al., 2020). In addition, prebiotic roles include facilitating the gastrointestinal transit, low caloric value, mechanisms to reduce the high levels of triglycerides and cholesterol in the blood, improvement in the absorption of calcium, magnesium, and iron, and also promotes the growth of beneficial bacteria in the colon (Shoaib et al., 2016).

Several studies deal with the impact of added probiotics and prebiotic ingredients in physicochemical, bacteriological, and sensory properties of cheeses, mainly in fresh and soft cheeses (Giri et al., 2017; Mushtaq et al., 2016;) and other dairy products to provide better nutritional and sensory characteristics, promoting the consumption of healthier dairy products (Salgado et al., 2020). However, little is known about the viability of adding inulin and L. plantarum in the technological processing of semi-hard and semi-cooked cheeses made from a mixture of milk from different species. In a previous study carried out by our group, we demonstrated that the mixture of both milk varieties, buffalo and cow milk, provided desirable sensory characteristics, facilitating the insertion of buffalo dairy products in the dairy industry and the diversification of the chain production (Rekowsky et al., 2020). Thus, this study aimed to develop a new Brazilian Coalho buffalo cheese made with cow milk (colocar a porcentagem) and the addition of inulin and L. plantarum, on physicochemical parameters and bacteria viability during 10 days of ripening period.

2 MATERIAL AND METHODS

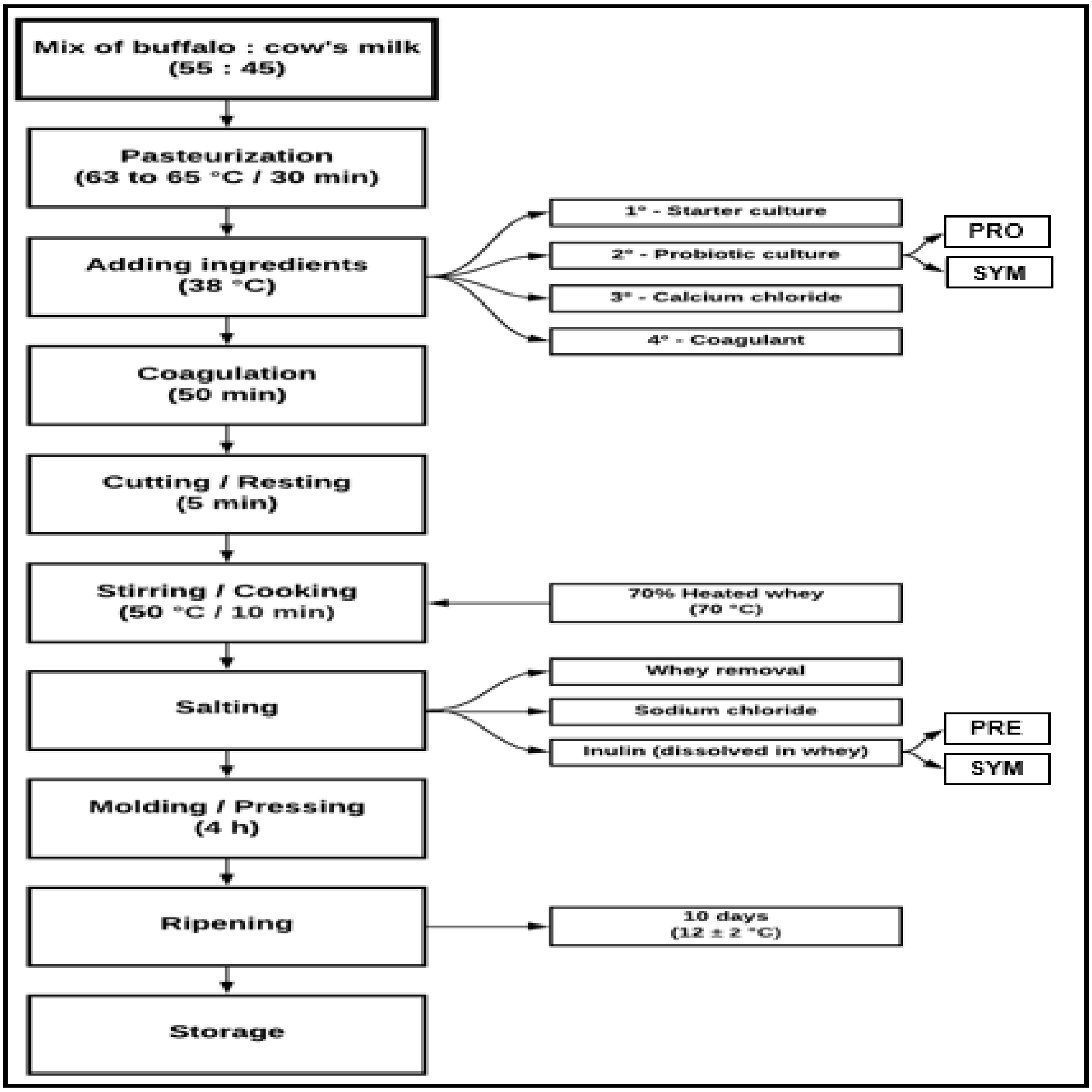
2.1 OBTAINING AND ANALYZING RAW MATERIAL

The raw material (Buffalo and cow milk) was obtained at the Experimental Farm of Entre Rios of Federal University of Bahia (UFBA), and all the analyzes took place at the Milk and Derivatives Inspection and Technology Laboratory (LaITLácteos), located at Universidade Federal da Bahia (UFBA), in Salvador - BA. The samples from both matrices (buffalo and cow milk) were subjected to physicochemical analyzes with an automatic ultrasound milk analyzer (Model LACTOSCAN SP®), and the measured parameters were total solids, solids-not-fat, fat, lactose, protein, ash, and density.

2.2 TECHNOLOGICAL CHEESE PROCESSING

Four formulations of Coalho cheese with buffalo and cow milk (55:45 ratio) were developed to include functional ingredients (Figure 1). CTL - control Coalho cheese (without the addition of probiotic or prebiotic); PRO - potentially probiotic Coalho cheese (with L. plantarum); PRE - Potentially prebiotic Coalho cheese (with inulin); SYM - potentially symbiotic Coalho cheese (with L. plantarum and inulin).

Figure 1. flowchart of elaboration of Brazilian Coalho buffalo cheese with functional potential



The buffalo and cow milk (55:45 ratio), previously mixed according to Rekowsky et al. (2020), was pasteurized (65ºC/30min) and cooled to 38 °C. The ingredients used were non-probiotic mesophilic homofermentative starter culture of Lactococcus lactis subsp. cremoris and Lactococcus lactis subsp. lactis (CR-704 from CHR HANSEN® at 0.02%), calcium chloride (0.02%), liquid coagulant CMAX® (according to the manufacturer's instructions), inulin 1% (Sweetmix®) and sodium chloride (0.7% Sal Lebre®). The first three ingredients were added in that order by constant agitation. Probiotic (PRO) and symbiotic (SYM) treatments received the probiotic mesophilic heterofermentative culture L. plantarum (Figure 1) to maintain a count of $5x10^9$ CFU / mL viable probiotic cells. The starter culture (Lactococcus spp.) was previously prepared and inoculated from the mother culture while the probiotic was added in lyophilized form.

After adding the liquid coagulant, the rest period was taken (50min) until obtaining the curd cut point.

The curd was cut into cubes of 1.5 to 2.0 cm, using a spatula followed by a resting period of5 min. The curd processing was carried out in two stages: (i) slow stirring for 3 min and resting for 3 min with the repetition of this process three times; (ii) removal of whey (60%) and heating (to 70ºC) to raise the temperature of the curd to 50ºC for 10min of cooking. Afterward, 90% of whey was drained, and sodium chloride (0.7%) was added to the curd. The inulin 1% (w/w) (Sweetmix®) was previously dissolved in heated whey (Karimi et al., 2015), added to the PRE and SYM treatments, and homogenized following the molding and pressing stages. Specific plastic molds for cheese were used and pressing was carried out for 4 hours. Soon after, they were submitted to the ripening process at 12 ± 2 ºC and approximately 85% humidity with turns every 12 hrs for ten days.

2.3 BACTERIOLOGICAL AND PH ANALYZES DURING RIPENING TIME.

Bacteriological analyzes were performed during ripening (Days 0, 4, 8, 10). For the count of L. plantarum, an acidified MRS culture medium (pH 5.4) enriched with 1% fructose was used (APHA, 2015). For the count of Lactococcus spp. M17 culture medium enriched with 0.5% lactose was used, incubating aerobically at 30º C for 24 to 48 hours (Moreira et al., 2019). Results were expressed as a log CFU/g.

2.4 PHYSICOCHEMICAL ANALYZES FOR DEVELOPED CHEESES

The pH determination was performed using a pH meter (AOAC, 2012) during the ripening process (Days 0, 4, 8, 10). The physicochemical analyzes were performed after ten days of ripening when the product was considered ready for consumption. So, on D0 of storage, the cheeses were analyzed for moisture content and dry matter in an infrared moisture analyzer (BEL MARK i-Thermo®), fat content by the Gerber method (AOAC, 2012), and the fat content in the dry matter was obtained by dividing the fat by the dry matter multiplied by 100 to obtain %FDM.

2.5 STATISTICAL ANALYSIS

The parameters of milk matrices were compared by analysis of unpaired T-test with a 95% confidence level ($P < 0.05$). All data about characterization parameters of cheese (pH, moisture, fat, fat in the dry matter), as well as bacteriological analyzes (Lactococcus spp. and L. plantarum) were organized and submitted to analysis of variance

(ANOVA one-way) with tests post hoc Tukey the 95% confidence level. The statistical analysis was performed using GraphPad Prism Software (version 5.02 for Windows).

3 RESULTS AND DISCUSSION

3.1 COMPOSITION OF BUFFALO AND COW MILK

The values obtained for fat, lactose, protein, and salts content were significantly higher ($P < 0.05$) in the composition of buffalo milk when compared to cow milk (Table 1), which is reflected in a higher total solids content ($P < 0.05$) for buffalo matrix. The results obtained for cow milk are following the recommendations of the legislation (Brasil, 2018), and, therefore, it was considered an adequate composition for the development of dairy products.

Table 1. Physicochemical composition (mean ± standard deviation) for buffalo and bovine milk

Parameters	**Type of milk**		**P-Value[1]**	**References[2]**	**References[3]**
	Buffalo milk	**Cow milk**			
Total solids (%)	15.27 ± 0.17	12.68 ± 0.09	< 0.0001	mín 11.4	15.60
Solids-not-fat (%)	10.12 ± 0.11	8.99 ± 0.09	< 0.0001	mín 8.4	9.72
Fat (%)	5.14 ± 0.23	3.69 ± 0.11	< 0.0001	mín 3.0	6.17
Lactose (%)	5.57 ± 0.06	4.94 ± 0.05	< 0.0001	mín 4.3	4.91
Protein (%)	3.69 ± 0.04	3.28 ± 0.04	< 0.0001	mín 2.9	4.25
Ash (%)	0.83 ± 0.01	0.73 ± 0.01	< 0.0001	ND	0.82
Density (%)	1.033 ± 0.00	1.031 ± 0.00	< 0.0001	1.028 a 1.034	1.034

ND = Not Determined
[1] Unpaired T-Test at confidence intervals of 95%
[2] According to Brazilian regulations for bovine milk (Brasil, 2018)
[3] Mean values reported in the literature for buffalo milk (Godinho et al., 2020; Rekowsky et al., 2020; Nayak, Ramachandra, and Kumar, 2020; Khalifa and Zakaria, 2019).

The composition of buffalo milk showed similar values compared to others observed in the literature (Table 1). The higher total solids content, mainly attributed to a more significant amount of fat, positively impacts the sensory and economic characteristics of buffalo milk (Verma et al., 2020), and the high content of protein and minerals are essential characteristics for the production of milk derivatives. These parameters can be associated with a higher yield in cheese production (Franceschi et al., 2020).

In addition to the amount of fat, the lipid profile also differs between the milk of both species and, according to Verma et al. (2020), about 187 lipid compounds are found only in buffalo milk and 170 only in cow milk. Buffalo milk has higher percentages of short- and long-chain fatty acids when compared to cow milk and is thus related to more beneficial health characteristics (Khan et al., 2017), which is a desirable feature for health-conscious consumers.

Buffalo milk showed a higher amount of protein ($P < 0.05$) when compared to cow milk. As for this protein profile, the more significant presence of alpha-s2-casein and k-casein is characteristic of buffalo milk and leads to faster clotting (Abd El-Salam and El-Shibiny, 2011). However, the casein micelles present in the buffalo milk are less hydrated and more mineralized when compared to cow milk, which can lead to the formation of a firmer- and drier-than-ideal curd during technological cheese processing (Hussain, Bell and Grandison, 2013).

Due to the differences related to the composition of the dairy matrices, several studies bring information to elucidate which is the best raw material for elaborating dairy products, either by the yield, composition, nutritional value, and sensory acceptance. Although cow milk is more widely marketed and used in dairy products, buffalo milk is associated with greater viability and resistance of probiotic bacteria, an alternative for vehiculating these beneficial microorganisms (Silva et al., 2020). Thus, strategies to diversify and enhance the use of milk of different species have been consolidated, for example, through the positive effect of the mixture of buffalo and cow milk in cheese-making (Rekowsky et al., 2020).

3.2 PHYSICOCHEMICAL CHARACTERISTICS OF DEVELOPED CHEESES

In the physicochemical characterization of cheeses, there was no significant variation ($P > 0.05$) regarding the moisture and the total solids content of all treatments, meaning the addition of the prebiotic and the probiotic ingredients did not affected these parameteres. As higher amounts of inulin are known for decreasing moisture and increasing total solids (Giri, Kanawjia and Singh, 2017), the addition of 1% inulin in coalho cheese is indicated, since low moisture is related to hardness problems in cheese (Fox et al., 2017). On the other hand, in other studies, the moisture content showed higher values between 37.46% and 51.34% for Coalho cheeses (Silva et al., 2021a) even in semi-hard cheese made with alternative milk species (Silva et al., 2021b) in accordance with characteristics for this type of cheese.

Table 2. Composition (mean ± standard deviation) of developed cheese and whey obtained on the process

Cheese Parameters	Treatments[1] CTL	PRE	PRO	SYM	P-value
Moisture (%)	$39.73^{a} \pm 0.42$	$40.49^{a} \pm 0.40$	$40.41^{a} \pm 1.27$	$38.09^{a} \pm 0.87$	0.063
Dry matter (%)	$60.27^{a} \pm 0.42$	$59.38^{a} \pm 0.26$	$59.59^{a} \pm 1.27$	$61.91^{a} \pm 0.87$	0.051
Fat (%)	$20.00^{b} \pm 0.82$	$25.33^{a} \pm 1.25$	$22.00^{ab} \pm 1.63$	$22.00^{ab} \pm 0.82$	0.012
FDM[2] (%)	$33.17^{b} \pm 1.12$	$42.66^{a} \pm 1.96$	$36.88^{b} \pm 3.5$	$35.53^{b} \pm 1.16$	0.002

CTL = Control Coalho cheese; PRO = Coalho cheese with probiotic; PRE = Coalho cheese with prebiotic; SYM = Coalho cheese with probiotic and prebiotic.
[1] Different letters in the same row point to a significant difference on the same treatment (p<0.05).
[2] FDM = Fat in dry matter.

Fat differed (P < 0.05) only in the treatment with inulin, presenting the highest content. Similarly, the fat in dry matter of PRE obtained higher values (P < 0.05) when compared to the CTL, PRO, and SYM treatments. These values were also lower when compared to the FDM found in other Coalho cheeses (between 47.71 and 60.01) in the market (Silva et al., 2021). The casein present in milk promotes a network structure that captures fat globules and other solids during the clotting process (Cecchinato et al., 2012). Indeed, although inulin does not directly influence the aggregation of casein micelles, it does favor the retention of solids and water depending on the variation of its loss through syneresis (Karimi et al., 2015). According to the analyzed variables, 1% inulin in PRE may have led to more excellent solids retention, such as fat. In this context, the differences between buffalo and cow milk casein ratio and other characteristics such as the amount of fat could lead to the observed variation in cheese parameters. Moreover, as there was a significant difference (P < 0.05) regarding the FDM between PRE and SYM, we can assume differences in the loss of inulin in whey during cheese-making occurred, which should be evaluated in future works.

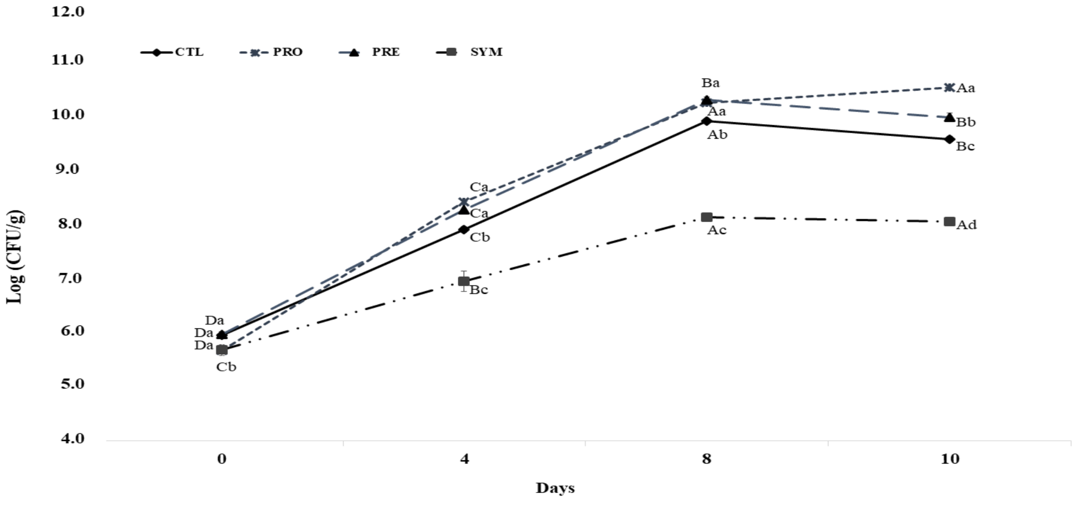
3.3 MICROBIOLOGICAL CHARACTERISTICS OF CHEESE

The viable counts of Lactococcus spp. in cheese treatments are displayed in figure 2. A similar pattern was observed, whereas from day 0 to 8, a constant and expressive growth (P < 0.05) was achieved in all treatments. In addition, the counts of Lactococcus spp. in PRO and PRE were higher (P < 0.05) than CTL and SYM, respectively, which suggests a synergistic effect of Lactococcus spp. in the presence of L. plantarum or inulin. Indeed, inulin has been linked to a progressive increase in lactococci viable cells in cheese

(Melilli et al., 2021). Also, a collaborative effect between L. lactis and L. plantarum has already been discussed in the literature (Requena, Pelaez and Fox, 1993).

On the other hand, the SYM treatment presented lower (P < 0.05) viability of Lactococcus spp. during all ripening period, which is probably attributed to an antagonistic effect while in the presence of L. plantarum and inulin together. Similar to our results, Minervini et al., (2017) reported lower viability of Lactococcus spp. in the presence of a co-culture of L. plantarum and L. rhamnosus in burrata cheese fortified with inulin. Finally, on the last day of ripening, the counts of Lactococcus spp. for CTL, PRO, PRE, and SYM were 9.6, 10.5, 10.0, and 8.1 Log (CFU/g), respectively. These values are higher than those find for Costa Lima and collaborators (2018), which presented counts between 5.85 and 6.55 Log (CFU / g) on the first day of storage but following those findings in Coalho cheese between 8.45 and 11.36 Log (CFU/g) market in Brazilian Northeast (Medeiros et al., 2016).

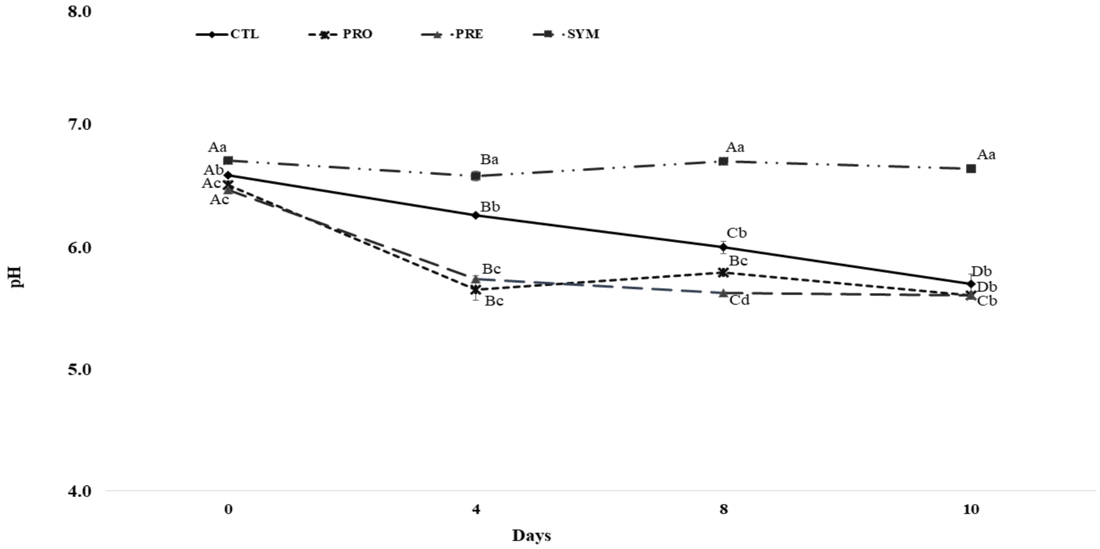
Figure 2. Growth variation of Lactococcus spp. during the ripening of potentially functional Brazilian mixed Coalho cheeses.
CTL = Control Coalho cheese; PRO = Coalho cheese with probiotic; PRE = Coalho cheese with prebiotic; SYM = Coalho cheese with probiotic and prebiotic. Different lowercase letter in the same column = significant difference on the same day (P < 0.05). Different capital letter on the same line = significant difference in the same treatment on different days (P < 0.05).



During the evaluated period, the viability of L. plantarum was not observed. Therefore, the pH variations were (Figure 3) mainly attributed to the fermentation process of the lactic acid bacteria inoculated as starter cultures.

Figure 3. Variation of pH values during the ripening period and Day 0 of storage of the potentially functional Brazilian mixed Coalho cheeses.

CTL = Control Coalho cheese; PRO = Coalho cheese with probiotic; PRE = Coalho cheese with prebiotic; SYM = Coalho cheese with probiotic and prebiotic. Different lowercase letter in the same column = significant difference on the same day (P < 0.05). Different capital letter on the same line = significant difference in the same treatment on different days (P < 0.05)



The pH of SYM remained high (6.6 to 6.7) when compared to the other treatments (P < 0.05) throughout the evaluated period, which corroborates with the lower viability of Lactococcus spp. in this treatment. However, it approached the characteristic pH of the Coalho cheese, between 6.0 and 7.2, observed by Costa Lima and collaborators (2018). On the last day of ripening, the pH showed values between 5.8 and 5.6 (P > 0.05) for the CTL, PRE, and PRO, reflecting the greater viability of Lactococcus spp. in these treatments.

The inclusion of probiotic cultures in dairy products is a challenge and can suffer competitive antagonism by the starter culture (Jesus et al., 2016). In Coalho cheese, even in the presence of other lactic acid bacteria such as Enterococcus spp., Leuconostoc spp., Lactococcus spp., and Lactobacillus spp., the presence of L. plantarum was commonly detected, indicating good chances of survival in this dairy product (Medeiros et al., 2016). Thus, strategies such as preparing probiotic cultures on a substrate before inoculation in the product (Cruz et al., 2009) and adapting technological processing by adding the probiotic only after the heating steps (Jesus et al., 2016) can assure greater chances of viability in functional Coalho cheeses during ripening and storage period. In vitro, the L. plantarum need to establish a short adaptation phase depending mainly on the temperature, amount of substrate, pH, and NaCl concentration (Agudelo, Ortega and

Hoyos, 2010; Dalcanton et al., 2018). Despite this ease of in vitro adaptation, the relevance of studies regarding this process to the best inoculation stage, adaptation time in milk, and the competitive presence of other microorganisms (starter culture) are directly influencing the probiotic viability at functional levels in the final product. Still, one can consider the possibility of individual addition of non-starter cultures considering the viability and the contribution to the sensory characteristics of the cheeses (Guarrasi et al., 2017). Thus, these results must be considered so that new strategies can be studied to allow the viability of the L. plantarum in ripened buffalo cheese.

4 CONCLUSION

In conclusion, the inulin addition as a prebiotic ingredient on the standard technology of coalho cheese did not cause significant changes in PRE and SYM physicochemical characteristics. This fact demonstrates the capability of inulin inclusion in cheese without impairing characterization parameters. However, the absence of *L. plantarum* counts points to the unattainability of using these bacteria as a probiotic ingredient by direct inoculation. Furthermore, studies to evaluate texture and rheology parameters must be performed as new tests to adapt the technological processing of Coalho cheese or select other probiotic cultures to enable proposals for functional buffalo dairy products.

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