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Image-Based Analysis of the Bactericidal Effect of *Lactobacillus acidophilus* Application in Cheeses Made from Raw Milk

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Coliforms have a high incidence as causative agents of foodborne illnesses. This study aimed to assess the bactericidal activity of *Lactobacillus acidophilus* at varying concentrations against total coliforms in fresh cheeses made from raw milk, employing imaging techniques and microorganism counts. Six milk samples were collected from the Model and Central markets in Barranca, Peru. Triplicate total coliform counts were conducted for each sample, and the most contaminated one was selected for further analysis. This sample underwent three treatments with different concentrations of *Lactobacillus acidophilus* (5, 6, and 7 log CFU 10 ml^{-1}) to produce fresh cheeses. Subsequently, triplicate total coliform counts were performed for each treatment. An image analysis of the cheese structure was conducted to quantify the cavities and their areas caused by coliform bacteria. Furthermore, a control cheese was prepared for subsequent comparison to determine the final reduction in population. The control cheese displayed a total coliform count of 6.9 ± 0.02 log CFU g^{-1}, 214 ± 12.03 cavities, and 0.4 ± 0.2 mm^2. Post-application of *Lactobacillus acidophilus* resulted in a maximum reduction of 3.79 ± 0.09 log CFU g^{-1}, yielding 44 ± 6.88 cavities with 0.1 ± 0.04 mm^2 compared to the control sample. Treatment with a concentration of 7 log CFU 10 ml^{-1} of *Lactobacillus acidophilus* exhibited the most significant bactericidal effect on the total microbial population of coliforms present in fresh cheeses made from raw milk obtained from the Model and Central markets in the city of Barranca.

1. Introduction

Food safety relies on the implementation of necessary measures to protect consumers from harm when preparing or consuming food (Le Coq et al., 2022). A significant portion of global public health issues is attributed to Foodborne Diseases (FBDs), where various biological, physical, or chemical agents may be involved (Martínez et al., 2017).
Enterobacteria, including certain pathogens like Salmonella and Escherichia coli, exhibit a higher incidence of foodborne diseases FBDs affecting millions of individuals and can lead to severe consequences such as hemolytic uremic syndrome (Tack et al., 2019). These bacteria can be transmitted through the consumption of raw or undercooked meat, vegetable, or raw milk, often originating from cross-contamination during food handling, processing, and/or storage (Antunes et al., 2019).
The high presence of total coliform microbial load in raw milk intended solely for dairy industry use serves as an indicator that this food is not adhering to proper hygiene measures during its collection. The presence of these microorganisms is crucial in determining its quality according to the sanitary standards established in the Ministerial Resolution No. 591-2008/MINSA (Ministerio de Salud del Perú, 2008), since these bacteriological agents are directly linked to handling practices, tools, storage, and the water used for extraction (Faruk and Akhter, 2011).
On numerous occasions, this same milk is used to craft fresh cheeses in unhygienic conditions for subsequent sale. Fresh cheeses, furthermore, represent potential reservoirs for bacterial contamination due to their favorable conditions for microbial growth, facilitated by their water content and rich nutrient composition (Gallegos et al., 2007). A swift means of assessing the sanitary quality of fresh cheeses is through the presence of cavities, which are generated by gases produced from lactose metabolism by

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contaminating microorganisms such as coliforms, swiftly revealing the inadequate hygiene employed during their production (Sheehan, 2011).

Research conducted to ascertain the bactericidal effect of *Lactobacillus acidophilus* (*L. acidophilus*) on pathogenic bacteria has demonstrated its substantial bactericidal effect against *Escherichia coli* (Durán et al., 2010), *Listeria monocytogenes*, *Staphylococcus aureus*, among other microorganisms (Shokryazdan et al., 2014). This bactericidal effect against certain pathogens is attributed to its ability to produce bacteriocins (Ozogul et al., 2022), which are antimicrobial compounds. These have garnered attention in the food industry owing to their potential application as additives, substituting chemicals, these applications could range from food bioconservatives to combatting foodborne pathogens (Garcha and Natt, 2012). Lactic acid bacteria like *L. acidophilus* are considered beneficial to humans as they confer benefits to intestinal microbiota and health, such as reducing gastrointestinal symptoms in lactose-intolerant individuals, alleviating constipation, and preventing traveler's diarrhea (Aimutis, 2014).

For proper preparation and preservation of fresh cheeses, it's necessary to employ methods for microbial inhibition, such as pasteurization (Macdonald et al., 2011), and the use of chemical additives like sodium or potassium sorbate. However, the use of chemical additives could lead to problems for consumers, such as allergies, nausea, etc., upon prolonged consumption (De la Rosa et al., 2020). Hence, the importance of seeking, promoting, and evaluating new alternatives, such as the use of lactic acid bacteria as potential natural bioconservatives. Continuously evaluating the bactericidal activity of *L. acidophilus* is recommended since the direct effect on foods is not widely understood. Therefore, this research aimed to evaluate, via imaging and microorganism count, the bactericidal activity of *L. acidophilus* at different concentrations against total coliforms in fresh cheeses made from raw milk.

2. Methodology

2.1. Sample Collection:

Raw milk samples from the Model and Central markets in the city of Barranca were used for the study, 4 and 2 samples respectively, obtaining 10 ± 0.03 L from each stall while maintaining them refrigerated at 4 ± 1 °C (Villegas et al., 2018). The milk exhibited a pH of 6.5 ± 0.1, a density of 1.030 ± 0.001 g/ml, and characteristic color, taste, and odor.

2.2. Microbiological Analysis:

To conduct the analysis of total coliforms in the collected milk samples, triplicate analyses were performed for each milk vending stall. Initially, 10 ml of milk sample was measured and mixed with 90 ml of sterilized water to achieve homogenization. Subsequently, an aliquot of 1 ml was extracted and transported in tubes containing 9 ml of sterile water each (Tascı, 2011). Dilutions were made up to 10^{-5} CFU/ml, and 1 ml aliquots were extracted for incubation on 3M™ PetrifilmTM Coliforms plates for 24 ± 2 hours at 32ºC ± 1°C, following the official method 986.33 of the Association of Analytical Communities (2000). Microbial loads were expressed as the average base 10 logarithm of colony-forming units per ml (log CFU ml^{-1}) (Torlak et al., 2013). Subsequently, the sample with the highest microbial load underwent both the control treatment (without *L. acidophilus*) and treatment with *L. acidophilus* at different concentrations (5, 6 y 7 Log CFU 10 ml^{-1}).

2.3. Preparation of Cheese with *Lactobacillus acidophilus*:

One liter of milk was used for each inoculation with *L. acidophilus* obtained from the Puritan´s Pride^{R} brand dispensed in tablets with concentrations of 8 Log CFU, previously activated (Guzmán et al., 2016). This initial concentration was adjusted to concentrations of 5, 6, and 7 Log CFU 10 ml^{-1} to be incorporated into each liter of milk (Taco et al., 2021), maintaining a temperature of 38 ± 2 °C. The milk was left at this temperature variation for 120 ± 3 minutes. For cheese production, the milk was at 38° ± 1 °C. Approximately 0.025 ± 0.005 g of rennet (Caglificio Clerici, Type P) was added, and it was kept at this temperature variation for about 25 ± 3 minutes until coagulation occurred. Then, the curd was cut into small cubes, followed by draining and manual pressing. The cheese was stored at 4 ± 1 °C.

For the microbiological analysis of the cheese, the same aforementioned steps were followed, and the results were expressed as the average base 10 logarithm of colony-forming units per gram (log CFU g^{-1}).

To determine the population reduction using equation (1), the initial control population count (P_0) was subtracted from the final population count (P) obtained after the treatment with *L. acidophilus* (Caro et al., 2021), (Gill et al., 2019).

$$\text{Population reduction} = \text{Log}_{10}(P_0) - \text{Log}_{10}(P) \quad (1)$$

2.4. Analysis of Cheese Hole Structure

For the image analysis, a 1600 mm^2 area of each cheese produced (Figure 1) was selected. The number of cavities and their respective areas (mm^2) were analyzed using Ilastik version 1.4.0 and ImageJ version 1.8.0 software. Images were captured using a smartphone (Apple, model 14, USA). An initial image treatment was conducted using the Ilastik program to enhance cavity visibility. The pixel classification tool was used to remove image backgrounds. Subsequently, the processed image was exported to ImageJ to establish a binary mask. The image was converted to 8 bits to facilitate analysis, a Gaussian Blur filter (0.5) was applied, brightness and contrast were adjusted, a threshold was set, and finally, particle analysis was performed (Li et al., 2021). The average area of each cavity in mm^2 was calculated by dividing the total area by the number of cavities.

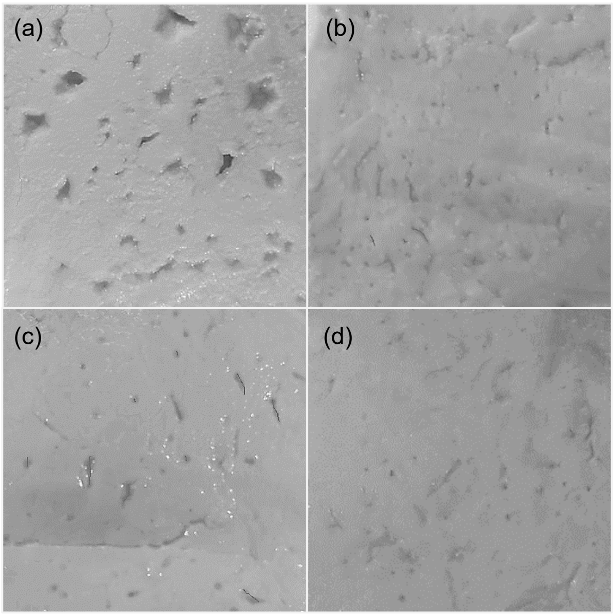

Figure 1. The different types of cheese studied were as follows: (a) Control cheese, (b) Cheese with a concentration of 5 Log CFU per 10 ml of L. acidophilus, (c) Cheese with a concentration of 6 Log CFU per 10 ml of L. acidophilus, and (d) Cheese with a concentration of 7 Log CFU per 10 ml of L. acidophilus.

2.1. Statistic analysis:

In this research study, Pearson's correlation coefficient and analysis of variance (ANOVA) were employed as statistical models to analyze the effect of *L. acidophilus* on total coliforms. Additionally, the study aimed to identify the most effective concentration. Statistical analysis was conducted using the JASP statistical software (version 0.17.2.1). A significance level of a p-value less than 0.05 was established to determine correlations and significant differences among treatments. A multiple comparisons test (Tukey's test) was also performed.

3. Results and discussions

The control cheese exhibited results of 6.9 ± 0.02 log CFU g^{-1} of total coliforms, displaying a structure with 214 ± 12.03 cavities and 0.4 ± 0.2 mm^2. This outcome was considered the initial population.

Total coliform counts in raw milk samples from the Central and Modelo markets in Barranca ranged from 4.78 ± 0.02 to 6.93 ± 0.02 log CFU ml^{-1} (Table 1). These results are alarming as the maximum accepted value according to Ministerial Resolution No. 591- 2008/MINSA, is 2 log CFU ml^{-1}, indicating a microbiological quality issue. They underscore the insufficient sanitary practices among milk producers and vendors (Pyz et al., 2015; Van et al., 2004).

Table 1: Total coliform count in raw milk from Central and Modelo markets in Barranca.

Sample	Total coliform counte (Log CFU ml^{-1})
M_1	4.78 ± 0.02^d
M_2	4.95 ± 0.03^c
M_3	4.82 ± 0.01^d
M_4	4.85 ± 0.04^d
M_5	5.56 ± 0.03^b
M_6	6.93 ± 0.02^a

The final results obtained after treatments at different concentrations with *L. acidophilus* are presented in Table 2. This allowed us to determine that the application of *L. acidophilus* at higher concentrations had a better effect on the reduction of total coliform populations, showing a positive correlation (0.98). This treatment achieved a maximum population reduction of 3.79 ± 0.09 log CFU g^{-1}. Additionally, there was a reduction in the number and area of cavities compared to the control sample, which initially had 214 ± 12.03 cavities and 0.4 ± 0.2 mm^2, reaching 44 ± 6.88 cavities and 0.1 ± 0.04 mm^2 with the highest concentration of *L. acidophilus*. Moreover, a strong positive correlation (0.98) was found between the concentration of *L. acidophilus* and the reduction in coliforms, between the reduction in coliforms and the number of cavities (-0.96), and a moderate correlation between the coliform population and the area of cavities (0.62).

These results are concordant with a reduction to undetectable levels after applying a combination of lactic acid bacteria (*L. acidophilus* and *Lactobacillus casei*) between 10^7 and 10^8 CFU/g in yogurt samples contaminated with *Escherichia coli* after 8 days of application (Calderón et al., 2007), additionally, a similar study evaluated the quality of yogurt after the application of *L. acidophilus*, achieving an improvement in its sensory profile and inhibiting the development of total coliforms, molds, and yeasts, thus extending its shelf life up to 39 days (Hussien et al., 2022). Another study conducted co-cultures of *L. acidophilus* strains (10^6 and 10^7 CFU/g) along with *Escherichia coli* in skim milk, obtaining a population reduction of 4 log CFU/g during the first 48 hours. Subsequently, none of the *Escherichia coli* cells were viable (Sreekumar & Hosono, 2000). This variation in the effectiveness of the bactericidal capacity can be explained by the fat content of the medium where it is applied. Studies have shown that bacteriocins can be affected by this factor, although not significantly with milk fat (da Silva Malheiros et al., 2012). In vitro tests also demonstrated inhibition diameters ranging from 10 to 11 mm for *Escherichia coli* (Durán et al., 2010; Shokryazdan et al., 2014). In one investigation, the application of *Lactobacillus paracasei* (10^6 CFU/ml) in the production of Argentine cream cheese was evaluated to control the formation of cavities. During the 30 days of maturation, it was able to reduce the metabolic activity of gas-producing microorganisms, thus preventing the formation of cavities, compared to the control group where cavities represented 0.31% of the total cheese volume (0.97 ml) (Giménez et al., 2021). Additionally, in another study, following the application of a bacteriophage cocktail (10^7 CFU/ml), a microbial reduction of 3 Log CFU/g was achieved in cheeses contaminated with *Escherichia coli* after 10 days of treatment. A decrease in the number and size of cavities was also observed, reducing them to less than 2 mm² (Tabla et al., 2022).

Table 2: Effect of the application of L. acidophilus on population reduction of total coliforms

Treatment	*L. acidophilus* concentration (Log CFU 10 ml^{-1})	Total coliform population reduction (Log CFU g^{-1})	Number of cavities	Area (mm^2)
T_0	0	-	214.00 ± 12.03^a	0.40 ± 0.20^a
T_1	5	2.66 ± 0.06^a	129.75 ± 17.99^b	0.45 ± 0.19^a
T_2	6	3.23 ± 0.07^b	73.63 ± 9.86^c	0.40 ± 0.10^a
T_3	7	3.79 ± 0.09^c	44.00 ± 6.88^d	0.10 ± 0.04^b

Additionally, it is important to mention that the final pH of the cheese was 5.28 ± 0.03 for the maximum concentration of *L. acidophilus*. This decrease in pH compared to raw milk wouldn't entirely explain the microbial reduction, as it is not a hindrance to the development of total coliforms (gram-negative bacteria), given that they have been reported to survive in acidic pH levels (Wahyuni, 2015). The population reduction could be explained by compounds formed by lactic acid bacteria such as bacteriocins; strains of L. acidophilus produce lactocidin bacteriocins (Cintas et al., 1998), which exhibit a wide range of activity against gram-negative bacteria (Coconnier et al., 1997). This action is due to a combination of hydrogen peroxide (Naidu et al., 1999), organic acids (lactic and acetic acid), and antibiotics (acidocin A and acidocin B) (Hamdan & Mikolajcik, 1974), (Ozogul et al., 2022). These bacteriocins are peptides that can cause the lysis of the cell wall through enzymatic actions (Aljohani et al., 2023), (Todorov et al., 2011).

4. Conclusions

The application of *L. acidophilus* at a higher concentration favored the reduction of contaminating microorganism populations. The treatment with a concentration of 7 log CFU 10 ml^{-1} of *L. acidophilus* had the most significant bactericidal effect on the total coliform microbial population. Moreover, it successfully reduced the number of cavities and their area in fresh cheeses made with raw milk. This demonstrates its potential application as a natural preservative, replacing chemical preservatives.

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References

Aimutis, W. R. (2014). Microflora of the intestine | Biology of Lactobacillus acidophilus, in C. A. Batt & M. L. Tortorello (Eds.), Encyclopedia of Food Microbiology (Second Edition). Oxford: Academic Press, (pp. 646-651).

Aljohani, A. B., Al-Hejin, A. M., & Shori, A. B. (2023). Bacteriocins as promising antimicrobial peptides, definition, classification, and their potential applications in cheeses. Food Science and Technology, 43, e118021.

Antunes, P., Novais, C. and Peixe, L. (2019). Food-to-Humans Bacterial Transmission, in Microbial Transmission. John Wiley & Sons, Ltd, pp. 161–193.

Association of Official Agricultural Chemist (AOAC). Official methods of analysis. 17th Edition. USA; 2000.

Calderón, O., Padilla, C., Chaves, C., Villalobos, L., & Laura Arias, M. (2007). Evaluation of the effect of the probiotic culture Lactobacillus rhamnosus added to natural yogurt and with commercial probiotics on populations of Staphylococcus aureus, Escherichia coli O157, Listeria monocytogenes, and Salmonella enteritidis. Archivos Latinoamericanos de Nutrición, 57(1), 51-56. (in Spanish)

Caro-Degollar, E. M., Fernández, F., Miranda, D. J., Vásquez, G. N., Bautista, F. A., & Nunja-García, J. V. (2021). Effect of ozonized water on the population reduction of Escherichia coli in minimally processed vegetables. Peruvian Agricultural Research, 3(2). (in Spanish)

Cintas, L. M., Casaus, P., Fernández, M. F., & Hernández, P. E. (1998). Comparative antimicrobial activity of enterocin L50, pediocin PA-1, nisin A and lactocin S against spoilage and foodborne pathogenic bacteria. Food Microbiology, 15(3), 289-298.

Coconnier, M. H., Liévin, V., Bernet-Camard, M. F., Hudault, S., & Servin, A. L. (1997). Antibacterial effect of the adhering human Lactobacillus acidophilus strain LB. Antimicrobial Agents and Chemotherapy, 41(5), 1046-1052.

De la Rosa-Alcaraz, M. de los Á., Ortiz-Estrada, Á. M., Heredia-Castro, P. Y., Hernández-Mendoza, A., Reyes-Díaz, R., Vallejo-Cordoba, B., & González-Córdova, A. F. (2020). Poro de Tabasco cheese: Chemical composition and microbiological quality during its artisanal manufacturing process. Journal of Dairy Science, 103(4), 3025-3037.

da Silva Malheiros, P., Sant'Anna, V., Utpott, M., & Brandelli, A. (2012). Antilisterial activity and stability of nanovesicle-encapsulated antimicrobial peptide P34 in milk. Food Control, 23(1), 42-47.

Durán Lengua, M., Montero Castillo, P., Flórez Díaz, W., Franco de la Hoz, V., & Coneo Rodríguez, R. (2010). Hygienic-sanitary evaluation and antagonistic action of commercial Lactobacillus strains against pathogenic microorganisms (Escherichia coli) present in layered cheese from the municipality of Mompox. Revista Científica, 20(3), 312-317. (in Spanish)

Faruk, M. O., and Akhter, M. Z. (2011). Presence of Coliforms and Fecal Coliforms in Fast Food Items of Local Restaurants and Fast Food Outlets of Dhaka City. Bangladesh Journal of Microbiology, 28(1).

Gallegos, J. G., Arrieta, G., Máttar, S., Poutou, R., Trespalacios, A., & Carrascal, A. (2007). Frequency of Listeria spp. in Colombian Costeño Cheese. Revista MVZ Córdoba, 12(2). (in Spanish)

Garcha, S. and Natt, N.K. (2012). In situ control of food spoilage fungus using Lactobacillus acidophilus NCDC 291. Journal of Food Science and Technology, 49(5), pp. 643–648.

Gill, A., Tamber, S., & Yang, X. (2019). Relative response of populations of Escherichia coli and Salmonella enterica to exposure to thermal, alkaline and acidic treatments. International Journal of Food Microbiology, 293, 94-101.

Giménez, P., Peralta, G. H., Guglielmotti, D., Audero, G., Páez, R., Hynes, E. R., & Bergamini, C. V. (2021). Preventing undesired eye formation in soft cheese. International Dairy Journal, 116, 104958.

Hamdan, I. Y., & Mikolajcik, E. M. (1974). Acidolin: an antibiotic produced by lactobacillus acidophilus. The Journal of Antibiotics, 27(8), 631-636.

Hussien, H., Abd-Rabou, H. S., & Saad, M. A. (2022). The impact of incorporating Lactobacillus acidophilus bacteriocin with inulin and FOS on yogurt quality. Scientific Reports, 12(1), 13401.

Le Coq, J.-F., Grisa, C., Guéneau, S., & Niederle, P. (Eds.). (2022). Public policies and food systems in Latin America. éditions Quae.

Li, C., Ma, X., Deng, J., Li, J., Liu, Y., Zhu, X., Liu, J., & Zhang, P. (2021). Machine learning-based automated fungal cell counting under a complicated background with ilastik and ImageJ. Engineering in Life Sciences, 21(11), 769-777.

Macdonald, L.E. et al. (2011). A Systematic Review and Meta-Analysis of the Effects of Pasteurization on Milk Vitamins, and Evidence for Raw Milk Consumption and Other Health-Related Outcomes, Journal of Food Protection, 74(11), pp. 1814–1832.

Martínez-Vasallo, A., Ribot-Enríquez, A., Villoch-Cambas, A., Montes de Oca, N., Remón-Díaz, D., & Ponce-Ceballo, P. (2017). Quality and safety of raw milk under the current Cuban conditions. Revista de Salud Animal, 39(1), 51-61.

Ministerio de Salud del Perú. (2008, 29 de agosto). Ministerial Resolution No. 591- 2008/MINSA: Sanitary Standard that establishes the microbiological criteria for sanitary quality and safety for food and beverages for human consumption. El Peruano. Retrieved from www.gob.pe/institucion/minsa/normas-legales/247682-591-2008-minsa. (in Spanish)

Naidu, A. S., Bidlack, W. R., & Clemens, R. A. (1999). Probiotic Spectra of Lactic Acid Bacteria (LAB). Critical Reviews in Food Science and Nutrition, 39(1), 13-126.

Ozogul, F., Yazgan, H., & Ozogul, Y. (2022). Lactic Acid Bacteria: Lactobacillus acidophilus☆, in P. L. H. McSweeney & J. P. McNamara (Eds.), Encyclopedia of Dairy Sciences (Third Edition) (pp. 187-197). Academic Press.

Sheehan, J. J. (2011). Cheese | Avoidance of Gas Blowing. En Encyclopedia of Dairy Sciences (pp. 661-666).

Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Faseleh Jahromi, M., & Ho, Y. W. (2014). Probiotic Potential of Lactobacillus Strains with Antimicrobial Activity against Some Human Pathogenic Strains. BioMed Research International, 2014, e927268.

Sreekumar, O., & Hosono, A. (2000). Immediate Effect of Lactobacillus acidophilus on the Intestinal Flora and Fecal Enzymes of Rats and the In Vitro Inhibition of Escherichia coli in Coculture. Journal of Dairy Science, 83(5), 931-939.

Tabla, R., Gómez, A., Rebollo, J. E., Molina, F., & Roa, I. (2022). Effectiveness of a bacteriophage cocktail in reducing cheese early blowing caused by Escherichia coli. LWT, 153, p. 112430.

Tack, D.M. et al. (2019). Preliminary incidence and trends of infections with pathogens transmitted commonly through food – Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2015–2018. American Journal of Transplantation, 19(6), pp. 1859–1863.

Taco, K. R., & García-Godos, P. (2021). Optimization of milk pasteurization and curd cutting time for artisanal enzymatic fresh cheese. Información Tecnológica, 32(1), 179-186. (in Spanish)

Tascı, F. (2011). Microbiological and Chemical Properties of Raw Milk Consumed in Burdur. Journal of Animal and Veterinary Advances, 10, 635-641.

Todorov, S. D., Furtado, D. N., Saad, S. M. I., & Gombossy de Melo Franco, B. D. (2011). Bacteriocin production and resistance to drugs are advantageous features for Lactobacillus acidophilus La-14, a potential probiotic strain. The New Microbiologica, 34(4), 357-370.

Torlak, E., Sert, D., & Ulca, P. (2013). Efficacy of gaseous ozone against Salmonella and microbial population on dried oregano. International Journal of Food Microbiology, 165(3), 276-280.

Villegas Soto, N. R., Hernández Monzón, A., & Díaz Abreu, J. A. (2018). Optimization of milk pasteurization and curd cutting time for artisanal enzymatic fresh cheese. Tecnología Química, 38(2), 386-397. (in Spanish)

Wahyuni, E. A. (2015). The Influence of pH Characteristics on the Occurance of Coliform Bacteria in Madura Strait. Procedia Environmental Sciences, 23, 130-135.