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# Effect of *Lactobacillus acidophilus* added to a starch coating related to the microbiological contamination, quality and acceptability of fresh cheese

## Efecto de *Lactobacillus acidophilus* añadido a un revestimiento de almidón en relación a la contaminación microbiológica, calidad y aceptabilidad de queso fresco

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## ABSTRACT

The fresh cheese produced in the province of Manabí is an Ecuadorian artisan cheese. The processing conditions commonly do not comply Ecuadorian regulations, resulting in the presence of pathogenic microorganisms such as *Salmonella*. The high number of cases of Salmonellosis in the province of Manabí justifies the need to identify and control the possible sources of this pathogenic microorganism. In the present work, the effect of the addition of *Lactobacillus acidophilus* to fresh cheese was studied, by immersing it in a starch solution with $1x10^8$ CFU $mL^{-1}$ of *L. acidophilus* with further storage for 30 days at 4 °C. The pH, acidity, weight loss, instrumental firmness, number of CFU of mesophilic aerobic bacteria and acceptability of fresh cheese were analyzed. At the same time, a duo-trio analysis was carried out, followed by a satisfaction analysis with the participation of semi-trained panelists. The presence of *L. acidophilus* reduced the pH and acidity in the fresh cheese in relation to the control sample. Satisfaction results, on a five-point hedonic scale, which ranged from 1 (I dislike it very much) to 5 (I like it very much), showed that the cheese treated with *L. acidophilus* and the control sample received a rating between "I neither like nor dislike" and "I like it moderately", with values of 3.63 for the treated sample and 3.50 for the control. The application of *L. acidophilus* did not affect the organoleptic acceptability of cheese and produced less changes in pH, acidity and weight loss during storage for 30 days at 4 °C in relation to the control sample.

**Keywords:**
Coated cheese
Inhibition
*Salmonella*
Satisfaction test
Sensory analysis

## RESUMEN

El queso fresco producido en la provincia de Manabí es un queso artesanal ecuatoriano, cuyas condiciones de procesamiento comúnmente no cumplen con la normativa ecuatoriana, dando como resultado la presencia de microorganismos patógenos como la *Salmonella*. El alto número de casos de Salmonelosis en la provincia de Manabí justifica la necesidad de identificar y controlar las posibles fuentes de este microorganismo patógeno. En el presente trabajo se estudió el efecto de la adición de *Lactobacillus acidophilus* al queso fresco, mediante la inmersión de este en una solución de almidón con $1x10^8$ UFC $mL^{-1}$ de *L. acidophilus* y su posterior almacenamiento por 30 días a 4 °C, durante los cuales se analizó el pH, acidez, pérdida de peso, firmeza instrumental, número de UFC de bacterias aerobias mesófilas y satisfacción del queso fresco. Al mismo tiempo se realizó un análisis dúo-trío y seguidamente uno de satisfacción con la participación de panelistas semientrenados. La presencia de *Lactobacillus acidophilus* redujo el pH y acidez en el queso fresco en relación con la muestra control. Los resultados de satisfacción, en una escala hedónica de cinco puntos, que varió desde 1 (me desagrada mucho) a 5 (me agrada mucho), mostraron que el queso tratado con *L. acidophilus* y la muestra control recibieron una calificación entre "ni me gusta ni me disgusta" y "me gusta moderadamente", con valores de 3.63 para la muestra tratada y 3.50 para el control. La aplicación de *L. acidophilus* no afectó el nivel de agrado de los panelistas por el queso fresco y produjo menores cambios de pH, acidez y pérdida de peso durante su almacenamiento por 30 días a 4 °C en relación a la muestra control.

**Palabras clave:**
Queso recubierto
Inhibición
*Salmonella*
Prueba de satisfacción
Análisis sensorial

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Fresh cheese produced in Manabí province is an Ecuadorian artisan cheese with a moisture of 37% (Minga and Pérez, 2019), with good acceptance among consumers, especially in the province of Manabí (Ecuadorian coast). Previous studies have shown that fresh cheese processing does not fulfill Ecuadorian regulations due to the presence of pathogenic microorganisms such as *Salmonella* (Zambrano, 2014). Additionally, during the commercialization the cold chain is affected in some points, since the cheese is exhibited at 25 °C, which increases the microbiological load affecting the health of consumers (Lobacz, 2020). Salmonellosis cases in the province of Manabí, Ecuador are around 600 annually (Ministerio de Salud Pública, 2015-2018). The presence of *Salmonella* and other pathogenic microorganisms has been detected in cheeses from Mexico (Plumb *et al*., 2019) and Egypt (El-Baz *et al*., 2017) among others.

Several authors have studied the control of pathogenic microorganisms in different varieties of cheese such as the control of *E. coli, S. enterica, B. cereus, S. scuiri* and *P. aeruginosa* using lactic acid bacteria (LAB) (Al-Gamal *et al*., 2019); the inoculation of *L. rhamnosus* in semi-hard goat cheese to inhibit the growth of pathogenic bacteria (Rodrigues *et al*., 2015) and the use of *L. acidophilus* to control the development of mesophilic aerobic bacteria and *Salmonella* in fresh cheese (Santacruz and Castro, 2018).

Coating is a layer that works as barrier against moisture, gases, loss of aroma and flavors of food. At the same time, it can act as carriers of antimicrobial microorganisms or substances (Guimarães *et al*., 2018). In fact, coatings have been used in cheese preservation, reducing water loss and spoilage damage (Costa *et al*., 2018). Moreover, the effect of coatings on several types of cheeses have been studied previously such as Gouda (Göksen *et al*., 2020); Port Salut (Ollé *et al*., 2014); Kashar (Kavas *et al*., 2015) among others.

The present work is a complementary study of a previous work where the growth of mesophilic aerobic bacteria and *Salmonella* in fresh cheese could be inhibited using *L. acidophilus.* The effect of a cassava starch + *L. acidophilus* as a coating on fresh cheese was studied, evaluating its acidity, pH, weight loss, firmness and counting of mesophilic aerobic bacteria during 30 days of storage at 4 °C. Additionally, the difference of satisfaction among panelists was evaluated for coated and uncoated cheeses.

## MATERIALS Y METHODS

Strains of *L. acidophilus* were obtained from Chr. Hansen A/S (Denmark). The fresh cheese was purchased in stores located in the center of the city of Manta, Ecuador.

### Preparation of coated cheese

The fresh cheese was coated with a starch + *L. acidophilus* (SL) film. A Cassava starch solution 1% (w/v) was prepared according to Santacruz *et al.* (2015), with slight modifications. The starch solution was heated with constant stirring from room temperature (approximately 25 °C) to 90 °C, keeping this temperature for 5 min. Afterwards, the mixture was homogenized with an ultraturrax (Polytron, Switzerland) at 11,000 rpm for 4 min. Free *L. acidophilus* was added to the starch solution guaranteeing a starch coating with an absorbance between 0.08 and 0.1, measured at 625 nm (Medina *et al*., 2005). This absorbance guaranteed an equivalent of $1x10^8$ CFU $mL^{-1}$ of *L. acidophilus* of starch solution.

The fresh cheese was immersed in the previous solution for 5 min, dried at room temperature and finally transferred to plastic bags before storage for 30 days at 4 °C. Every day during storage, cheese was taken out from refrigerator and kept at room temperature for 8 h, then placed again into the refrigerator. This procedure allowed to simulate commercialization conditions.

### Physical, chemical and microbiological analyses of fresh cheese

Cheese samples previously coated with a SL film were analyzed after 0, 10, 20 and 30 days of storage at 4 °C. Moisture content, titratable acidity, pH, and firmness of the cheese were examined. Moisture content was determined according to method 15.259 (AOAC, 1984), titratable acidity with method 16.276 (AOAC, 1984), pH was measured using a potentiometer (HANNA, Germany), firmness was measured as the maximum force (N) required to penetrate cheese cubes with a side length of 5 cm, using a texturometer (SHIMADZU EZ-XL, Japan). For the penetration test, a 2 mm diameter penetration probe was used at a speed of 20 mm $s^{-1}$, with a penetration

depth of 15 mm (Santacruz, 2021). The determination of mesophilic aerobic bacteria was carried out according to Castro *et al.,* (2014). Uncoated cheese was used as a control sample for all analyzes.

### Sensory analysis

Sensory analyses were done at 0 day of storage only, with cheese containing no *Salmonella*. This avoided health problems to panelists due to the growth of mesophilic aerobic bacteria in cheese during storage. Presence of *Salmonella* was analyzed by the Ecuadorian regulation for microbiological food control (INEN, 2015). For this stage, eight semi-trained panelists were involved in both tests (UPAEP, 2014). The panelists were previously chosen based on their ability to identify basic flavors (Sharif *et al*., 2017). First, a duo-trio test was carried out in two sessions, which aimed to determine if there was a difference between the sample coated with SL and the control sample. In this case, the number of correct responses was determined for a probability level of 5%. Afterwards, a satisfaction test was performed using a 5-point verbal hedonic scale (Wadhwani and McMahon, 2012), with a scale ranging from "I like it a lot" (5) to "I dislike it a lot" (1).

### Statistical analyses

Comparisons of control sample with coated cheese with a SL film were analyzed by means of an IBM SPSS Statistics software, using a one-way ANOVA (significance level of 5%). All measurements were made in triplicate.

## RESULTS AND DISCUSSION

The presence of *L. acidophilus* in cheese caused a lower pH and acidity compared to the control sample after 30 days of storage. Similar results were obtained by Mozuriene *et al.* (2016). The lower presence of mesophilic aerobic bacteria due to the inhibitory action of *L. acidophilus* may trigger a lower production of acidic metabolites and thus a lower acidity. Additionally, the increase of *L. acidophilus* may be responsible of a higher production of organic acids that act as buffer, controlling the increase of pH during storage. Regarding the weight loss, coated cheese showed a lower loss compared to control sample. The coating may act as a barrier against moisture loss.

Results of microbiological analyzes showed that control cheese had a higher quantity of mesophilic aerobic bacteria than the sample coated with *L. acidophilus* ($P$<0.05, Table 1). Santacruz and Castro (2018) showed that viability of *L. acidophilus* decreased along storage, however the viable cells inhibited the development of mesophilic aerobic (Mozuriene *et al*., 2016) and *Salmonella*. Coatings were able to control the release of the antimicrobial agents on the cheese surface improving the microbiological quality of the cheese (Santacruz and Castro, 2018; Krishnan *et al*., 2015) and *Salmonella* spp. in fresh cheese. Previous studies showed the inhibition of *E. coli*, *L. monocytogenes* and *S. enteritidis* by LAB (Winkowski *et al*., 1993; Lord, 2002). The presence of LAB could inhibit microorganisms through the production of bacteriocins (Aymerich *et al*., 2000).

**Table 1.** pH, acidity, weight loss, firmness and mesophilic aerobic bacteria counting in cheese stored at 4 °C during 30 days.

| | | Days of storage | | | |
|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 |
| pH | LAB[1] | 5.95±0.07 | 6.57±0.09 | 7.00±0.28 | 7.10±0.08 |
| | Control | 5.98±0.08 | 7.31±0.11 | 7.46±0.21 | 7.62±0.09 |
| Acidity | LAB[1] | 0.17±0.01 | 0.18±0.02 | 0.21±0.01 | 0.26±0.01 |
| | Control | 0.17±0.00 | 0.23±0.02 | 0.32±0.03 | 0.62±0.04 |
| Weight loss (%) | LAB[1] | 0.00±0.00 | 1.69±0.28 | 2.77±0.35 | 4.74±0.26 |
| | Control | 0.00±0.00 | 4.57±1.18 | 8.72±1.21 | 11.81±0.34 |
| Firmness(N) | LAB[1] | 0.87±0.02 | 1.34±0.01 | 1.34±0.10 | 1.12±0.14 |
| | Control | 0.89±0.02 | 1.17±0.01 | 1.16±0.01 | 0.97±0.11 |
| Mesophilic aerobic bacteria (Log CFU $g^{-1}$) | LAB[1] | 8.60±0.01 | 4.10±0.06 | 6.10±0.08 | 6.40±0.11 |
| | Control | 8.60±0.02 | 9.20±0.09 | 10.23±0.07 | 10.80±0.08 |

n=3. Results were expressed as means±SD (standard deviation). [1]LAB: fresh cheese coated with a SL film.

### Sensory analysis

Results of the duo-trio test showed that the number of correct responses was 12 of 16, which showed a difference between the cheese samples coated with *L. acidophilus* compared to control sample (Wittig, 2001). Another aspect to evaluate was the sensorial acceptation by the panelists. The results showed that there was no difference in panelists satisfaction between coated and control sample ($P<0.05$). Both received a rating between "I neither like nor dislike" and "I like it moderately", with values of 3.63 and 3.50 for coated and control sample, respectively. Previous works showed similar results; in fact, LAB strains increased the acceptability and shelf-life of unripe curd cheese (Mozuriene *et al*., 2016). Coelho *et al.* (2014) found no significant differences in overall sensory evaluation of fresh cheese. Even after the addition of *L. acidophilus,* the semi-hard goat cheese showed better sensory scores in the acceptance test (Fernandes *et al*., 2012). The SL film could be used as an agent to control the growth of mesophilic aerobic bacteria in fresh cheese with no changes on the satisfaction among consumers.

## CONCLUSIONS

The use of a cassava starch +*L. acidophilus* film inhibited the development of mesophilic aerobic in fresh cheese showing no difference in panelists satisfaction between coated and control sample. SL film-coated cheese could act as a barrier against moisture loss and may be a promising option to control mesophilic aerobic bacteria in fresh cheese during storage under refrigeration conditions with no changes in consumers satisfaction.

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