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ABSTRACT:

The use of edible film can improve the quality and increase the shelf life of food. The objective of this study was to evaluate the application of films containing acacia gum and different percentages of lactic acid in ricotta cheese. Samples were coated with the film-forming solution (T2 containing 15 mL lactic acid and T3 containing 30 mL lactic acid in the film-forming solution) and uncoated samples (T1 control samples) were stored for 5 days under refrigeration. The water activity (Aw), pH, titratable acidity and counts of aerobic mesophilic bacteria and fungi were evaluated at 0, 2 and 4 days. There was a significant difference for Aw between the evaluated times ($p < 0.05$), with higher Aw values at two days of storage. The titratable acidity was significant for treatment ($p < 0.05$), with treatments T1 and T2 being the most effective and for the time, there was a decrease in values over the evaluated period. The microbiological evaluation of mesophilic aerobes and fungi was significant for the evaluated times ($p < 0.05$). Therefore, the proposal to develop an edible film resulted in similarity in microbiological efficiency in relation to the control treatment.

KEYWORDS: dairy products, packaging, shelf life.

INTRODUCTION

Over the last few years, the search for use of natural antimicrobials for food preservation, due to the consumers rejection of synthetic additives commonly used for inhibit microbial growth in food (Artigas-Artigas, Fani, & Beloso, 2017).

In addition, the use of biodegradable polymers from natural sources can be used as substitutes to synthetic polymers (Lopez et al., 2017). Edible films and coatings are prepared from materials which act as a barrier to external elements such as water, steam, moisture or temperature (Mei, Yan, Wu, & Li, 2013).

This can not absorb oxygen, forming a selective barrier to gases such as carbon dioxide. The use of alginate provides oxygen barrier and protects against lipid oxidation (Tavassoli-Kafrani, Shekarchizadeh, & Masoudpour-Behabadi, 2016). However, the increasing consumer demand for food by reducing the use of preservatives and additives may eventually result in products with shorter shelf life. The development of new materials and particularly innovative formulations from biopolymers can meet these requirements of food protection and preservation, besides replacing other forms of packaging and providing intelligent communication with consumers (Flores et al., 2017). Different natural and/or synthetic substances that polymerize and isolate the food may be used and are considered to be innocuous because they are not metabolized by the human organism during passage through the gastrointestinal tract (Maia, Ferreira, & Abreu, 2004).

Acacia gum has been extensively studied and used commercially due to its lower viscosity, even at a higher concentration, high water solubility, and emulsion property. Over 900 species of Acacia trees are available worldwide where most are able to produce gum (Verbeken, Dierckx, & Dewettinck, 2003). It is widely used in food industries including dairy products, confectionery, soft drink, encapsulation of flavors and phenolic compounds, as edible coating material, baking and in frozen foods (Ali, Maqbool, Ramachandran, & Alderson, 2010). Gum is used as a stabilizer and emulsifier in the food industry and has an influence on nutrient absorption. The physiological effects of its ingestion include the complete fermentation in the colon by bacteria belonging to the beneficial microbiota (Gee, Blackburn, & Johnson 2000). Ricotta is a product whose shelf life is short due to high moisture content (70 to 73%) and nutrients, such as mineral salts and lactose (Maia et al., 2004). These conditions, along with high pH, contamination by microbiota, either deteriorating or pathogenic, which present inappropriate actions on cheeses, alter sensory properties and may cause risks to the health of consumers (Pintado, Macedo, & Malcata, 2001). The development of edible films will extend the shelf life of a highly perishable food.

This study evaluated an edible film made from acacia gum, lactic acid and glycerol (lactic acid, organic acid, water soluble having the ability to increase the acidity of the product and glycerol used as plasticizer to improve the characteristics of the packaging) and evaluated its efficiency in controlling the development of bacteria and fungi in ricotta cheese to investigate the possibility of implementing this methodology in dairy products in order to increase the shelf life of this product.

MATERIAL AND METHODS

Samples of ricotta cheese were obtained directly from a dairy under Municipal Inspection Service, belonging to the city of Iguaraçu, State of Paraná, collected at the date of production, all from the same lot. Three treatments were tested, using three cheeses for each, with five replicates: with T1 being the control treatment, without immersion of cheeses in solution, for the T2 and T3, cheeses were immersed in a film-forming solution elaborated according to Moritz, Rolim, Tomás, and Aguiar (2009) modified due to the inclusion of acacia gum (Fibregum®) and lactic acid. A solution for each treatment (T2 and T3) was prepared with 10 g acacia gum (Fibregum®) powder dissolved in 500 mL distilled water at 50°C, adding 7.5 mL glycerol and the addition of lactic acid varied according to the treatment. For T2 solution, it was added 15 mL lactic acid and for T3, 30 mL. Cheeses were directly immersed in the coating formulation (in aqueous medium), the excess was removed by forming a film on the surface of the product.

Samples were refrigerated at 4°C for 5 days, evaluated at 0, 2 and 4 days, being analyzed in triplicate for the following laboratory analyses.

Analysis of water activity: using the Labswift (Novasina) equipment;

Titratable acidity: according to Silva, Pereira, Oliveira, and Junior (1997), where 5 g cheeses samples of each treatment were used which were mixed in 10 mL distilled water at a temperature of approximately 30 to 40°C forming a homogeneous paste. After cooling, cold water was added and the mixture was allowed to

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stand for 5 min., then the mixture was filtered through cotton wool and 3 drops of 1% neutralized alcoholic phenolphthalein (m v^{-1}) in the total volume of the filtrate was added and the solution was titrated with 0.1 mol L^{-1} sodium hydroxide solution to the final point detectable by the pink coloration.

pH analysis: with a digital pH meter according to Silva et al. (1997) where 10 g cheese samples from each treatment were ground in 10 mL distilled water to complete homogenization. A further 30 mL distilled water was added and allowed to stand for 5 minutes. After, the mixture was filtered through cotton wool and the bulb of the measuring electrode was plunged into the filtrate for pH reading.

Microbiological analyses: Petri dishes were counted for mesophilic aerobes (weighed 25 g sample, homogenized by manual shaking). Dilutions were made up to 10^{-5} inoculating the surface dilution of the PCA agar plates where they were incubated in an oven at 35ºC for 48 hours for counts of mold, fungi and yeasts in BDA agar where they were incubated in an oven at 28ºC for 5 days (Silva et al., 2007).

Analysis of variance (ANOVA) was run with significance level of 0.05% in a 3x3 factorial arrangement, with three treatments and three evaluation times. Tukey's test was applied to compare the means and for the time, the regression analysis was performed (Universidade Federal de Viçosa, 1999).

RESULTS AND DISCUSSION

For the parameter water activity (Aw), there was no significant difference for the treatments evaluated nor for the interaction Treatment x Time ($p > 0.05$), but there was a significant difference between the evaluated times ($p < 0.05$), with low r2.

The mean observed for this analysis in all treatments and times was 0.947, with a standard deviation of 0.036. Values similar to these were obtained by Sousa et al. (2014) ranging from 0.911 to 0.963 for rennet cheese. Spanu et al. (2017) observed values for Aw between 0.990 (T0) and 0.997 (T21) in samples of ricotta under refrigeration.

Values of water activity also vary according to the relative humidity of the air, that is, if the relative humidity is lower than the Aw values of the food, this will tend to dehydrate until reaching equilibrium, as is the case of the cheese under refrigeration when it begins to release water (Fellows, 2006).

In turn, authors such as Maruyama, Cardarelli, Buriti, and Saad (2006) evaluated the shelf life of petit suisse cheeses,and found that pH values decreased and the moisture values remained constant during the 21 days of storage under refrigeration. The titratable acidity was significant for treatment and for time ($p < 0.05$) but non-significant for the Treatment x Time interaction ($p > 0.05$); however, the regression equation was not estimated, since the value of r2 was considered low (r2 = 0.15), but we can observe that there was a tendency to decrease values over the time evaluated.

The titratable acidity mean values, expressed in Dornic degrees, were higher and statistically similar ($p > 0.05$) for T1 = 13.94 and T2 = 13.22, with T3 = 11.10, which corresponds to 0.11; 0.14 and 0.13 g lactic acid-1.

However, di Piero et al. (2011) evaluated ricotta cheeses with and without film coating based on chitosan and whey proteins, and reported different behavior in the control samples, with higher values of titratable acidity provided by the higher growth of lactic acid bacteria when compared to coated cheeses.

Fritzen-Freire et al. (2013) evaluated ricotta samples for 60 days and verified that the acid values increased and consequently pH decreased ($p < 0.05$) along storage. According to Buriti, Cardarelli, Filisetti, and Saad (2007), the decrease in pH values observed during storage of fresh cream is a natural process caused by the continuous production of lactic acid and other organic acids by lactic and/or probiotic cultures. Higher acidity values for ricotta cheese can be explained by the processing of the product, given the addition of lactic or citric acid, which coagulate serum proteins (Modler & Emmons, 2001).

According to Scott (2002), in the elaboration of cheeses, due to the size of the grains obtained after the curd cut, the variation in the amount of lactic acid per cheese sample obtained may be due to the activity of the lactic bacteria present in the milk, method and quantity of salt as well as time and temperature of the cheeses pressed. As the concentration of lactic acid rises, the pH values decline.

The formation of lactic acid, which is essential for the taste of cheeses, is also related to shelf quality and shelf life, however, at high amounts it can lead to an intense acidic taste and characterize the cheese (Saboya, Oliveira, Furtado, & Spadoti, 1998).

For pH values, there was no significant difference for treatment, time and for interaction of Treatment x Time ($p > 0.05$). The mean obtained among all treatments was 5.45, with a standard deviation of 0.672. The mean values of pH obtained for ricotta in by Esper, Bonets, and Kuaye (2007) ranged from 4.95 to 6.26. For treatment 1 (control treatment), the mean observed at all times was 5.45, in treatment 2 (including 15 mL lactic acid), the mean for all times was 5.50 and for the treatment 3 (30 mL lactic acid), the mean was 5.43, values that are close to the ideal required for microbial growth (Franco & Landgraf, 2003).

Values similar to these were obtained by Rosa, Porto, and Fillet (2005) who, when studying the best conditions for processing ricotta, obtained better production conditions at pH 5.0. According to Cecchi (2003), pH values indicate the degree of deterioration of cheeses, to check the suitability for consumption.

Low pH represents a safety factor for cheeses, according to Khanipour et al. (2016), decreases in Aw and pH have been applied to inhibit the growth of any microorganism in foods; however, the addition of salts (> 2%) and various acidulants (acetic > lactic > malic > tartaric) that reach inhibitory values for most spore-forming bacteria is difficult because these levels can affect organoleptic attributes. Buriti, Rocha, and Saad (2005) evaluated Minas frescal cheese for 21 days at 8°C also found a pH drop from 6.16 to 5.38 between the 1st day and the 21st day of storage and an increase in titratable acidity of 0.36%, expressed as a percentage of lactic acid.

Sangaletti et al. (2009) found that the increase in acidity over the shelf life in cream cheese was proportional to the increase in the population of mesophilic bacteria, mainly lactic and psychrotrophic bacteria, since these are the main microorganisms related to the conversion of lactose into lactic acid. However, in the present study, there were reductions in the acid values observed over the shelf life.

For the microbiological evaluation of aerobic mesophilic bacteria, differences were not significant between treatments neither for the treatment x time interaction ($p > 0.05$), but were significant between the evaluated times ($p < 0.05$). The regression equations are presented in Table 1.

High bacterial counts may be associated with lower acidity of the product and high Aw observed, leading to a higher level of deterioration of cheeses. Most of the bacteria require minimum Aw values for their development at 0.90-0.94 (Franco & Landgraf, 2003).

TABLE 1.
Regression equations of the microbiological counts as a function of the evaluated times

Tratament 1	R^2	P
Mesophilic aerobic	0,69	0,07771
Y = 5,83125+0,481250x		
Fungi	0,76	0,06884
Y = 2,55083+0,732500x		
Tratament 2	R^2	P
Mesophilic aerobic	0,83	0,26385
Fungi	0,54	ns
Tratament 3	R^2	P
Mesophilic aerobic	0,97	0,21298
Fungi	1	0,02980
Y = 8,39725-6,3415x+1,75300x^2		

*ns = no significative.

The phases of growth and microbial metabolism occur rapidly, where microorganism pass through the period of adaptation to the environment and cell growth followed by the phase that begins the process of cell division occurring the highest metabolic activity, then occurs the stability phase, the speed of growth decreases. The population becomes stable, and finally there is the phase of decline where cell death occurs and there are no more nutrients (Gava, Silva, & Frias, 2008).

The microbial growth curve explains the values observed in the evaluated times, since, up to four days, adaption period still occurring. Although there is no standard in mesophyll aerobic legislation, the counts ranged from 3.84 to 4.92 $\log_{10}$, but the lower the number of aerobic mesophilic bacteria found in cheeses, the better the microbiological quality. It was observed that the averages obtained in the present study are in agreement with the values cited in the literature. Rosa, Porto, and Spoto (2005) evaluated fresh cheese samples packed under modified atmosphere and obtained mean counts of aerobic mesophilic bacteria of 6.4 $\log CFU g^{-1}$.

For Ribeiro, Marques, Sodré, Abreu, and Piccoli (2005), the results obtained ranged from 2.30 to 4.39 $\log_{10}$ in ricotta. According to the mentioned authors, the low number of microorganisms found can be explained by two factors, process temperature and conservation method. Ricotta presents excellent conditions for microbial growth, whether pathogenic or deteriorating. In the microbiological evaluation of fungi, molds and yeasts (BDA) the treatment and the interaction treatment x time were not significant ($p > 0.05$), only for the evaluated times there was significance ($p < 0.05$).

For fungi, there was an increase in the values of the counts over the evaluated period; with the pH values obtained in this study, the growth of fungi could be potentiated (Table1). Although there are no limits in the legislation for the presence of molds and yeasts in cheese, they may cause sensory changes in the product, which may represent a risk to public health, in addition to economic losses, as potentially pathogenic mycotoxigenic fungi may develop (Ledendach & Marshall, 2009).

The averages obtained for the fungal count were 3.67; 3.24 and 4.85 log; such values may be related to Aw, whose mean values at time 2 were higher. Most of the molds have Aw values as minimum for their development of 0.70 - 0.80, which are values lower than the minimum required by bacteria. According to Carrijo et al. (2001), the counts in their study were considered high, ranging from 7.92 to 10.55 $\log_{10}$, averaging 9.84 $\log_{10}$.

In accordance to Pinto, Souza, Saling, and Moura (2011), for Minas frescal cheese, the highest values for molds and yeasts were obtained for samples of artisanal cheeses reaching values of up to 6.70 $\log_{10}$ while for the samples inspected, the values were only 2.70 $\log_{10}$.

Spanu et al. (2017) tested commercial biopreservatives (*Enterococcus, Lactobacillus* and *Carnobacterium*) against spoilage microorganisms, and reported that yeast and molds were occasionally reported, with maximum values around 4.0 $\log_{10}$ at 21 days storage and total bacterial count in control samples at time 0 was < 3.0 log 10 and at 21 days, under refrigeration, above 7.0 $\log_{10}$, while lactic bacteria were below the detection limit ($\geq 10 CFU g^{-1}$) and reached 5.0 log10 values at 21 days.

The importance of the presence of yeasts depends on the specific type of cheese. Yeasts may contribute to spoilage in some cheeses, or aid in the development of a characteristic flavor during the ripening process of other cheeses. The equipment surfaces, floor and the brine bath were mainly responsible for the incidence of various contaminating yeast species (Viljoen, Khoury, & Hattingh, 2003).

Kure, Skaar, and Brendehaug (2004) report that, during parts of the production process, the cheeses are in direct contact with equipment and are also exposed to air. Mold growth can be seen during ripening, storage and distribution to the consumer. According to the authors, the mold levels at some of the air control points had high counts while the mold levels in milk and brine, on equipment and on plastic film, were generally low. Molds growth on dry-ripened foods has been associated with off-flavors and unpleasant appearance (Delgado, Peromingo, Nunez, & Asensio, 2016).

Conclusion

Although the edible cover tested was not efficient in extending the shelf life tested, the product maintained adequate conditions for consumption. However, studies are needed on the combination of coatings that may be a more effective barrier.

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