Behavior Assesment of Methylcellulose Coatings as Packaging Material for Fresh Costeno Cheese

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Abstract

Objective: In this research work, properties of the methylcellulose films were studied to be applied as coatings on Costeno cheese. **Methods /Analysis:** Methylcellulose (A4M) solutions were prepared at 1.5% w/v in 0.25, 0.25 and 0.75% sorbitol concentrations; and 1, 5 and 10% (w/w MC) citric acid. Nine formulations obtained from a 3² factorial experimental design were prepared to determine effect of sorbitol and citric acid addition on physicochemical and mechanical properties of films; the effect of addition of sorbitol as a plasticizer, and citric acid as a crosslinker of the polymer matrix. **Findings:** Transparent and flexible films were obtained for all cases. The 1.5 g MC, 0.25% sorbitol and 10% citric acid formulation was selected as the coating forming solution to be applied in Costeno cheese. A decline in weight loss and conservation of color of stored cheese was observed for 15 days under refrigeration once this formative solution was applied to Costeno cheese. **Application /Improvements:** Thus, the development of this research contributes to conservation of intermediate moisture products, such as Costeno cheese by using materials from renewable sources as an alternative to plastic materials.

Keywords: Costeno Cheese, Metilcellulose, Packaging Material

1. Introduction

Edible films and coatings are mainly used to protect products from mechanical, physical, chemical and microbiological activities causing damage on them. Films can help, depending on their characteristics, reduce these damages in food by slowing their deterioration, increasing quality and improving safety. This last, thanks to the coating natural activity against organisms or antimicrobial compounds incorporation in formulation. Films and edible coatings are defined as substances applied outside the food so that the final product may be suitable for consumption. Edible coatings have been used in the food industry, with the priority goal to prevent moisture loss in food. Coatings must be legal, safe for

consumption, acceptable for consumers and must provide food added value³.

Costeno cheese is a typical dairy product from the Colombian Caribbean region⁴. It is a variety of unripen cheese, i.e., fresh, made with unpasteurized cow milk, not acid, with a salty flavor between moderate and strong. It has 65% moisture and 45% fat in dry extract; classified as semi-hard, with a high fat content according to the FAO and WHO. It is shaped in rectangular section blocks with a 15 to 20 cm size and 6 to 40 Kg weight. Its external appearance is cream, dull and with uneven surfaces. It internally has open texture and hard consistency⁵. Due to its characteristics, it is a highly perishable product and susceptible to significant changes in temperature. For this reason, it must be marketed under refrigeration.

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Nonetheless, prolonged refrigerated storage affects its organoleptic conditions and contributes to weight loss, due to loss of water.

Polyethylene bags, which prevents deterioration and dehydration, are the main containers for transport. However, this packaging does not prevent food water loss, reflected in the product weight loss which directly affects food price.

On the other hand, it has been observed that edible coatings coming from renewable sources can reduce mechanical, physical and chemical damages the environment generates to coated product¹. Studies antimicrobial films effect on survival of *salmonellas pp.* and *staphylococcus aureus* in Costeno cheese made with different NaCl concentrations, found favorable results in terms of weight loss, color retention, taste and inhibition of fungal contamination⁶.

Raw materials from biological origin, including polysaccharides, proteins, and lipids alone or in mixtures, have been proposed for edible films or coating preparation, both for container type applications as well as for the use as coatings^Z. Regarding technical-economic factor, raw materials must be inexpensive, and their processing technology must be simple. Methylcellulose is a long-chain substituted cellulose, derived from plant fibrous material of natural strains, in which approximately 27-32% hydroxyl groups are in the methyl ether form. From MC aqueous solutions, high transparency and strength films can be obtained⁸. These are water soluble and resistant to most organic solvents, fats and oils.

MC films can be made more elastic and flexible by adding a plasticizer, such as sorbitol, glycerol or polyols². As starch, methylcellulose has many free hydroxyl groups, giving the obtained films, a highly hydrophilic character¹⁰. ¹¹. Additions of plasticizers to biodegradable materials generally increase their permeability and affect their mechanical properties¹². ¹³. Effect of water plasticization on carbohydrates is produced through complex mechanisms involving both, formation of hydrogen bonds, and interruption and changes in the matrix free volume¹⁴. Polyols such as sorbitol, are generally used for films obtained from cellulose and starch¹⁵⁻¹⁷.

Sorbitol is a pharmaceutical product commonly used as an excipient to improve stability of proteins and other molecules during freezing, drying and storage¹⁸. Chemical modifications such as crosslinking can provide viable routes to improve mechanical properties and stability

and reduce their dissolution in water for use as controlled release agents or edible containers $\frac{19}{20}$.

Polymeric matrices can be modified by chemical, physical or enzymatic crosslinking reactions²¹⁻²³. Crosslinking is an effective alternative in improving properties of films obtained from biopolymers, because it provides greater stability and mechanical strength, allowing to obtain a matrix with swelling capacity, good mechanical properties, thermal stability and greater resistance to water dissolution²⁴⁻²⁶; and useful for active agents release and as a support for cell growth^{27, 28}.

Citric acid is an organic tricarboxylic acid present in most fruits, especially lemon and orange. It is non-toxic and has a wide application in the food industry as a safe natural additive, since it is used as a preservative. This acid can crosslink hydroxyl groups in the cellulose and it has been reported that the crosslinking reaction occurs at temperatures from 165 to 175°C^{29, 30}. Certain concentrations of citric acid can also act as a plasticizer³¹.

For the afore-mentioned, the objective of this research was to develop an edible coating from methylcellulose, citric acid and sorbitol, which would prevent weight loss in Costeno cheese and contribute to preserve of this product's organoleptic properties. Also, characterization was carried out through films as its model system, on which physicochemical properties, water vapor permeability and mechanical properties were evaluated.

2. Materials and Methods

2.1 Materials

For the biodegradable coatings and films preparation as a model system, A4M Methylcellulose (Dow Chemical, Argentina), citric acid (Crosslinker) (JT Baker, USA) and sorbitol (Plasticizer) (Sigma Aldrich, USA) were used.

2.2 Preparation of Formulations

Nine formulations from a 3² factorial experimental design were prepared to determine effect of sorbitol and citric acid addition on physicochemical, mechanical and thermal properties of the films to be obtained. A design with 2 experimental factors and 3 levels was used to evaluate factors effect on the response variables: concentration of sorbitol (0.00, 0.25 and 0.75% w/v) and

Table 1. Experimental design for preparing film forming solutions

	Concentrations		
Sample	Sorbitol (% w/v)	Citric Acid (w/w MC)	
A	0.25	1	
В	0.25	5	
С	0.75	1	
D	0.75	5	
E	0.00	1	
F	0.00	5	
G	0.25	10	
I	0.75	10	
K	0.00	10	

citric acid (1.0, 5, 0 and 10.0 p/p based on polymer) is shown in Table 1. Methylcellulose concentration was standardized with a 1.5 p/v number. Variable responses were water vapor permeability and thickness. An ANOVA was applied using the InfoStat 2009 statistical program (InfoStat, Argentina) to determine the factors significance on the response variables.

2.3 Obtainment of the Forming Solutions and Films as a Coating Model System

Methylcellulose, citric acid and sorbitol were weighed for the preparation of the forming solutions. Then, 50 ml distilled water was added at 80°C and subject to continuous stirring for two hours. After an hour, an additional 50 ml of water was added at room temperature up to a 100 ml volume was completed.

30 g of solution obtained in Petri dishes were placed and dried at 37°C for 48 continuous hours to obtain the films. After 48 hours, the films were removed from the Petri dishes and stabilized in desiccators at a 65%, relative humidity to continue with the determination of the different physicochemical and mechanical tests of the material.

2.4 Thickness Determination

Films' thickness was measured at 4 different points and then averaged to obtain more accurate data. Coating thickness tester ERASMUS ECT-204 equipment was used to measure formed films.

2.5 Determination of Moisture Content

Determination of films' humidity values was carried out with the oven drying method at 105°C, until the sample was brought to constant weight. Results were expressed as grams of water per 100 g of dry sample.

2.6 Permeability to Water Vapor

Water vapor permeability was measured using the ASTM E9632 method with some modifications. Once dried, films were removed from the plates and conditioned in 65% relative humidity environments at 20°C, for 48 hours before the permeability determinations. Samples were placed in the permeability cells, of known mouth area, containing silica gel33.

2.7 Mechanical Properties of Obtained **Films**

A DMA TA Instruments Q800 equipment with a voltage clamp was used to evaluate the mechanical properties. Samples were evaluated at 25°C until rupture. Tests were carried out at the facilities of the Center for Research and Development in Food Cryotechnology (CIDCA-UNLP).

2.8 Determination of Weight Loss and Color in Costeno Cheese during Storage

2.8.1 Weight loss

To perform weight loss test over time of coated and uncoated Costeno cheese samples, fresh Costeno cheese (obtained from the local market) was cut into $5 \times 5 \times 2$ cm cubes and distributed in 5 trays, each with 5 cheese portions covered with film-forming solution and the remaining 5 without film to control and identify changes in weight for 20 days, monitoring from day zero with a time interval from 5 days up to day 20.

2.8.2 Color determination

A CR-10 PLUS colorimeter (Konica Minolta Sensing, Inc.) was used for color study of cheese samples with and without coating, calibrated, with a portion of fresh Costeno cheese, as a reference system, using the CIELAB color coordinates (L*, a* and b*).

The portions of cheeses from the weight loss characterization were used and monitored in the same time intervals to identify color variation in the Costeno Creole cheese with respect to time.

2.9 Statistical Analysis

All tests were carried out in duplicate. The factors effect on the response variables was determined by ANOVA. Fisher's test (LSD) was used to compare means (P < 0.05).

3. Results and Discussion

Films obtained by the molding or casting method were transparent and could be easily unmolded. Regarding thickness, differences were observed among samples when they contained sorbitol compared with those to which the plasticizing agent was not used is shown in Table 2. Regarding films thickness determination, differences were found for the three used concentrations of plasticizers (P < 0.05). Also, when the citric acid concentrations were analyzed (P < 0.05) is shown in Table 2.

Concentrations E, F and K were the ones registering the lowest thickness values, respectively (44.00 $\mu m, 40.00~\mu m, 55.00~\mu m)$. The samples did not contain sorbitol, which would indicate that the plasticizer is an agent that significantly increases thickness of films made with biodegradable materials. Samples with higher sorbitol content showed similar thickness. This behavior was similar for chitosan and gelatin films obtained 26 . Thickness, however, can also vary depending on initial concentration of the forming solution in the plate.

3.1 Moisture Content in Films Obtained

Table 3 shows that variations of citric acid concentrations increase moisture content in the samples, showing

Table 2. Average thickness of formed films

Run	Sorbitol Content (%w/v)	Citric Acid Content (w/w MC)	Thickness (µm)
A	0.25	1	58.25 ± 6.75
В	0.25	5	69.50 ± 1.73
С	0.75	1	$63.50 \pm 3,42$
D	0.75	5	$63.50 \pm 5{,}20$
E	0	1	$44.00 \pm 4{,}32$
F	0	5	$40.00 \pm 4{,}55$
G	0.25	10	65.25 ± 7.04
I	0.75	10	64.00 ± 5.32
K	0	10	55.00 ± 4.55

Table 3. Humidity percentage in the films formed

	Sorbitol Content	Citric Acid Content	
Run	(%w/v)	(%w/w MC)	% Humidity
A	0.25	1	6.56 ± 8.97
В	0.25	5	7.56 ± 0.51
С	0.75	1	7.38 ± 0.95
D	0.75	5	8.13 ± 0.32
E	0.00	1	5.13 ± 0.75
F	0.00	5	6.72 ± 0.02
G	0.25	10	10.23 ± 1.14
I	0.75	10	11.58 ± 2.27
K	0.00	10	9.69 ± 0.87

significant differences for the three concentrations analyzed (P < 0.05) This would indicate that, according to the case, the acid citric acid acts as a plasticizing agent, which compromises the capacity of water retention in the films formed. As the sorbitol and CA concentrations increased, an increase in moisture content of the analyzed samples was observed. When plasticizer concentrations were statistically analyzed, differences between concentrations 0 and 0.75% of sorbitol and 0.25% w/v were observed, as the other analyzed concentrations. This behavior was corresponded to that obtained by $\frac{34}{2}$ in methylcellulose films.

When influence of sorbitol addition in the samples was analyzed, it was observed that the greatest influence concentration is 0.75%, which correlates with the increase in the capacity to plasticize the polymer network that sorbitol has according to the added concentration. No significant differences were found among concentrations A, E and F (P < 0.05).

3.2 Permeability to Water Vapor

Results of water vapor permeability are presented in Table 4, showing that there are differences in the samples, being the G sample with a 1.5% (w/v) methylcellulose content, 10% (w/w MC) citric acid and 0.25% (w/v)sorbitol, the concentration that resulted with lower permeability values, which would indicate that this film would be the most adequate among the other concentrations analyzed for the Costeno cheese application. Significant differences were found both for the plasticizer concentration and for the citric acid

Table 4. Water vapor permeability values (gms/pa) in the films obtained

Sample	Sorbitol Content (%w/v)	Citric Acid Content (%w/w MC)	Permeability to Water Vapor
A	0.25	1	1.05422E ⁻¹⁰
В	0.25	5	9.45014E ⁻¹¹
C	0.75	1	1.0665E ⁻¹¹
D	0.75	5	1.038877E ⁻¹⁰
E	0.00	1	5.1984E ⁻¹⁰
F	0.00	5	3.66444E ⁻¹¹
G	0.25	10	5.33699E ⁻¹¹
I	0.75	10	9.22472E ⁻¹¹
K	0.00	10	5.02181E ⁻¹¹

content and its interaction (P < 0.05). All the formulations showed differences between them (P < 0.05).

Other researchers indicated that there is a critical plasticizer concentration allowing to obtain flexible films with good barrier properties34. Another factor that varies films permeable capacity is the hygroscopic character and the affinity of the plasticizer for water, as well as the film thickness. Similarly³⁵ concluded that rise in permeability is directly proportional to the thickness. Concentration G showed a 65.2 µm thickness, compared with the other analyzed films. This concentration resulted in high thickness values compared to concentrations without added sorbitol. Nonetheless, presence of plasticizer and crosslinking agent could influence permeability values to drop. These results are comparable with those obtained.

3.3 Mechanical Properties of Metilcellulose **Films**

Flexibilities of polymers are improved with plasticizers addition. They tend to increase extensibility and reduce fragility of the polymeric matrix, preventing formation of pores and fissures 12. This behavior is important when it is required to completely cover a surface and the coating acts as a barrier between material and environment. When sorbitol was added to films, there was a change in the films mechanical profile. This could be verified when the resulting elastic modulus values were obtained as a function of plasticizer concentration is shown Table 5 here.

The higher sorbitol concentration, the lower the elastic modulus value, indicating that the plasticizer interacts with the methylcellulose structure. As a result,

Table 5. Elasticity Modulus (Mpa) of MC films 1.5 with Added Sorbitol (0-0.75%) at 25°C

Comple	Sorbitol Content	Citric Acid Content	Elastic Modulus
Sample	(%w/v)	(%w/w MC)	(Mpa)
A	0.25	1	2020.5
В	0.25	5	1989.5
С	0.75	1	870.6
D	0.75	5	865.4
E	0.00	1	3850.5
F	0.00	5	3720.2
G	0.25	10	1980.7
I	0.75	10	850.6
K	0.00	10	3689.3

the obtained films were more flexible than those without added plasticizer. No differences were observed in values of the elastic modulus with the citric acid addition.

Once the previous results were obtained and, considering moisture content, presence of citric acid in the matrix, permeability to water vapor and mechanical properties of films; sample G was selected. This sample contains 1.5 g methylcellulose, 0.25% sorbitol and 10% citric acid, as the coating forming solution to be used in Costeno cheese as a container.

3.4 Weight Loss Assessment in Coated **Costeno Cheese Samples**

Weight loss for 20 days is shown in Figure 1. The difference among the portions of cheese coated with the film of methylcellulose, citric acid and sorbitol, showed less weight loss compared to portions of uncoated cheese. However, on day 20, no differences were observed in the samples with and without coating.

3.5 Color Determination in Costeno Cheese

Figure 2 shows evolution of color coordinates (ΔL^* ; Δa^* , Δb^* and ΔE) in cheeses coated with film containing 1.5 g methylcellulose, 0.25% sorbitol and 10% CA, with its respective control (cheeses without coatings) throughout 20 days, stored with an average temperature of 6°C. According to the results, an increase in the of a* and b* values was observed for the samples with the coatings, while color variation turned out to be similar for both samples analyzed.

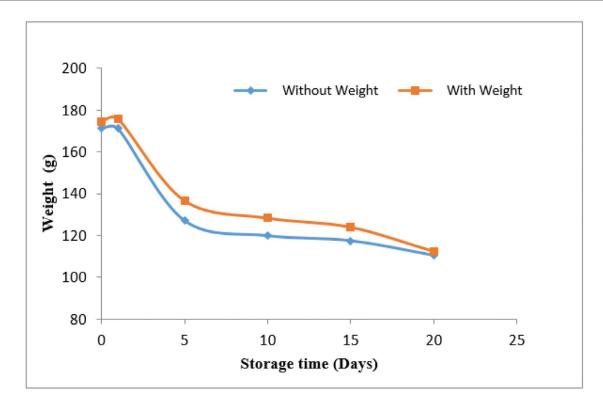


Figure 1. Weight loss of Costeno cheese samples with and without coating over time

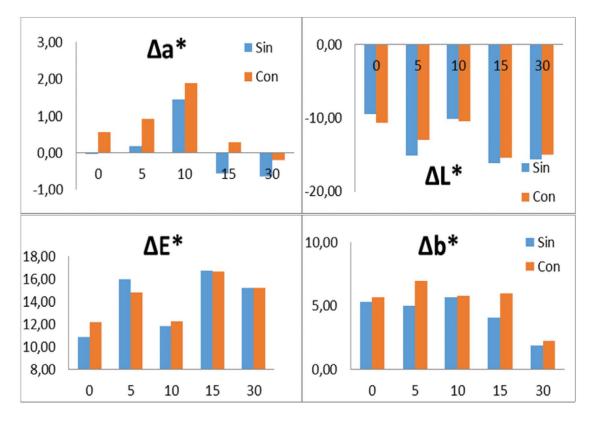


Figure 2. Color parameters $(\Delta a^*, \Delta E^*, \Delta L^*, \Delta b^*)$ for Costeno cheese with and without coatings

Figure 2 shows evolution of color coordinates (ΔL^* ; Δa^* , Δb^* and ΔE) in cheeses coated with the film containing 1.5 g methylcellulose, 0.25% sorbitol and 10% AC, and its respective control (cheeses without coating) throughout the 20 days, stored with an average temperature of 6°C. According to the results, an increase in a* and b* values was observed for the samples with the coatings, while color variation turned out to be similar for both analyzed samples. Considering that a* defines the redgreen component, red for positive values and green for negative values, results show that the coatings had a darkening effect on the samples, which may be since the film is affected by temperature during drying.

A significant rise in the *b values (yellow-blue component) from the first day shows that cheeses with the coating had a yellow color compared to the control samples, which could be due to the temperature effect.

On the other hand, ΔE (total color difference) allows to describe color changes perceived in the cheeses. In this case, total color difference was not significant, not much affecting cheese color tonality and this is due to the light brightness contributed by the coating that would make it more appetizing.

4. Conclusions

Biodegradable coating use in intermediate humidity products such as Costeno cheese, allowed to obtain a weight loss reduction of food stored for 15 days. The 1.5 g methylcellulose, 0.25 g sorbitol, and 10% citric acid formulation, was the most suitable for Costeno cheese conservation, since it allowed to obtain adequate organoleptic characteristics in the cheese. It is essential to obtain and characterize films as coatings model systems since their obtained properties allowed to design the MC coating, with the most suitable characteristics for its final requirement.

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