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Optical and mechanical properties of active biofilms made with citral microparticles

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Abstract

The effect of the sodium caseinate:sorbitol (CS:Sb) and CS:citral microparticle (CS:MC) ratio on the optical and mechanical properties of active biofilms was studied. The application of a 3x3 3x3 factorial arrangement was followed with three CS:Sb ratios (1:0.5, 1:1.0 and 1:1.5) and three CS:MC ratios (1:0.5, 1:1.0 and 1:1.5). It was made by casting method, pouring the formulation solution into Petri dishes and dried 24 hours at 25 ± 0.5 °C and 55 ± 2.0% relative humidity. Optical and mechanical properties were evaluated. The studied variables showed significant differences in the interaction of the factors and between the levels of the factors. Biofilms between 121 and 192 µm thick were obtained. The significant increase in thickness was directly related to higher CS:Sb and CS:MC ratios. It was observed that the best results regarding the active biofilms optical properties were found for formulations with lower levels of CS:Sb and CS:MC, obtaining less color difference (7.6), less opacity (14.8%) and less transparency value (1.4 A_{600}/mm). Regarding the biofilms mechanical properties, the best results were found for formulations with high sorbitol concentrations and low MC concentrations, showing greater deformation (63%) and less tensile strength (1.9 MPa).

Keywords: Deformation; active package; elastic modulus; opacity; transparency value.

Propiedades ópticas y mecánicas de biopelículas activas elaboradas con micropartículas de citral

Resumen

Se estudió el efecto de la relación caseinato de sodio: sorbitol (CS:Sb) y relación CS: micropartícula de citral (CS:MC) en las propiedades ópticas y mecánicas de biopelículas activas. Se siguió la aplicación de un arreglo factorial 3x3 con tres relaciones de CS:Sb (1:0,5; 1:1,0 y 1:1,5) y tres relaciones CS:MC (1:0,5; 1:1,0 y 1:1,5). Se elaboraron mediante método de *casting*, vertiendo la solución de formulación en placas de Petri y se secaron 24 horas a 25±0,5 °C y 55±2,0% de humedad relativa. Se evaluaron las propiedades ópticas y mecánicas. Todas las variables evaluadas mostraron diferencias significativas en la interacción de factores y entre los niveles de factores. Se obtuvieron biopelículas entre 121 y 192 µm de espesor. El incremento significativo del espesor tuvo relación directa con el aumento de las relaciones CS:Sb y CS:MC. Se observó que los mejores resultados, con respecto a las propiedades ópticas de las biopelículas activas, fueron encontradas para formulaciones con menores niveles de relación CS:Sb y CS:MC, obteniendo menor diferencia de color (7,6), menor opacidad (14,8%) y menor valor de transparencia (1,4 A_{600}/mm). Respecto a las propiedades mecánicas de las biopelículas, los mejores resultados se encontraron para formulaciones con altas concentraciones de sorbitol y bajas concentraciones de MC, observándose mayor deformación (63%) y menor resistencia a la tracción (1,9 MPa).

Palabras clave: Deformación; envase activo; módulo elástico; opacidad; valor de transparencia.

Introduction

Container and packaging industry is one of the most dynamic in the world, mainly due to its importance in the various value chains. For this reason, this sector reached, in 2018, a world production of 975 billion dollars [1].

Containers and packaging are widely used in different industrial sectors, such as the food industry. In this sector, the most commonly used primary packaging materials are paper, cardboard, plastics, metal and glass [2,3]. Of these, plastic packaging has increased its share in recent years, occupying 45% of the total packaging market, due to its light weight, versatility and low cost [1,4]. However, plastic containers are manufactured from petroleum products,which are non-renewable, non-biodegradable sources;they take more than a hundred years for their degradation and, therefore, cause environmental pollution; the main ones being polyethylene, polystyrene and polypropylene [5,6].

Faced with this problem, biopolymers are considered as biotechnological resources with unique properties such as the absence of toxicity, degradation and biological compatibility; moreover, they constitute a source for the development of biodegradable biofilms [3]. The main biopolymers used for this purpose are obtained from starch, cellulose, seaweed, chitosan, fish scales, protein sources, fruit seeds, among others; to which other materials such as lipids, plasticizers, active agents and solvents are added [7–10]. Among the biopolymers from protein sources, those obtained from dairy products such as casein stand out [11,12].

Casein biofilms are transparent, biodegradable, and have good oxygen barrier properties; in addition, they can be used to support antimicrobial or antioxidants compounds (biocomposites), giving the biofilm a functional property known as active biofilm or active packaging [13–15]. The addition of these biocomposites is enhanced when incorporated protected, for example, by microencapsulation [16,17]. These active biofilms are very useful for the packaging of perishable foods such as dairy, meat, fruit and vegetables, among others processed foods. In some case studies, the following benefits are shown: prevention ofwater loss, oxygen permeability, lipid oxidation delay, texture and flavorpreservation, microbial count reduction and, in general, the improvement of foodstuff shelf life from the interaction of the biocomposites with its container [8].

The production of active biofilms with antimicrobial and/or antioxidant properties can be formulated with the incorporation of natural substances, such as extracts or active agents (compounds or secondary metabolites) from essential oils (EA), in free or microencapsulated form [18–21]. Of the active agents (AA), menthol, geraniol, thymol, eugenol, carvacrol, citral, among others, stand out [22–25]. From the formers, citral (3,7-Dimethyl-2,6-octadienal) is an acyclic monoterpene aldehyde, composed of two geometric isomers: geranial (citral *A* in its *cis* form) and neral (citral *B* in its *trans* form) [26,27];which mainly possesses antimicrobial activity [28].

The literature reports some studies where microencapsulated citral was incorporated into the formulation of active biofilms, among the most recent, the reported by Alarcón-Moyano et al. [16], who added it to a sodium alginate matrix, obtaining stable biofilms and microbial reduction from the*in vitro* tests. On the other hand, there are studies where sodium caseinate was used in the production of active biofilms with the incorporation of AA in free form, such as carvacrol [14,15], maize germ EA [29]ortung oil EA [30]. Most of the aforementioned works agree that the addition of different levels of the biopolymer, plasticizer and active agent (AA) significantly influence the physical, optical and mechanical properties of the biofilm. However, no evidence was found for the use of citral microencapsulated with sodium caseinate and sorbitol for the production of active biofilms.

Therefore, the present study aimed to evaluate the effect of sodium caseinate, sorbitol and citralmicroparticles on the optical and mechanical properties of active biofilms.

Experimental

Reagents

For the preparation of the active biofilm is require: sodium caseinate, sorbitol (Sigma-Aldrich, Germany) and citralmicroparticles (average size of 7.08 µm) obtained in previous studies [31,32], prepared with citral and soy lecithin (Sigma-Aldrich, Germany) and dextrin (Ingredion, Peru).

Preparation of active biofilms

The biofilms were prepared following the methodology proposed by Arrieta et al. [15], with modifications. The solutions were formulated in distilled water with 5 % m/v sodium caseinate (CS). Sorbitol (Sb) was added to obtain CS:Sbdifferent ratios (1:0.5, 1:1.0 and 1:1.5). They were mixed for 10 minutes under continuous stirring at 1000 rpm on a magnetic stirrer (Thermo Scientific, SP131015 Cimarec, USA)at 50 °C,and then cooled to room temperature. The average

pH of the CS-Sb solutions was 6.48 ± 0.01. Subsequently, citralmicroparticles (MC) were added in ratios of CS:MC (1:0.5; 1:1.0 and 1:1.5),then mixed at 1000 rpm for 5 minutes at 35 °C. The average pH of the final solutions was 6.39 ± 0.04. Finally, all solutions suffered ultrasonic degassing (QSonica Q55, Newtown CT, USA) at 35% intensity for 10 minutes and room temperature, to eliminate foam and air bubbles.

Films were made by a casting method, pouring 9 mL of these solutions in Petri dishes of 9 cm diameter (EULab, Germany); and casting a total of four petri dishes per solution(see treatment column in table 1). They were conditioned for 24 hours at 25 ± 0.5 °C and 55 ± 2.0% relative humidity (HR) in a drying chamber with forced airflowVenticell-VC222 (MMM Group, Germany), equipped with aTraceable® hygrometer (Thermo Scientific, USA). The resulting active biofilms were stored at room temperature, inside duly labeled polyethylene bags, for subsequent evaluation within the next 48 hours; with the exception of the transparency value that was evaluated 14 days after forming them, looking for significant differences between the treatments.

Active biofilm evaluations

a) **Thickness**

The average film thickness was measured with a micrometer Digimatic IP-65, series 293-240 (Mitutoyo, Japan) ± 0.001 mm, in five random positions on the surface of three different biofilm per treatment.

b) **Optical properties**

- **Color**

Biofilm color measurement was performed as described by Pires et al. [33], they were applied on the surface of a white standard plate(L^* = 93.11; a^* = -0.63 and b^* = 3.82). Color parameters (L^*, a^* and b^*) were measured with a chromameterCR-400 (Konica Minolta Co., Ltd., Osaka, Japan). The whiteness (W) of the biofilms were calculated using equation (1):

$$W = 100 - ((100 - L^*)^2 + a^{*2} + b^{*2})^{1/2} \quad (1)$$

The color of the film was reported as a difference(ΔE^*) calculated through equation (2) [33].

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

whereΔL^*, Δa^*, Δb^* are the differentials between the film samples color parameter and thewhite standard used as the film background. Color tests were performed at four different positions of three different biofilms per treatment.

- **Opacity**

For the measurement of opacity, the Hunterlab Method [34] and the methodology proposed by Pires et al. [33] were used, employing a CR-400 chromameter (Konica Minolta Co., Ltd., Osaka, Japan). Opacity percentage of the samples was calculated through equation (3) from reflectance measurements of each sample on a black background ($Y_{blackbackground,}$ L^* = 21.84; a^* = 0.29 and b^* = 1.70) and on a white background ($Y_{whitebackground,}$ L^* = 93.12, a^* = -0.65 and b^* = 3.99).

$$Opacity = \frac{Y_{Black\ Background}}{Y_{White\ Background}} x100 \quad (3)$$

Where Y is the tristimulus value; considering that the concept of tristimulus values (X, Y, Z) is based on the theory of the three components of color vision, which establishes that the eye has receptors for the three primary colors (red, green, blue) and that all the other colors seem like mixtures of these three primary ones. These XYZ tristimulus values are the basis for defining the CIELab* color system [35,36]. Opacity tests were performed at four different positions of three different biofilms per treatment.

- **Transparency value (VT)**

Transparency value (VT) of the biofilms samples was calculated through equation (4), as reported by Hamaguchi et al. [37], Pires et al. [33] and Shiku et al. [38], their measurement was performed employinga Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, USA), for which 10x80 mm strips (width x length) were cut and placed in quartz cuvettes. Empty cuvettes were measured as blank.

$$Transparency\ value\ (VT) = \frac{A_{600}}{x} \quad (4)$$

Where A_{600} is the absorbance at 600 nm wavelength and x is film thickness (mm). According to this equation, higher transparency values indicate lower real transparency. Transparency values (VT) were measured 14 days after the forming process, to show the differences between treatments, because in the first few days they were not evident; it was performed on three different biofilms per treatment.

c) **Mechanical Properties**

- **Elastic Modulus (E)**

Tensile tests were carried out at room temperature and 50% RH employing a3365 Instron

Instrument texturometer (Fareham Hants, United Kingdom), according to ASTM D882-01 standard [39] and the methodology proposed by Arrieta et al. [15] with modifications. The tests were performed on rectangular strips (10×80 mm^2), initial grip separation of 40 mm and crosshead speed of 25 mm/min. The average elastic modulus (E) measured in MPa was calculated from the resulting stress (N) - strain (mm) curves resulting as the average of three different biofilms per treatment.

- **Elongation at break (ε_B) andTensilestrenght (RT)**

Elongationat break (deformation) and tensile strength were reported in % and MPa, respectively, calculated from stress (N) - strain (mm) curves, resulting as the average of three diferentbiofilms per treatment and according to the methodologies proposed by Arrieta et al. [15] and ASTM D882-01 [39].

In the present study, for the selection of the best treatment, the one that shows the lowest ΔE^*, opacity value and *VT*for optical properties, as well as the highest percentage of whiteness, was taken into account; as for mechanical properties, the choosen would bethe one that shows lower *E* and RT, and greater percentage of ε_B.

Experimental design and data analysis

In this study, a completely randomized design (DCA) with a 3x3 factorial structure was used. Factors evaluated corresponded to three levels of CS:Sbratios (1:0.5, 1:1.0 and 1:1.5) and also to three levels of CS:MC ratios(1:0.5; 1:1.0 and 1:1.5).From this combination, nine treatments emerged with three replicates each (Table 1). The experimental unit corresponded to an active biofilm of 9 cm in diameter. The residuals obtained from the data of the evaluated variables (optical and mechanical properties) were subjected to a test of normality and homogeneity of variance. After verifying the assumptions, an analysis of variance (ANDEVA) was carried out with 5% significance.

When significant interaction was evidenced among the factors (treatments), a Tukey mean difference test ($\alpha \leq 5\%$) was applied fot suchtreatments. But when significant differences only existed among the levels of at least one factor, theTukey mean difference test($\alpha \leq 5\%$) was appliedfor the levels of this factor.

The results were statistically analyzed using the Minitab® 17.1, free access statistical software for Windows, available at: http://www.minitab.com.

Table 1. Distribution of factors and treatments for the casting of active biofilms.

Factor A CS:Sb ratio	Factor B CS:MC ratio	Treatment
1: 0.5	1: 0.5	T1: 0.5 + 0.5
1: 0.5	1: 1.0	T2: 0.5 + 1.0
1: 0.5	1: 1.5	T3: 0.5 + 1.5
1: 1.0	1: 0.5	T4: 1.0 + 0.5
1: 1.0	1: 1.0	T5: 1.0 + 1.0
1: 1.0	1: 1.5	T6: 1.0 + 1.5
1: 1.5	1: 0.5	T7: 1.5 + 0.5
1: 1.5	1: 1.0	T8: 1.5 + 1.0
1: 1.5	1: 1.5	T9: 1.5 + 1.5

*/ CS: Sodium caseinate; Sb: Sorbitol; MC: Citralmicroparticles.

Results and Discussion

The results for all the variables evaluated showed significant differences not only inthe interaction among factors, but also in their levels.

Thickness

Table 2 shows thickness values found for the active biofilms, which varied between 121.3 and 192.0 µm and were higher than those reported by Arrieta et al. [14,15], for CS biofilms with free carvacrol active agent (AA) (88 ± 16 µm). This is mainly due to the incorporation in the present study, of citral microencapsulated AA with dextrin and soy lecithin, solutes that influenced thickness increment.

As expected, it was observed that the addition of some solutes contributed to the increase in the biofilms thickness. It was also observed that the CS:Sb ratio and CS:MC ratio, both with level 1:0.5, result in thinning biofilms. Likewise, it was found that T1 and T4, both with the lowest content of plasticizer and MC, reported the thinnest biofilms with 121.3 and 143.8 µm, respectively. Significant change was observed with the addition of plasticizer and MC. These results are similar to those reported by Pereda et al. [30] for biofilms with CS and tung oil AE, and that prove a direct relationship between biofilm thickness and the content of solutes in the formulation.

Optical Properties

The whiteness of the active biofilms ranged from 73.1 to 85.4 (Table 3), which is lower than thereported by Pires et al. [33], for biofilms made with hake protein

Table 2. Active biofilm thickness for the evaluated treatments

Treatment (T)	Thickness (µm) X ± SD			
T1: 0.5 + 0.5	121.271	±	6.20	a
T2: 0.5 + 1.0	154.53	±	10.63	bc
T3: 0.5 + 1.5	159.47	±	1.21	bc
T4: 1.0 + 0.5	143.80	±	12.93	ab
T5: 1.0 + 1.0	164.78	±	3.38	bcd
T6: 1.0 + 1.5	170.73	±	22.15	bcd
T7: 1.5 + 0.5	179.07	±	1.75	cd
T8: 1.5 + 1.0	189.93	±	0.99	d
T9: 1.5 + 1.5	192.00	±	8.34	d

[1]Values indicate average (n=3). X: average; SD: standard deviation.
Different letters in the column, indicate statistically significant differences according to the Tukeymean difference test ($p<0.05$), for each treatment (T).

and thyme oil EA. The biofilms with CS:Sbratio (1:0.5 and 1:1.0) and CS:MC ratio (1:1.0), i.e. T1 and T4, had the highest whiteness values, being these treatments also the most transparent. On the other hand, when evaluating the interaction among treatments, it was observed that T3, T6 and T9, all with higher MC content, showed lower whiteness values; results that show that these treatments were less transparent, mainly due to the increase of MC in the formulation to obtain the active biofilms. These results were different from those obtained by Pires et al. [33], who observed that the different concentrations of thyme oil EA, added to the hake protein, did not significantly influence the whiteness of the biofilm, probably due to the fact that it was added in free form. In the present study, instead, AA was added microencapsulated, where in addition to citral; it contained dextrin and soy lecithin (encapsulant and emulsifier, respectively), solutes that, due to their own biochemical, physical and optical characteristics could be responsible for low whiteness values.

Table 3. Optical properties of the active biofilms for the evaluated treatments

Treatment (T)	Optical Properties											
	Whiteness X ± SD			ΔE*[2] X ± SD			Opacity (%) X ± SD			*VT*[3] - 14 days (A_{600}/mm) X ± SD		
T1: 0.5 + 0.5	85.371	±	0.56 f	7.61	±	0.58 a	14.82	±	0.90 a	2.40	±	0.40 ab
T2: 0.5 + 1.0	78.32	±	0.29 c	15.09	±	0.30 c	17.38	±	0.60 abc	1.36	±	0.42 a
T3: 0.5 + 1.5	73.05	±	0.85 a	20.59	±	0.86 e	19.99	±	1.11 c	1.99	±	0.10 ab
T4: 1.0 + 0.5	84.88	±	0.49 ef	8.03	±	0.50 a	16.34	±	0.90 abc	5.80	±	1.14 c
T5: 1.0 + 1.0	79.72	±	0.70 cd	13.61	±	0.69 bc	15.71	±	0.31 ab	2.63	±	0.22 ab
T6: 1.0 + 1.5	75.54	±	1.71 ab	18.11	±	1.68 de	16.20	±	0.90 abc	3.32	±	0.57 b
T7: 1.5 + 0.5	82.20	±	0.68 de	11.07	±	0.75 b	33.66	±	2.57 e	8.29	±	0.69 d
T8: 1.5 + 1.0	77.26	±	1.06 bc	16.16	±	1.09 cd	25.37	±	2.64 d	7.11	±	0.80 cd
T9: 1.5 + 1.5	75.01	±	1.44 ab	18.64	±	1.25 de	19.21	±	0.11 bc	6.83	±	0.72 cd

[1]Values indicate average (n = 3). X: average; SD: standard deviation.
[2]Total color differences.
[3]Transparency value.
Different letters in the column, indicate statistically significant differences according to the Tukey mean difference test ($p<0.05$), for each treatment (T).

The difference of color Δ*E** ranged between 7.6 and 20.6 (Table 3), values higher than those reported by Pires et al. [33], for biofilms with hake protein and thyme oil EA. When evaluating the interaction among levels, it was observed that the CS:Sb ratio with level 1:1.0 and the CS:MC ratio with level 1:0.5 showed lower Δ*E** values. When evaluating the interactions among factors, it was observed that treatments T1 and T4, both with lower MC content, showed the lowest values of Δ*E**.

Opacity varied between 14.8 and 33.7% (Table 3), values that were within the ranges reported by Pires

et al. [33], for biofilms with hake proteins, glycerol and thyme oil EA (15 and 16%). On the contrary, they were higher than those reported by García and Sobral [40] in biofilms of tilapia protein and glycerol (4 and 10%), and more transparent than the biofilms of the present study. This could be mainly due to the addition of an active agent (AA) in the formulation; similarly as in other studies, where the opacity of the biofilms was influenced mainly by AA, which gave it a more opaque appearance[29]. The biofilm with the lowest opacity value, and therefore the most transparent, was achieved with T1.

On the other hand, the transparency value (VT), evaluated 14 days after forming the biofilm, varied between 1.36 and 8.29 A600/mm (Table 3), with T2 having the lowest VT. These values were within the ranges reported for biofilms with CS and maize germ oil [29] and with soy protein [41]. The behavior of the VT was similar to the other optical properties (Whiteness, opacity and ΔE^*), which were influenced by the incorporation of solutes in the formulation, such as Sb and MC.

In general, biofilms in the present study were more opaque than those prepared with CS and EA of free cinnamon and ginger [42], hake proteins [33], wheat protein [43] and soy protein [44]. These differences could be due to the type of protein, origin and form of EA incorporation, which influence the optical properties of biofilms, particularly transparency and color [43,45]. In the present study, it was observed that biofilms with lower levels of CS:Sb and CS:MC ratio showed the best optical properties.

Mechanical Properties

Mechanical properties are considered one of the most important attributes of food packaging materials. The maximum capacity of the films to resist the applied stress is measured by the tensile strength (RT), while the elongationat break (ε_B) is a mechanical property that provides information on the capacity of materials to resist changes in shape before break. These properties are important in packaging material to protect packaged foods from deterioration due to mechanical damage and to maintain their integrity during storage in logistics processes [46]. The mechanical properties of the active biofilms are summarized in Table 4.

Elongation at break(ε_B) varied between 0.55 and 62.97%. When evaluating the interationamong levels, it was observed that the increase in Sb in formulations also increased the% of ε_B, on the contrary, the increase in MC in formulations reduced this parameter. The treatments with the highest percentage of deformation were T4 and T7, where it was noted that the higher levels of plasticizer and lower levels of MC favored the increase in εB. Similar results were reported for biofilms with CS, glycerol and carvacrol [14,15], CS, glycerol and maize germ oil EA[29], in which a direct relationship between the levels of plasticizer and εBwas observed; Furthermore, a reduction in εB with the incorporation of AE was showed.

The elastic modulus (*E*) varied between 52.7 and 493 MPa. The incrementof sorbitol in the formulation reduces*E*, whereas the incrementof MC increases *E*. When evaluating the interaction among factors, it was observed

Table 4. Mechanical properties of the active biofilms for all the treatments evaluated

Treatment (T)	Mechanical Properties		
	Elongationat Break - ε_B (%) X ± SD	ElasticModulus– *E* (MPa) X ± SD	Tensile strength - RT (MPa) X ± SD
T1: 0.5 + 0.5	41.221 ± 4.18 b	239.47 ± 24.56 c	8.42 ± 0.96 e
T2: 0.5 + 1.0	2.42 ± 0.41 e	407.46 ± 42.19 d	5.65 ± 1.10 d
T3: 0.5 + 1.5	0.55 ± 0.13 e	492.96 ± 62.49 e	4.43 ± 1.14 bcd
T4: 1.0 + 0.5	62.97 ± 11.23 a	72.91 ± 7.09 a	5.99 ± 0.72 d
T5: 1.0 + 1.0	33.50 ± 2.14 bc	100.24 ± 8.50 ab	4.64 ± 0.22 bcd
T6: 1.0 + 1.5	15.58 ± 1.45 de	163.24 ± 12.05 bc	4.85 ± 0.58 bcd
T7: 1.5 + 0.5	61.14 ± 9.91 a	52.72 ± 2.85 a	2.88 ± 0.08 abc
T8: 1.5 + 1.0	39.09 ± 5.96 b	55.32 ± 2.57 a	1.90 ± 0.18 a
T9: 1.5 + 1.5	22.70 ± 1.06 cd	68.04 ± 4.32 a	2.76 ± 0.23 ab

[1] Values indicate average (n = 3). X: average; SD: standard deviation.
Different letters in the column, indicate statistically significant differences according to the Tukey mean difference test ($p<0.05$), for each treatment (T).

that treatments T4, T7, T8 and T9, mostly with a higher content of plasticizer, showed lower *E* values. On the other hand, tensile strength (RT) varied between 1.9 and 8.4 MPa. It was observed that an increment in Sb and MC levels, significantly reduce RT. The treatments with the lowest RT values were T7, T8 and T9; all of them with the highest sorbitol content. Both *E* and RT showed similar behaviors, noticing that sorbitol plasticizer is the main responsible for the reduction of these mechanical properties, the same ones that are affected, when other solutes such as free AA or microencapsulated are included. These results were similar to those reported by Arrieta et al. [14] and Akhter et al. [46] for CS, glycerol and AA biofilms.

On the other hand, previous studies report that AAs, such as citral, carvacrol, thymoloilamong others, affect in some ways the interactions between the macromolecular chains in the polymer matrix. This effect may be related to electrostatic interactions between CS and AA due to the different charge distributions in proteic chains. It can be stated that caseinates act as macroanions at the experimental pH (6.3 - 6.6); while AAs such as citral, could be a carrier of protons, because it is an acyclic aldehyde, with a formyl functional group (-CHO),exchanging its proton for another cation, such as sodium, with a positive charge [15]. Another way in which AA incorporation affects the mechanical properties of biofilms could be attributed to the fact that lipophilic AAs embedded in the CS matrix can act as deformable filler particles, improving the traction properties of biofilms [29]. The positive effects of AAs on the elasticity of biofilms are greater when they are incorporated in free form; but they are significantly reduced when added microencapsulated[16].

Most of the investigations where CS plasticizers and lipophilic AAs are evaluated, coincide in that the mechanical properties of biofilms are positively influenced by the incorporation of plasticizer; non-plasticized films have high *E* and RT at the expense of low εB; the opposite behavior (lower *E* and RT, and higher εB) is reported for samples with glycerol [15,29,30,47] and sorbitol [48], which confirms the role of the plasticizer.

Generally, food packaging films are known to require great flexibility at room temperature to avoid unnecessary breakage during use [49]. In this sense, it was demonstrated that the biofilms of the present study, with high levels of CS:Sb ratio and low levels of CS:MC ratio, had adequate mechanical responses for food packaging.

From the results obtained for both the optical and mechanical properties, it could be stated that the treatment that showed the more adequate results was at a medium level of CS:Sb(1:1.0) and a lower level of CS:MC (1:0.5), such a biofilm photography is shown in Figure 1.

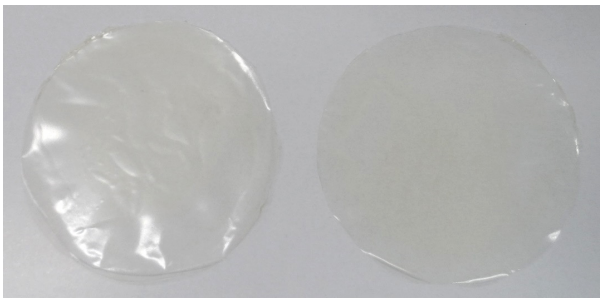

Figure 1. Active biofilms with citralmicroparticlesformulated with CS:Sb ratio (1:1.0) and CS:MC ratio (1:0.5), photography taken immediately after of its processing.

Conclusions

The levels of CS:Sb and CS:MC used in the present work, significantly influenced all the evaluated variables. Increasing levels of CS:Sb and CS:MC significantly increased thickness and negatively influenced the optical properties of biofilms. Regarding the mechanical properties, the active biofilms showed greater elasticity with the increase in Sb, in contrast, they were negatively affected with the increase in MC.

Therefore, if the forming of biofilms with the best mechanical properties (i.e. higher $\%\varepsilon_B$ lower RT and lower *E*) is considered without affecting their thickness and optical properties, among all the formulations tested, the outstanding results were found for biofilms with mean level of CS:Sb (1:1.0) and lower level of CS:MC (1:0.5). This formulation could ensure the conditions for the biofilm processing, as well as the significant presence of the antimicrobial (MC) to obtain an active packaging system. Consequently, these biofilms show potential for future use in preserving fresh food. Moreover, further studies on functional properties related to food contact materials (i.e. microstructure, antimicrobial properties, biodegradability, permeability to oxygen and water vapor) should be needed.

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