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Chemistry

Characterization of Edible Coatings Based on Carboxymethylcellulose with Oregano Essential Oil

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Abstract | **Original Research Article**

The agri-food industry has been closing the technological gap in terms of new food preservation techniques that respect the environmental balance, allowing to extend the shelf life of food. Edible Coatings (EC) provide an additional protective cover to fresh fruits and vegetables, simulating a modified atmosphere. The objective of the present study was to characterize the physico-mechanical and water vapor barrier properties of EC based on Carboxymethylcellulose (CMC) (1.5-2.0%) additivated with oregano (Origanum vulgare) at concentrations of 5, 10, 20 and 40 ppm The statistical analysis of the mechanical properties of the coatings was carried out with a statistical software STATGRAPHICS Centurión XVI.I; where the pH variables that range between 6.986 ± 0.023 and 7.394 ± 0.031 for EC of CMC, density and barrier properties were considered. Ten isolated coatings were obtained, which were characterized for Water Vapor Permeability (WVTR) and mechanical properties of the edible films dried at 40 ° C by natural convection. The results showed that the increase in the concentrations of EOs decreased the WVTR and the capacity of water absorption. The CMC films showed a significant difference in WVTR between the EO.

Keywords: Physical-mechanical properties, rheology, permeability, shelf life, biodegradable packaging.

INTRODUCTION

A coating is a solution applied to a food that forms a surface layer when dried [1, 2]. These films adhere to the surface of the food and take the form it has, separating the product from different food components [3]. This type of EC can be consumed and, in turn, can house different compounds that contribute to the improvement of the final product [4].

Biopolymers are the most used hydrocolloids as coatings and are part of most of the formulations that exist in the agri-food industry, due to their good mechanical properties, gas barrier and adhesion [3]. These films can incorporate various additives such as plasticizers, generally added to improve their elastic properties, surfactants or emulsifiers [5]. In addition, antioxidants or antimicrobial compounds, flavors and pigments can be incorporated that improve the effectiveness of the coating [2].

The edible containers can be grouped according to the polymeric base used, being hydrocolloids, lipids, mixtures or multicomponent systems and proteins (whey, soya, ovalbumin, among others) [6]. Lipids include waxes, acylglycerols, fatty acids and mixtures containing lipid and hydrocolloid components [7].

The use of natural polymers such as polysaccharides and proteins for the production of packaging in food is the trend of the moment, being used for the production of coating films in fruits and vegetables that reduce respiration and transpiration speeds [8]. In another way, they allow to extend the useful life and improve the conditions of structural integrity and mechanical handling of the fruit [4].

The Edible Coatings (EC) used in fruits and vegetables allow to reduce the growth of microorganisms that cause the deterioration of the product, improving the surface brightness and increasing the commercial value of the fruits [9].

The use of chitosan and solutions of coatings with starches where preservatives are incorporated for salmon have allowed to increase the useful life and to avoid the attack of microorganisms, being the chitosan

the best treatment for the processes of conservation and stability for pH and weight loss [10].

The microbial action on the surface of the product depends on the application technique, because some compounds have greater interaction in the films, affecting the physicochemical properties of the coatings [5]. With the incorporation of natural compounds in the coatings, which act as antioxidants or antimicrobials, it can reduce the speed of deterioration of the products and increase their useful life [11].

Agribusiness comes at an accelerated pace to replace chemical additives with compounds of natural origin. The most used are complexes or substances of plant origin such as Essential Oils (EOs) through microencapsulation of the compounds in the polymeric gel network of the coating. The EOs have proven to be potent antimicrobials and recognized as safe foods.

One of the EO with greater antimicrobial efficiency are those obtained from oregano to add RC that can be incorporated in horticultural, meat and cheese foods [12]. Among the lipids added to the formulation are the properties of the coating, the characteristics of the food, the application technique, drying of the coating and the storage conditions that condition the quality of the coated product [13].

The potential of the EC to protect the food from deterioration depends on its barrier and mechanical properties. The first is determined in isolated films preformed on plates that include the permeability to water vapor, gases, volatile compounds and solutes. The second, determines the ability of the coating to form a continuous layer [14]. These barrier and mechanical properties depend on the internal factors of the coating, such as the composition, thickness and preparation technique [15]. In addition, other factors linked to drying and storage conditions can determine the shelf life of the product [16].

The ingredients that form a structural part of the coatings, the thickness and the final drying conditions have a direct influence on the functional properties of the coatings [17]. The chemical characteristics of lipids determine the barrier properties of composite films, such as water vapor permeability [18].

Properties such as polarity, length of the hydrocarbon chain, physical state, degree of saturation and polymorphism are characteristics that affect the water vapor permeability of films [3]. Similarly, the distribution of the lipid in the films (bilayer films or emulsified films), modify their properties [1]. The lipid content, the size of the particle, the stability of the emulsion and the mechanical properties of the lipids are some of the factors that determine the functionality of the emulsified films [19].

The water vapor resistance of the lipid is inversely proportional to its polarity [9]. Water molecules are attracted to these groups by reducing the effectiveness of lipids against the water vapor barrier [20]. In contrast, non-polar groups confer hydrophobicity to the molecule and reduce the water vapor permeability of the resulting film [21].

The characterization of some properties of the film is a contribution when evaluating the behavior of dry film properties [22]. The surface charge and particle size distribution are properties of the coating during its drying process [23].

The objective of this work is to evaluate the effect of the incorporation of essential oil of oregano in the properties of the formulations of the coating and of the dry films of CMC.

MATERIALS AND METHODS

Edible Coatings

To obtain the EC, Merck sodium CMC was used at concentrations of 1.5% and 2.0%, and essential oil from oregano leaves from the municipality of Vijes, Valle del Cauca; obtained by Microwave-Assisted Hydrodistillation (MWHD) at concentrations of EOO (5,10,20 and 40) ppm according to (Table-1).

Table-1: Formulations of edible coatings at two concentrations of CMC additivated with oregano essential oil

Solution	EC-CMC	Essential Oil	Concentration Biopolymer CMC w/v	Plasticizer Glycerol (w/w)	Concentration Essential Oil (ppm)
Control	Control 1	-	-		
	Control 2	-	-		
C-CMC	C-CMC1	Control 1	1,5	1,0	-
	C-CMC2	Control 2	2,0		-
O	CMC1-EOO1	Oregano	1,5	1,0	5
	CMC1-EOO2				10
	CMC1-EOO3				20
	CMC1-EOO4				40
	CMC2-EOO1	Oregano	2,0	1,0	5
	CMC2-EOO2				10
	CMC2-EOO3				20
	CMC2-EOO4				40

Source: self made

Preparation of the EC

Distilled water was used, which was put to cooking. At 37 ° C the biopolymer (CMC) was added very slowly according to the nature of the coating, until reaching a temperature of 80 ° C with constant agitation. Once the temperature was reached, stirring was continued for two hours. The concentrations of the AEs were incorporated when the coatings were at 34 ° C and were homogenized with an ultraturrax T25 at 13000 rpm for 5 minutes and degassed with a vacuum pump at room temperature.

Physicochemical Properties of the Formulations

Particle Size

Determined using a laser diffractometer. The samples were dispersed in distilled water at 2000 rpm to a level of obscuration between 5-10%. To apply Mie's theory, we considered a refractive index of 1.46 and an absorption of 0 for the dispersed particles.

The particle size was determined in triplicate of each of the RCs. The average particle size was expressed in terms of parameters $d_{3,2}$ and $d_{4,3}$ according to equations 1 and 2.

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad \text{Eq. 1}$$

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad \text{Eq. 2}$$

The following properties were measured for each formulation: Density (Picnometer), pH and The water content of the films was determined by drying at 110 ° C for 24 hours, A.O.A.C, 1995. The water content was expressed as a percentage of the total weight.

Obtaining the Dry Films

Each EC was served on methyl-methacrylate plates (MMP) of 15 x 15 cm in diameter that were leveled and dried at 40°C and a relative humidity of 60% for 48 hours in an oven. dried by natural convection. The thickness of the films was controlled by adjusting the same density of surface solids to 62g/m^2

Thickness of the films

The thickness of the films was measured with a FLOWER 54-850 / 860 electronic micrometer, with an accuracy of 1.27 μm in 5 randomly selected sites.

Mechanical Properties

The mechanical tests were carried out using the TA-XT Plus Texture Analyzer brand, with the Tensile Grip A / MTG accessory, according to the standardized method ASTM D882-09. Strips or sheets of 2 x 7 cm were cut for the respective analyzes. The fracture stress (σ), the percentage of deformation at the moment of rupture (% E). Thus the Elasticity Module (EM) of the films formulated from CMC. The fracture stress was calculated by dividing the maximum force required for the rupture by its cross section. E was calculated by dividing the deformation of the film at the moment of rupture by the initial length (50 mm). The modulus of elasticity is obtained with the curve tensile stress against deformation in the initial linear stretch.

The parameters of length, width, weight and thickness were considered for each strip; required for the calculation of the maximum breaking stress and maximum relative elongation. The Force Vs distance curves obtained in the mechanical tests were transformed into Hencky strain vs. deformation curves [24] using the following equations:

$$\delta = \frac{F*(L_0+L(t))}{A_0*L_0} \quad \text{Eq. 3}$$

$$\varepsilon_H = Ln\frac{L_0+L(t)}{L_0} \quad \text{Eq. 4}$$

Where: σ, effort, Pa; F, force, N; *L (t),* length of the film at time *t, m; L_0* Initial length of the film, m; A_0 Initial cross-sectional area of the film m^2; ε_H deformation of Hencky.

Water Vapor Permeability (WVP)

The samples used were stored in desiccators at 54.4% RH and 20 ° C in a climatic chamber for 15 days before making the determinations. This was determined by the gravimetric method recommended by Astm E96 [25] and modified by McHugh *et al.,* (1993), using the laws of Fick and Henry. Permeability was measured using the moisture gradient of 0-100 RH and at room temperature.

Color

The color of the samples was expressed in terms of coordinates *L**, *a**, *b** of the CIELab space, calculated using the KubelkaMunk theory considering an infinite thickness for the films and using as reference the observer 10 ° and the illuminant D65 (Eq. 16,17,18) and from the values of the tristimulus coordinates X, Y, Z (Eq. 19,20,21).

$$L* = 116\left[\frac{Y}{Yn}\right]^{1/3} - 16 \quad \text{Eq. 5} \qquad a* = 500\left[\left(\frac{Y}{Xn}\right)^{1/3} - \left(\frac{Y}{Yn}\right)^{1/3}\right] \quad \text{Eq. 6}$$

$$b* = 200\left[\left(\frac{Y}{Yn}\right)^{1/3} - \left(\frac{Z}{Zn}\right)^{1/3}\right] \quad \text{Eq. 7} \qquad X = \sum[S_\lambda R_\lambda \bar{x}_\lambda] \quad \text{Eq. 8}$$

$Y = \sum[S_\lambda R_\lambda \bar{y}_\lambda]$ Eq. 9 $Z = \sum[S_\lambda R_\lambda \bar{z}_\lambda]$ Eq. 10

Where:

Xn, Yn, Zn = tristimulus coordinates of the standard light source
S (λ) = relative spectral distribution of the illuminant at each wavelength
R (λ) = reflectance of a sample of infinite thickness at each wavelength R_∞
x¯(λ), and¯(λ), z¯(λ) = spectral tristimulus values of the observer 10 ° at each wavelength

From the coordinates L *, a *, b * the tone (Eq. 11), chroma (Eq. 12) and whiteness index of the films were calculated (Eq. 13).

$h *_{ab} = \tan^{-1}\left(\frac{a*}{b*}\right)$ Eq. 11

$C *_{ab} = \sqrt{a *^2 + b *^2}$ Eq. 12

$WI = 100 - \sqrt{(100 - L *)^2 + a *^2 + b *^2}$ Eq. 13

Where:

h*$_{ab}$= tone
C*$_{ab}$= chroma
WI= whiteness index

Statistical Analysis

The results were analyzed by means of an analysis of the variance (ANOVA) with the software STATGRAPHICS Centurión XVI.I, with a level of significance of 5% (Pv <0.05) to evaluate the effect caused by one or more factors in each of the mechanical properties and their interactions

RESULTS AND DISCUSSION

Physicochemical Properties of the Formulations

Density and pH

The density and pH values for EC of CMC additivated with Oregano EO show in (Table-2).

Table-2: Average values of pH and density for EC of CMC additivated with EO

Edible Coatings	Density (kg/m^3)	pH -
C-CMC1	1003,8 ± 0,3	7,22 ± 0,09
C-CMC2	1005,6 ± 0,6	7,12 ± 0,15
CMC1-EOO1	1005,3 ± 0,4	7,10 ± 0,34
CMC1-EOO2	1005,1 ± 0,6	7,16 ± 0,56
CMC1-EOO3	1005,4 ± 0,8	7,02 ± 0,42
CMC1-EOO4	1005,8 ± 0,5	7,17 ± 0,23
CMC2-EOO1	999,21 ± 0,6	6,99 ± 0,12
CMC2-EOO2	998,52 ± 0,2	6,88 ± 0,29
CMC2-EOO3	996,47 ± 0,1	6,93 ± 0,21
CMC2-EOO4	992,19 ± 0,3	6,92 ± 0,45

[a, b, c, d] Different superscripts indicate significant differences (p <0.05).
Source: self made

The incorporation of EO of oregano generated a significant decrease of pH in EC; being lower for films with higher concentration of polymer and essential oil. The decrease in pH may be related to the disintegration of some secondary metabolites present in the EO; as natural acids present in the matrix [23]. With regard to density, a slight decrease was observed, although it was not significant (p> 0.05).

Particle Size

Figure-1 shows the distributions of monomodal and polydispersed particle sizes, with average sizes between 1 and 14 µm for all formulations. The ECs with higher concentrations of EO of oregano had larger particle sizes without significant differences. Table-3 shows the values in terms of $d_{3,2}$ and $d_{4,3}$. The average of the size in surface $d_{4,3}$ as a function of the specific surface per unit of volume, facilitating the characterization of small and spherical particles.

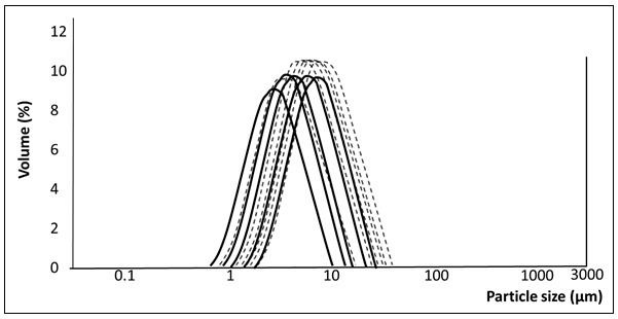

Fig-1: Distribution of particles present in RC additivated with AE of oregano

Table-3: Mean values of particle size $d_{3,2}$, $d_{4,3}$ surface area and particle number of EC with Oregano EO

Edible Coatings	$d_{3,2}$ (µm)	$d_{4,3}$ (µm)	$A_N \times 10^{-3}$ (m^2)	$N \times 10^{-13}$ (Particle/m^3)
C-CMC1	2,69 ± 0,02[a]	3,66 ± 0,01[a]	0,74 ± 0,01[a]	1,28 ± 1,8E^{-2}
C-CMC2	2,76 ± 0,03[a]	3,75 ± 0,01[a]	0,77 ± 0,02[a]	1,54 ± 8,1E^{-2}
CMC1-EOO1	3,78 ± 0,02[b]	4,85 ± 0,02[b]	1,23 ± 0,03[b]	2,16 ± 4,4E^{-2}
CMC1-EOO2	3,81 ± 0,03[b]	4,93 ± 0,03[c]	1,68 ± 0,04[b]	2,27 ± 3,8E^{-2}
CMC1-EOO3	3,86 ± 0,03[bc]	4,86 ± 0,03[c]	1,91 ± 0,06[b]	2,38 ± 1,6E^{-2}
CMC1-EOO4	3,92 ± 0,03[cd]	5,99 ± 0,01[d]	2,12 ± 0,01[c]	2,58 ± 2,5E^{-2}
CMC2-EOO1	3,72 ± 0,02[b]	4,89 ± 0,02[b]	1,35 ± 0,03[b]	2,27 ± 4,7E^{-2}
CMC2-EOO2	3,83 ± 0,01[b]	5,02 ± 0,02[b]	1,64 ± 0,01[b]	2,35 ± 7,2E^{-2}
CMC2-EOO3	3,88 ± 0,02[b]	5,10 ± 0,02[c]	1,98 ± 0,05[b]	2,56 ± 3,4E^{-2}
CMC2-EOO4	3,96 ± 0,02[bc]	5,22 ± 0,01[cd]	2,27 ± 0,02[c]	2,78 ± 1,1E^{-2}

[a, b, c, d] Different superscripts indicate significant differences ($p < 0.05$).

Source: self made

The average values of particle size distribution in volume ($d_{3,2}$) represents the average size based on the weight unit of the particles, and represents the largest particles and irregular shapes, such as aggregates. Likewise, the average surface area of droplets exposed to the continuous phase per unit volume of dispersion (A_N) related to the parameter $d_{3,2}$ and the number of particles per m^3 of the EC (N) from the area was calculated. superficial and $d_{4,3}$

Drying kinetics of the CMC EC additivated with Oregano EO

The CMC films added with EO of oregano showed a drying time between 40 and 48 hours at 40 ° C with a RH of 27% (Table-4). The formulations of CMC with 1.5% of the polymer with the highest concentration of AE used (40 ppm) showed a lower process speed during drying due to a higher moisture content, compared to films made with 2% CMC and with the same volume of EO (Figure-2). The humidity of the films was between 0.80 and 1.76%; It being found that the higher the concentration of the polymer, the greater the drying speed.

Table-4: Drying kinetics of the CMC EC at two concentrations additivated with oregano EO

Days	CMC3-EOO	CMC4-EOO
6	23,0	23,4
9	23,5	20,8
12	18,1	17,1
15	19,9	16,9
24	16,1	12,7
27	12,6	9,18
30	10,8	7,25
36	7,45	7,29
40	4,74	0,80
45	1,71	-
48	1,76	-

Source: self made

The formulations of CMC with 1.5% of the polymer with the highest concentration of EO used (40 ppm) showed a lower process speed during drying due to a higher moisture content, compared to films made with 2% CMC and with the same volume of EO (Figure-2).

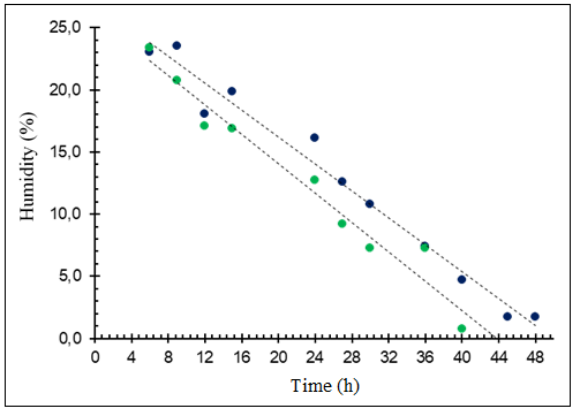

Fig-2: Drying kinetics of the CMC edible coatings added with oregano EO

Characterization of Dry Films

10 types of CMC dried films were obtained, added with Oregano EO adjusted to the same solids surface density, for each coating, in the preparation of the formulations; all the films had the same behavior and sensory aspect.

The addition of EO of oregano in the CMC formulations produced a decrease in modulus of elasticity and tensile stress at the time of rupture caused by a weakening in the structure when interruptions were generated in the gelation network of the polymer (Table-5). Similarly, the percentage of elongation also decreased, resulting in less rigid films and less deformable to incorporate higher oil concentration. These results coincide with those obtained by other authors for CMC based films with essential oils [18].

Table-5: Modulus of elasticity (ME), tensile stress (σf) and deformation at the moment of fracture (ε_f) of CMC films with oregano EO

Edible film	ME (MPa)	σ_f(MPa)	ε_f
C-CMC1	2,69 ± 0,02^{a}	3,66 ± 0,01^{a}	0,74 ± 0,01^{a}
C-CMC2	2,76 ± 0,03^{a}	3,75 ± 0,01^{a}	0,77 ± 0,02^{a}
CMC1-EOO1	3,78 ± 0,02^{b}	4,85 ± 0,02^{b}	1,23 ± 0,03^{b}
CMC1-EOO2	3,81 ± 0,03^{b}	4,93 ± 0,03^{c}	1,68 ± 0,04^{b}
CMC1-EOO3	3,86 ± 0,03^{bc}	4,86 ± 0,03^{c}	1,91 ± 0,06^{b}
CMC1-EOO4	3,92 ± 0,03^{cd}	5,99 ± 0,01^{d}	2,12 ± 0,01^{c}
CMC2-EOO1	3,72 ± 0,02^{b}	4,89 ± 0,02^{b}	1,35 ± 0,03^{b}
CMC2-EOO2	3,83 ± 0,01^{b}	5,02 ± 0,02^{b}	1,64 ± 0,01^{b}
CMC2-EOO3	3,88 ± 0,02^{b}	5,10 ± 0,02^{c}	1,98 ± 0,05^{b}
CMC2-EOO4	3,96 ± 0,02^{bc}	5,22 ± 0,01^{cd}	2,27 ± 0,02^{c}

$^{a, b, c, d}$ Different superscripts indicate significant differences (p <0.05).

Source: self made

Water Vapor Permeability (WVP)

The values of Water Vapor Permeability (WVP) at 20 ° C for a relative humidity gradient of 100/54 are shown in table 6. With the addition of oregano EO, the WVP of the films decreased when higher concentration in the formulation; finding significant differences (Pv <0.05) for the formulations where 40 ppm of oregano EO was used. This phenomenon occurs when essential oils are used in hydrophilic films. WVP is increased by plasticizing the polymer network due to water sorption, which results in a less dense structure, with greater molecular mobility, allowing better water permeability and diffusivity [1].

Table-6: Water Vapor Permeability *values for CMC films with EO of oregano*

Edible film	WVP (g/Pa*m*s)
C-CMC1	73,60 ± 0,04^{a}
C-CMC2	74,18 ± 0,06^{a}
CMC1-EOO1	63,12 ± 0,12^{b}
CMC1-EOO2	62,35 ± 0,08^{b}
CMC1-EOO3	61,78 ± 0,14^{bc}
CMC1-EOO4	59,52 ± 0,05^{d}
CMC2-EOO1	61,45 ± 0,02^{b}
CMC2-EOO2	60,06 ± 0,16^{b}
CMC2-EOO3	59,17 ± 0,01^{c}
CMC2-EOO4	58,34 ± 0,14^{d}

$^{a, b, c, d}$ Different superscripts indicate significant differences (p <0.05).

Source: self made

Color

The obtained spectra were calculated for a sample of infinite thickness to avoid the influence of the depth of measurement [26]. The CIE Lab* coordinates were calculated and from these the chromatic parameters tone (h^*_{ab}) and chroma (C^*_{ab}) were obtained as well as the whiteness index (WI) of the samples which are shown in Figure 3 and 4.

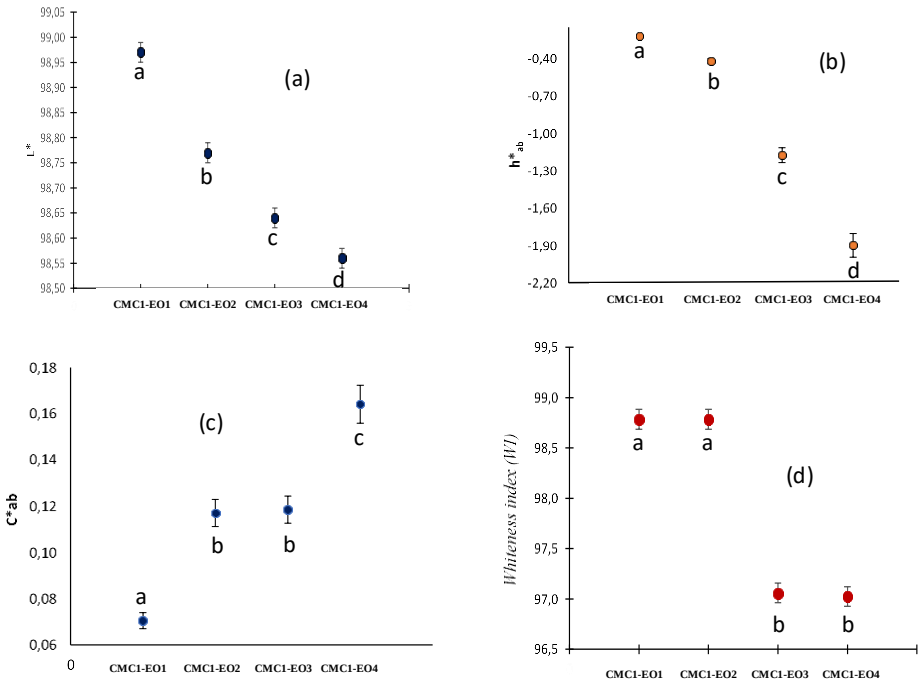

Fig-3: (a) Luminosity, (b) Chroma, (c) Tone and (d) Whiteness index for 1.5% CMC films with Oregano EO

$^{a, b, c, d}$The different index indicate significant differences

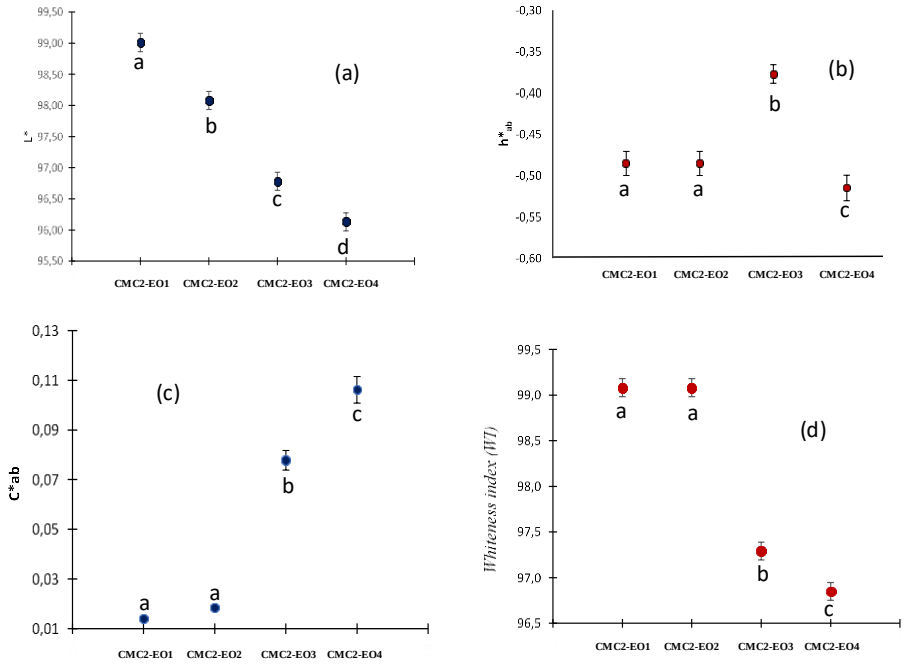

Fig-4: (a) Luminosity, (b) Chroma, (c) Tone and (d) Whiteness index for 2.0% CMC films with Oregano EO
[a, b, c, d]The different index indicate significant differences

With the addition of oregano EO, a greater color saturation (chroma) occurs. Similarly, an increase in the tone, taking increasingly green tones. This is consistent with the decrease in the whiteness index of the films, which is due to the selective absorption of the essential oil of oregano. The quantified changes between the films were not visually appreciated for any of the established formulations.

CONCLUSIONS

The addition of oregano essential oil in CMC matrices allowed to encapsulate secondary metabolites characteristic of oregano in relation to the controls and had a significant effect depending on the properties of the coatings and the dry films becoming more rigid and porous with the increase of the volume of the EO.

The particle size distribution was affected by the EO content of oregano, generating larger particles with the highest oil concentration; which could exhibit stability problems in the EC over time.

The increase in EO of oregano in the dried films caused an improvement of the barrier properties when there was a decrease in the water vapor permeability. The mechanical properties were affected by having a lower mechanical strength and less deformable.

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