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Research Paper

Oregano essential oils: Antimicrobial activity and its application to films based on cornstarch and glycerol

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ABSTRACT

This trend has led to research on the development of new biodegradable packaging materials from natural polymers also suggests that several materials may be incorporated into edible films and have influence on the mechanical, protective and sensory properties. The objective of this work was to characterize oregano essential oils and incorporated edible films made with cornstarch and glycerol. The oregano essential oil obtain the raw material used was the variety Italian Oregano or *Origanum majorana* using a Clevenger-type apparatus. The study included the refractive index, chemical composition, color at day 0 and day 10 and antimicrobial activity of *Salmonella Enteritidis* and *Escherichia Coli* O157:H7. The films were prepared according to the method of casting, cornstarch, glycerol (plasticizer) and water. To assess the effect of the composition on the physical properties of films, the experiment was arranged in a 2^2 factorial design, with one central point. It analyzed the transfer of water vapor (Method ASTM E96-92) and color. The film which showed the best characteristics was selected; subsequently oregano essential oil was added. The refractive index of the oregano essential oils obtained from the various extractions varied between 1.4875 and 1.4981. There was a significant difference ($P<0.05$) between the parameters a* and b* between day 0 and day 10. According to gas chromatography analysis, 52 compounds were identified in oregano essential oil, thymol (31.96%) and carvacrol (0.66%) phenols. The test antimicrobial activity, showed that *Salmonella Enteritidis* and *Escherichia Coli* O157:H7 were extremely sensitive to pure essential oil. The films obtained were transparent, presented homogeneous and compact surfaces. The values of permeability to water vapor were between 1.93 x 10^{-12} and 9.85 x 10^{-12} (g/m.s.Pa). The Analysis of Variance (ANOVA) indicated a significant differences between the different formulation ($P<0.05$). The incorporation of oregano essential oil at the formulations influenced the permeability presenting more or less resistant to the passage of water vapor. About the color, incorporating essential oil did not affect the appearance and did not show strong antibacterial activity at the concentration at which it was decided to work.

Key words: Oregano essential oil, edible film, cornstarch, permeability.

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Abbreviations: OEO: oregano essential oils, **RH:** relative humidity, **WVP:** water vapor permeability, **MIC:** minimum inhibitory concentrations, **MBC:** minimum bactericidal concentrations.

INTRODUCTION

Essential oils are rich in phenolic compounds, such as flavonoids and phenolic acids. In general, the ones that possess the strongest antibacterial properties against the foodborne pathogens contain high concentrations of these

compounds such as carvacrol, eugenol, phenol and thymol that also have a wide range of biological effects including antioxidant and antimicrobial properties (Burt, 2004). The mode of action has been described as the alteration of the cytoplasmic membrane, disrupting the proton motive force, the flow of electrons, active transport, and /or coagulation of the cellular content (Burt, 2004). Fractions of essential oil of oregano and pepper are efficient against various food borne bacteria such as *Salmonella*, *E. coli* O157:H7. Some essential oils of spices incorporated into the packaging materials can control microbial contamination in meat muscle, reducing the growth of *Escherichia Coli* O157:H7 and *Pseudomonas spp*. (Oussallah et al, 2004).

The development of new biodegradable packaging materials from natural polymers, in order to achieve a partial alternative to the plastic containers is important. Edible films can be obtained from various sources (polysaccharides, lipids, proteins) (Tharanathan, 2003), which in many cases are waste products of fishing, agriculture or livestock. In recent years, research has focused on edible films made from protein sources of edible plants and animals, such as corn zein, wheat gluten, soy and peanut protein, cottonseed, albumin, gelatin, collagen, casein and whey proteins (Tharanathan, 2003). Guilbert (1986) also suggests that several materials may be incorporated into edible films and have influence on the mechanical, protective and sensory properties. This author also mentions that to improve the organoleptic properties in the food or nutritional products flavoring agents, pigments or nutritional additives in may be incorporated in edible films. The potential influence of the additive in the film properties will depend on the degree of concentration, in the chemical structure, the degree of dispersion on the film and on the interaction with polymers (Okhamafe and York, 1954).

The essential oils can be applied to the development of edible films, providing them with additional antioxidant and/or antimicrobial properties, which can extend the life and reduce or inhibit foodborne pathogens (Oussallah et al., 2004; Zivanovic et al., 2005). There are some studies on the antimicrobial properties of films based on milk proteins, chitosan or alginate incorporated with various essential oils (oregano, rosemary, garlic, paprika, cinnamon) (Oussalah et al., 2004, 2006; Zivanovic et al., 2005; Seydim and Sarikus, 2006) which have been successful. Essential oils have been widely used in food products due to their antibacterial, antifungal and antioxidant properties (Viuda-Martos et al., 2009). Edible films can serve as a carrier of antimicrobial compounds in order to maintain a high concentration of preservatives in food surfaces. Application of natural antimicrobials provides ample opportunities to develop new food preservation systems, as compared to the direct application of antimicrobials to food surface; antimicrobial edible films are more effective. Additionally forming characteristics and application of edible films can improve the organoleptic, mechanical, nutritional and personal protection properties of small pieces of food delaying microbial growth and extend the life of food. Hence the importance of further studying of the properties and applications of films with added antimicrobial, giving specific emphasis to the potential use of specific natural antimicrobial.

The objective of this work was to characterize oregano essential oil (OEO) and its incorporation to the edible films made with cornstarch and glycerol, and to study physical and antibacterial properties.

MATERIALS AND METHODS

Oregano essential oil

Extraction

To obtain the essential oil of oregano, the raw material used was the variety known as Italian Oregano or *Origanum majorana*. The essential oil was extracted from the aerial parts (leaves) of the plants selected by the method of hydro-distillation using a Clevenger-type apparatus according to the method of Dadalioglu and Evrendilek (2004). The sample was collected in a separating funnel, which was collected with a syringe and the mixture of water-oil, it was centrifuged, dried over anhydrous sodium sulfate and stored in sealed glass jars covered with aluminum foil at 4°C until assayed.

Physical properties

Essential oil refractive index was measured in triplicate using an ABBE refractometer (PZO, Poland). The measurement temperatures were 20 ± 2°C. Essential oil color was measured using a colorimeter HunterLab (Minoltta, USA) in the CIE L* a* b*. The measurement was made to the product recently obtained and it was stored for ten days in refrigeration and protected from light. For the calibration of the equipment a porcelain white plate was used. The color measurement of the sample was performed in triplicate.

For the chemical composition analysis of oregano essential oil were performed with a Agilent 6890 Series Plus gas chromatograph equipped with a HP-5 capillary column (30m × 0.25 mmid; coating thickness 0.25 µm), an Agilent 7683 Series automatic Injector and a flame ionization detector. Analytical conditions were: injector temperature 250°C, oven temperature programmed from 40 to 190°C at 5°C /min; carrier gas nitrogen at 1 ml/min. injection of 1 µl (diclorometano DCM), split ratio 1:50, detector temperature 250ºC (flame ionization detector). The identification of the main components was performed by using standards compounds (thymol and carvacrol).

Table 1. Design matrix used in the film preparation.

Experiment	Coded variables		Original variables	
	Glycerol contents	Starch contents	Glycerol contents* (%)	Starch contents* (%)
1	0	0	2.5	5
2	-1	-1	1.5	3
3	-1	1	1.5	7
4	1	-1	3.5	3
5	1	1	3.5	7

*Percentages are given as g/100 g film

Antimicrobial activity

Bacterial strains: Two test microorganisms were used, a strain of *Salmonella Enteritidis* isolated from poultry origin and a strain of *Escherichia Coli* O157:H7 isolated from animals confined in the province of Entre Rios, Argentina. The strains were stored at -30°C glycerol solution (50% v/v). For these studies were recovered in brain heart infusion broth (BHI) at 37°C for 24-48 h and were reincubated in nutrient agar before using them in experiences.

Agar diffusion method: The adjustment of the concentration of the inoculum was determined by performing several dilutions in peptone water and measuring the absorbance with spectrophotometer until a specified value was achieved. After the inoculum a massive sowing was performed in Petri dishes. There were four replicates per microorganism. On each plate disks (of approx 6 mm) were placed impregnated in undiluted oregano oil (as is) and the following dilutions: 1/5, 1/10, 1/20, 1/30, 1/40 and 1/50. A sterile disc was used as control. As a positive control a nitrofurantoin disc, an antibiotic that was resistant to the strain of *Salmonella Enteritidis* and one nalidixic acid, an antibiotic that was resistant to the strain of *Escherichia Coli* O157: H7. The diameters of the inhibition zones were measured in milimeters. The sensitivity of the microorganism to the essential oil of oregano relates to the size of the zone of inhibition of bacterial growth (Ponce et al., 2008). Measurements were performed in quadruplicate.

Tween-80 (0.5% v/v) was used as an emulsifying agent in a concentration that did not affect the growth of microorganisms due to the hydrophobic nature of essential oils.

Determination of minimum inhibitory concentrations (MIC) and bactericidal (MBC)

The method dilution was used in trypticase soy broth medium for the bacteria. Dilutions of 50, 100, 200, 300, 400, 500 and 600 mg/l were prepared, 0.5 ml of each culture of the bacteria to be tested is added to each concentration, and the tubes were incubated at 35°C for 24 h. Subsequently, the tubes were examined visually for turbidity in the test medium. The lowest concentration at which turbidity is observed and the MIC was established. From the concentrations that do not exhibit microbial growth turbidity, they are confirmed by inoculation on plates with trypticase soy agar by streak and incubated at 35°C for 24 h. The lowest concentration at which the plates exhibit no bacterial growth was determined as MBC. Measurements were performed in quadruplicate.

Edible films

Preparation

The films were prepared according to the method of casting or emptying. Films solutions were prepared with cornstarch, glycerol (plasticizer) and water based on starch gelatinization process. An aqueous solution of corn starch was heated to a temperature between 70- 80°C for 30 min with continuous magnetic stirring; glycerol was added later, while maintaining solution at pH 6.0. Once the solution was completely homogeneous, it was spread on polyacrylic petri dishes and dried at 55°C for 6 h with a forced convection dryer. Once dried, all samples were conditioned inside desiccators containing saturated saline solutions of NaBr which provided relative humidity (RH) of 57.5 – 57.7% for 48 h at temperature of 25°C, before testing.

Experimental design

To assess the effect of the composition on the physical properties of films, the experiment was arranged in a 2^2 factorial design with one central point, it was replicated two times. The contents of glycerol and starch were the two factors. The levels were 1.5, 2.5 and 3.5%, for glycerol, and 3, 5 and 7%, for starch, resulting in a total of five experiments (Table 1).

Water vapor permeability

The transfer of water vapor through the film was determined using the gravimetric method ASTM E96-92 (1990). The film was mounted in a permeation cell (8.4 cm of diameter) with silica gel (0% RH), the head space of the cell was 1.0 cm and placed in a desiccator with distilled water (95% RH) at 25°C and we proceeded to record the weight loss kinetics every half hour. The thickness was measured with a micrometer analog (0.01 mm).

Calculation of water vapor permeability (WVP): the weight gain for the different films along the time was graphed, which gives the slope of the line (Δm/t), the linear portion of the graph that best fits a straight line (R^2, close to 1) will represent the stabilizing diffusion of water vapor through the film per unit time (g/h), which is then normalized by the area of films used. The data of the value of the slope was used to calculate the water vapor transmission (WVT). The area (A) of film exposed to the exchange (the effective area of the cell) was 55.4 cm^2. By using the equation 1, we calculated the water vapor permeability (WVP, g/msPa):

$$WVP = \frac{WVT}{\Delta p} = \frac{WVT}{A * P * (R_2 - R_1)} * e \qquad (1)$$

Where Ap is the difference in water vapor pressure between inside and outside cell pressure (desiccator controlled humidity and temperature). P is the saturation vapor pressure at the working temperature (2333.1 Pa), R_1 is the relative humidity desiccator and R_2 relative humidity inside cell, where P_x (R_2-R_1) = 2216.445 Pa, and e is the thickness in mm. The samples were analyzed in triplicate.
The films color was measured using a colorimeter HunterLab (Minoltta, USA) obtained the values of L *, a * and b *. With the data obtained, the whiteness index was calculated according to Equation 2:

$$\text{Whiteness index} = 100 - \sqrt{(100 - L^*) \cdot 2} \cdot a^* + b^* \qquad (2)$$

The edible film samples were analyzed in triplicate.

Incorporation of oregano essential oil to edible films:

The film which showed the best characteristics was selected, subsequently OEO was added. To the perfectly homogeneous solution, without starch gelatinization, the OEO was added, with stirring using Ultra-Turrax (Precytec, Argentina) at 6500 rpm and Tween 80 (0.02% v/v) as emulsifying and surfactant that allows the formation of uniform film. Then there was the starch gelatinization and film formation by the process described earlier.

Color change and antimicrobial activity

The films color change of the film with and without oregano essential oil was measured according to whiteness index calculated a colorimeter HunterLab (Minoltta, USA).

The determination of the antibacterial activity of the films against *Salmonella Enteritidis* and *E. coli* O157: H7 was performed according to the method of Duan et al. (2008) with modifications. In an initial culture of about 10^5 UFC/ml in 100 ml of buffered peptone water with Tween 80 (0.02% v/v), 0.03 g of film with a proportion of OEO concentration was placed and it was cultured at room temperature and at 8°C by stirring. As control, a bacterial film without the OEO was taken. Bacterial counts were performed every 3 h in trypticase soy agar supplemented with 0.6% yeast extract at 35°C for 24 h. Measurements were performed in quadruplicate.

Statistical analysis

Tukey's test was chosen for simultaneous pairwise comparisons. Differences in means were considered significant when P<0.05, were computed using the SYSTAT 12 software (SYSTAT, Inc., Evanston. IL).

RESULTS AND DISCUSSION

Oregano essential oil

The calculated performance of the hydro-distillation of the extraction was 0.02 ml of the essential oil per gram of dry material from the plant. The essential oil obtained after distillation was the characteristic nice, strong, pungent, warm and herbaceous smell.

The refractive index of the oils obtained from the various extractions varied between 1.4875 and 1.4981 which is attributable to the drying method with anhydrous sulfate in which oil can be kept with different water contents. The average value of 1.4928 value measurements are similar to those reported by Bayramoglu et al. (2008) and Albado Plaus et al. (2001). The refractive index value for the majority of essential oils is relatively close and varies between 1.43 and 1.61 at 20°C. In general, those with lower refractive index of 1.47, have a high percentage of terpene hydrocarbons or aliphatic compounds. By contrast, a refractive index greater than 1.47 indicates the possible presence of aliphatic oxygen in essence. In some cases, this determination is used to reveal the presence of impurities.

For oregano essential oil obtained, the measurements in CIE L* a* b* in Day 0, there were high values of L* (79.18 ± 0.52), which indicates a light color, negative low values of a* parameter (-5.47 ± 0.67) which would be within the green range, and b* parameter (15.98 ± 2.43) presents positive values approaching yellow hue. The values parameters a* and b* were increased in day 10, at - 8.92 ±

0.67 and 24.07 ± 2.43 respectively, which may be due to reactions between compounds, oxidation or auto oxidation that could change the color during storage.

According to gas chromatography analysis, 52 (100%) compounds were identified in oregano essential oil (Table 2), two of which were identified by their respective standards, thymol and carvacrol phenols, being the former the major component (31.96%). These results agreed with the OEO analyzed by Baratta et al. (1998) which contained a high percentage of thymol and carvacrol (32.4 and 16.7% respectively), these results not so with those obtained by Viuda-Martos et al. (2007) which reported the essential oil of oregano (*O. vulgare L.*) analyzing 32 compounds, representing 88.5% of the total essential oil, the major constituent being carvacrol (61.21%).

The antimicrobial activity of OEO is related to its chemical composition, especially with the concentrations of phenolic compounds carvacrol and thymol, which in turn depends on the species and the harvesting time.

The antimicrobial activity carried out by the agar disc diffusion method shows values of the essential oil resulted discs with halos around them which indicate susceptibility to the essential oil, predicting that both bacteria were inhibited by the essential oil of pure oregano presenting larger halo diameter, which reduced in size to the dilution 1/30 (Table 3). Depending on the size of the zone of inhibition both microorganisms are extremely sensitive to pure essential oil and as the dilutions increase, they lose sensitivity, but at the 1/5 dilution they are very sensitive which would be helpful when the essential oil is added to a biodegradable film used for packaging food.

The minimum inhibitory concentrations (MIC) determined for *S. Enteritidis* equal to 483.33 ± 98.32 mg/l and for *E. Coli* O157: H7 equal to 566.66 ± 136.62 mg/l and minimum bactericidal concentrations (MBC) for *S. Enteritidis* equal to 566.3±183.5 mg/l and for *E. Coli* O157: H7 equal to 600 ±178.9 mg/l. Santoyo et al. (2006) reported values of 750 mg/l for the bacterium *E. coli* when using Carvacrol obtained from the oregano essential oil of the same range studied (*O. majorana*). Dadalioglu and Evrendilek (2004) reported a strong inhibitory effect of the direct application of essential oil of oregano for *E. coli* O157:H7, *L. monocytogenes, S. typhimurium* and *S. aureus*. In other research work, it was found that gram negative bacteria such as *E. Coli*, *P. aeruginosa, S. typhimurium*, *S. cholerae* suis and *V.* cholerae and gram-positive bacteria such as *S. aureus* and *B. cereus*, showed different degrees of sensitivity and only *P. aeruginosa* showed resistance (Albado Plaus et al., 2001).

Properties of edible films

The films obtained, which were transparent, presented homogeneous and compact surfaces. The color attributes of the containers is an important factor in terms of overall appearance as it directly influences consumer acceptability (Srinivasa et al., 2004). All formulations showed high values of the parameter of brightness (L*> 88) indicating that the formulations exhibit light color. It was observed that there were significant differences between the different formulations. The coordinate a* showed negative values near neutrality (-1.03<a*<-0.90), indicating that there would be no green hue and the coordinate b * values presented positive but small (0 <b * <3.5), giving evidence that it would present a slight yellowish color (Table 4). By the results of analysis of variance it can be concluded that there were significant differences between formulations for both coordinates, resulting in both the content of starch and glycerol were influential variables. It can be observed that the whiteness index of the films was modified by the composition, and its lowest value was when the concentration of glycerol and starch was high. In turn there were no significant differences ($P<0.05$) between formulations containing a lower concentration of glycerol regardless of the starch content.

The water vapor permeability can be described as the volume or mass of gas or water vapor that penetrates the surface of a film in a given period of time. Permeability is an important concept within the food packaging industry. The testing of the WVP of the film allowed us to determinate how much permeable is the developed film, that is, the material's ability to allow the passage of water vapor, therefore a material more resistant to the passage of water vapor has a low permeability, which would be very convenient to preserve fresh food longer. The values of permeability to water vapor (Table 5), it can be observed that they are between 1.93×10^{-12} and 9.85×10^{-12} (g/m·s·Pa), there were significant differences between the formulations ($P<0.05$), it can be observed that the highest value corresponds to the formulation containing a greater amount of glycerol and minor amounts of starch (Formulation 4). High permeability values in high concentrations of glycerol may be because there was an increased flow of water through the polymeric matrix attributable to the hygroscopicity of glycerol, which generates more paths to transport water molecules through polymer matrix (Flaker et al., 2010). Despite the fact that glycerol is an effective plasticizer and its efficiency is determined by their low molecular weight, high ability to interact with water facilitates solubilization and permeation through the film (Cuq et al., 1997).

Moreover the film that had the lowest permeability value was the one with the highest starch and glycerol content. This could be attributed to the fact that the higher starch content in the matrix decreases the effect of the ability to interact with the water of glycerol and not allowing it to modify the molecular organization of the matrix, so it generates a denser structure and as a consequence a less permeable one. Garcia et al. (2000) reported that WVP depends on many factors such as the ratio of crystalline and amorphous region, the mobility of the polymer chain and

Table 2. Chemical composition of the essential oil of oregano.

Peak number	Name	Rt [min]	Área [pA*s]	%Área [gr/100gr]
1	DCM (svte)	1.929	2119	
2	DCM (svte)	2.204	39874	
3	DCM (svte)	2.271	89378	
4	DCM (svte)	2.295	330356623	
5	DCM (svte)	2.455	16067	
6		8.162	114275	1.223829696
7		8.356	47070	0.504096817
8		8.771	5855	0.062704204
9		9.448	66738	0.714731536
10		9.554	88944	0.952547001
11		9.784	11849	0.12689703
12		9.907	113239	1.21273464
13		10.019	8433	0.09031333
14		10.309	22013	0.235748529
15	NI	10.659	466688	4.998001605
16	NI	10.884	374620	4.011998083
17		11.015	132774	1.421944993
18		11.097	5672	0.060744363
19		11.238	83170	0.890710268
20		11.54	7706	0.082527514
21	NI	11.872	824200	8.826781324
22		12.11	98760	1.057671589
23		12.728	157418	1.685870253
24	NI	13.005	614023	6.575887829
25		13.347	43909	0.470244044
26		13.675	94416	1.011149461
27		14.187	64942	0.695497249
28		14.965	24649	0.263978807
29	NI	15.284	1609212	17.23387822
30		15.471	5942	0.063635931
31	NI	15.64	339107	3.631671117
32		15.78	12903	0.138184857
33		15.834	2115	0.022650622
34		16.107	34114	0.365344356
35		16.829	31744	0.339962808
36		17.085	119572	1.280557991
37		17.371	20745	0.222168865
38	Timol	18.348	2984693	31.96461105
39	Carvacrol	18.596	61477	0.658388784
40		20.653	10632	0.113863551
41		20.904	10974	0.117526205
42		21.782	175492	1.879434006
43		22.004	8304	0.088931803
44		22.265	12401	0.132808681
45		22.622	6939	0.074313317
46		23.143	24365	0.260937305
47		23.284	78279	0.838330036
48		23.66	62785	0.672396828
49		23.853	73533	0.787502683
50		24.054	18109	0.193938587

Table 2 Cont.

51	24.248	52175	0.558768886
52	25.56	52225	0.559304361
53	25.709	31420	0.336492926
54	26.303	6527	0.069900997
55	27.255	7642	0.081842105
56	27.615	4865	0.052101785
57	27.966	7838	0.08394117
		Total	100

NI: Unidentified peaks. a: identification of components based on standard compounds.

Table 3. Measurement of inhibition halos produced by *Salmonella Enteritidis* and *Escherichia Coli* O157: H7 at the different dilutions studied of the oregano essential oil.

	Microorganisms	
	Salmonella enteritidis	***Escherichia coli* O157: H7**
	Diameter of inhibition zone (mm)*	**Diameter of inhibition zone (mm)***
Control positive	12	8
Control negative	without halos	without halos
Essential oil of pure oregano	24.2±6.18[a]	24.2±6.0[a]
Dilution 1/5	14.4±3.05[a]	19.8±6.2[a]
Dilution 1/10	13.0±2.5[a]	13.6±1.8[a]
Dilution 1/20	9.8±1.7[a]	9.0±2.3[a]
Dilution 1/30	8.6±1.1[a]	7.6±1.3[a]
Dilution 1/40	without halos	without halos
Dilution 1/50	without halos	without halos

*including disk diameter of 6 mm. The results are the mean values ± standard deviation. Means with the same letters in rows indicate there are no significant differences between treatments (P <0.05) Tukey's test was chosen for simultaneous pairwise comparisons.

Table 4. Variations of permeability, thickness and parameters L*,a*, b* of the films.

Formulation	**Thickness (m)**	**WVP(g/smPa)**	**L***	**a***	**b***	**Whiteness index**
1	$2.99x10^{-4}$±$1.89x10^{-5}$[a]	$4.60x10^{-12}$±$4.7x10^{-13}$[a]	89.3±0.34[a]	-0.96±0.02[ab]	2.69±0.06[a]	103.6±0.10[b]
2	$1.66x10^{-4}$±$1.89x10^{-5}$[b]	$3.44x10^{-12}$±$4.7x10^{-13}$[ab]	92.4±0.34[c]	-0.97±0.02[a]	2.49±0.06[a]	102.9±0.10[b]
3	$2.94x10^{-4}$±$1.89x10^{-5}$[a]	$3.75x10^{-12}$±$4.7x10^{-13}$[ab]	89.8±0.42[ab]	-0.96±0.02[ab]	3.21±0.07[b]	103.0±0.12[b]
4	$3.47x10^{-4}$±$2.31x10^{-5}$[a]	$9.85x10^{-12}$±$5.7x10^{-13}$[c]	90.9±0.34[b]	-0.90±0.02[b]	2.54±0.06[a]	102.8±0.10[b]
5	$2.72x10^{-4}$±$1.89x10^{-5}$[a]	$1.93x10^{-12}$±$4.7x10^{-13}$[b]	92.4±0.34[c]	-1.03±0.02[a]	3.30±0.06[b]	102.4±0.10[b]

The results are the mean values ± standard deviation. Means with the same letters in columns indicate no significant differences between treatments (P <0.05). Tukey's test was chosen for simultaneous pairwise comparisons.

Table 5. Variation of the thickness. water vapor permeability and parameters L*, a *, b * of the films with and without essential oil.

Formulation	Thickness (m)	WVP(g/smPa)	L*	a*	b*	Whiteness index
Without oil	$2.99 \times 10^{-4} \pm 1.89 \times 10^{-5a}$	$4.6 \times 10^{-12} \pm 5.8 \times 10^{-13a}$	89.3±0.34[a]	-0.96±0.02[a]	2.69±0.06[a]	103.6±0.10[a]
With oil	$2.72 \times 10^{-4} \pm 2.7 \times 10^{-5a}$	$2.6 \times 10^{-12} \pm 5.8 \times 10^{-13b}$	91.9±0.19[b]	-1.05±0.02[b]	3.30±0.13[b]	102.7±0.09[b]

The results are the mean values ± standard deviation. Means with the same letters in columns indicate no significant differences between treatments (P <0.05) Tukey's test was chosen for simultaneous pairwise comparisons.

the specific interaction between functional groups of polymers and gases in the amorphous region. The slight differences in the values of WVP may be related to this difference in the diffusion of water molecules and the hydrophilic and hydrophobic ratio (Arvanitoyannis et al., 1994; Garcia et al., 2000).

If it refers to packaging typical polymers including polyvinylidene chloride which has a permeability between 0.7-2.4 $\times 10^{-13}$ (g/m·s·Pa), high density polyethylene (2.4 $\times 10^{-13}$ g/m·s·Pa), developed films without oil would be within the order of magnitude but if compared with the cellophane (7.7 $\times 10^{-11}$ to 8.4 $\times 10^{-11}$ g/m·s·Pa) commonly used in food packaging (sweets, dried and crystallized fruits, baked goods), they would be two orders of magnitude lower (Gennadios et al., 1994b; Krochta et al., 1994).

The values of the thicknesses of the films are shown in Table 5, there are varied records in the literature as to the thickness of films obtained by casting method based on starch, authors as Garcia et al. (2000) developed corn starch films unplasticized, with an average thickness of 1.20×10^{-4} m and plasticized with glycerol or sorbitol, with thicknesses of 1.04×10^{-4} and 1.11×10^{-4} m, respectively, It was significantly (P<0.05) affected by the composition of the formulations. Being the highest values, that corresponds to the formulation with the highest permeability, there being a positive relationship between permeability and the thickness of the film obtained (Figure 1). Bertuzzi et al. (2002) exhibited this behavior indicates that the films would be provided not only as a simple barrier but that there was an association of water molecules and functional polar groups of the starch, resulting in swelling of the grain and structural changes that facilitate diffusion of water vapor.

Incorporation of oregano essential oil

To incorporate the essential oil, formulation 1 was chosen because it was the one that presented an intermediate permeability and an increased thickness.

Color and water vapor permeability

Films with essential oil had a pale yellow color, regarding the whiteness index of the films without essential oil was 103.6±0.10 and with essential oil was 102.7±0.09, which were significantly different (P <0.05). The variables of L*, a * and b * of the films with and without OEO showed, a significant increase in value when the essential oil was incorporated into the matrix of the film (Table 5), the increase of these variables may be due to some interactions between components with starch chains. According to Kunte et al. (1997), the color of a film may influence the acceptability of a product by consumers. The addition of essential oil promotes a pale yellow film that not affects the appearance of the food product when in use.

The addition of essential oils significantly reduces (P <0.05) the WVP of the films, Table 5 shows the values, thickness and water vapor permeability in the films with and without OEO, for the films containing no oils the following values were observed $4.06 \times 10^{-12} \pm 5.87 \times 10^{-13}$ (g/m·s·Pa) and the films containing oil this value was reduced to $2.56 \times 10^{-12} \pm 5.8 \times 10^{-13}$ (g/m·s·Pa) the reduced vapor transmission was 57%. This effect may be related to the fact that the OEO gives the matrix structure of the film a hydrophobic character. It can be emphasized that it improves the moisture barrier. Hernández (1994) indicated the transfer process of the steam into the films depends on the ratio of the hydrophilic-hydrophobic constituents of the film. The addition of essential oil did not significantly affect (P>0.05) film thickness. Comparing the results obtained with literature data was difficult because there were many factors that affect the phenomenon of permeability of the films. Romero-Bastida et al. (2011) mentioned that the addition of the essential oil of cinnamon to films oxidized banana starch decreased the water vapor permeability of 18.34×10^{-10} to 5.07×10^{-10} (g/m·s·Pa). Also, investigations based on films in zein with essential oil of oregano, clove, eugenol, carvacrol and where the water vapor permeability was significantly affected by the concentration of oil and not by the type of oil (Marzo, 2010). Other investigators indicate that incorporating essential oils may affect the hydrophilic/hydrophobic property of the film due to their hydrophobic nature (Ojagh et al., 2010). In general, edible films based on corn starch have important applications for use in foods, however, they have a moderate gas barrier due to its hydrophilic properties, but the addition of a hydrophobic substance (oil) as an additive helps increase the water vapor resistance of the films.

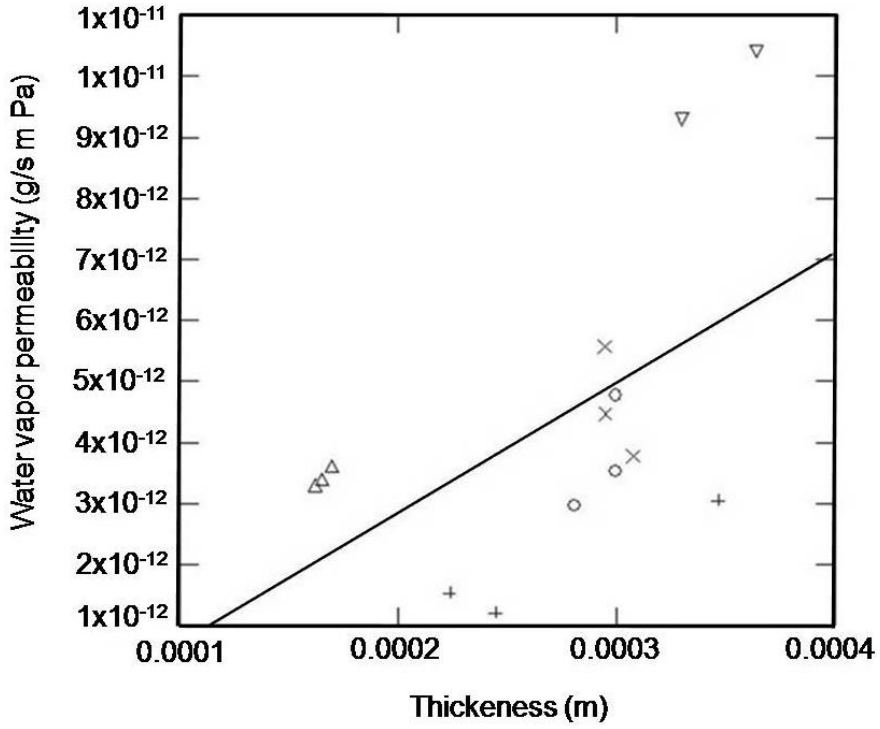

Figure 1. Ratio of water vapor permeability and thickness of the films developed (× Formulation 1; △Formulation 2; ○Formulation 3; ▽Formulation 4; + Formulation 5).

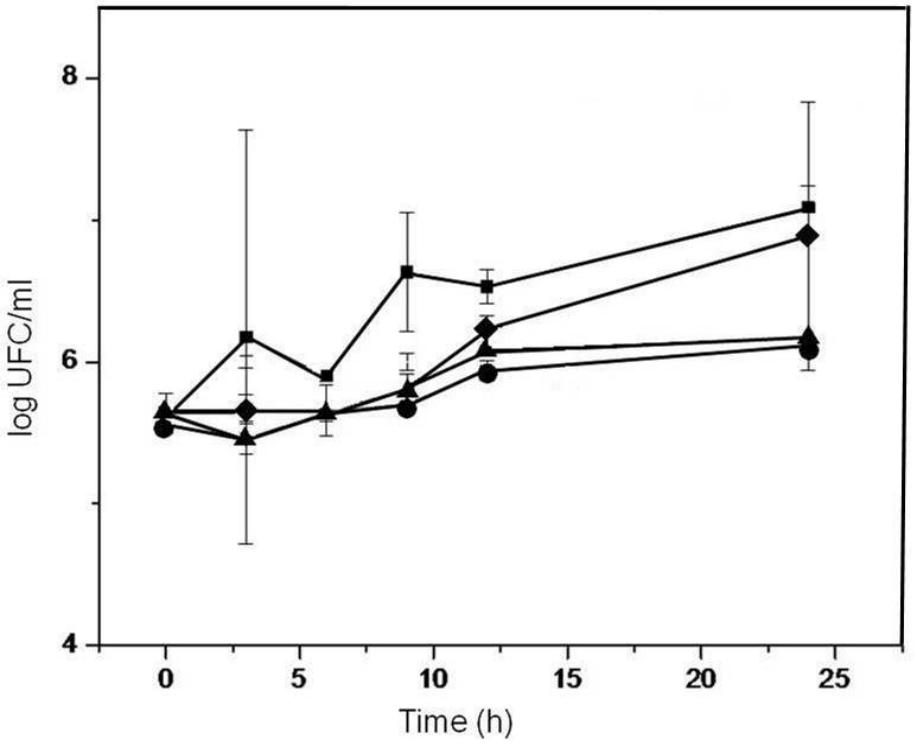

Figure 2. Survival charts of the strains studied. ■*S Enteriditis* (20°C) ▲ *S Enteriditis* (8ºC) ◆*E. coli* O157:H7 (20ºC) ● *E. coli* O157:H7 (8°C)

Determining antibacterial activity of the films with essential oil

The concentration of the film with oregano essential oil was 0.5%. Figure 2 shows the curves of the bacterial counts in trypticase soy agar for bacteria and at the two different operating temperatures chosen. It can be seen that the concentration chosen for the test did not produce inhibition of both cultures. In turn, the bacterium *E. coli* O157: H7 can be considered as more inhibited than *S. enteritidis* in the two selected temperatures. In the literature, contradictory results are expressed for films formed with different matrices added with essential oil of oregano. One of the cases was for the alginate films different degrees of crosslinking obtained by internal gelation where antimicrobial activity was determined using the method of diffusion agar on which showed antibacterial effect against *S. aureus*, *L. monocytogenes*, *E. coli* and *S. enteritidis* after the incorporation of 1.0% of the essential oil of oregano (Benavides et al., 2012). Seydim and Sarikus (2006) reported that in whey protein films with different concentrations of OEO (1 - 4% w/v) against *E. coli* O157: H7 and *Salmonella Enteritidis*, didn´t showed any inhibition at concentrations of 1% but they did when the concentration was increased. So we can say that the antimicrobial activity can be influenced by the structure of the film in which the oregano essential oil is dispersed and we know that this activity is also dependent on the concentration of phenolic compounds present which in turn depends on the origin of the plant, harvest and prior treatments.

Conclusions

The preferred method for obtaining the essential oil of oregano was the most suitable as compared with the distillation by steam. It was observed the refractive index of the essential oil varies between 1.4875 - 1.4981 and the color changes after ten days of storage. The chemical composition determined by gas chromatography showed that the essential oil contains fifty-seven compounds two of which were identified by their respective standards, phenols Thymol (31.96%) and Carvacrol (0.66%). Its antimicrobial activity, showed that the microorganisms tested that was extremely sensitive to pure essential oil. The development of glycerol corn starch films, using the chosen design allowed us to determine that the composition is a factor influential in the permeability, in parameters L*, a* and b* and its whiteness index. The incorporation of an essential oil in one of the formulations influenced the permeability and does not affect the appearance, resulting in a pale yellow. The film with oregano essential oil in this trial did not show strong antibacterial activity at the concentration at which it was decided work.

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