Effect of oregano essential oil (Lippia berlandieri Schauer) inclusion on physicochemical properties of buffalo gourd (Cucurbita foetidissima Kunth) root starch films

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Research Article

Keywords: Starch, oregano essential oil, oregano, buffalo gourd, physical characterization

Posted Date: May 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1630683/v1

Abstract

In the present assay buffalo gourd root starch (BGRS) and Mexican oregano essential oil (OEO) (which are considered alternative botanical sources) were used to obtain biodegradable films. The effect of different concentrations of OEO (0.1, 0.3, 0.5 and 1.0% w/w respect to starch) on the film's physicochemical properties *vs* a control sample (without OEO) was evaluated. The additive inclusion significatively impacted on optical, mechanical, permeability, thermal and morphological properties with respect to the control, mainly at OEO low concentrations (0.1 and 0.3%), due to its component's plasticizer capacity. FTIR analysis evidenced the different degrees of material's components mixtures integration through the possible formation of hydrogen bonds. SEM micrographies showed an appropriated components integration for 0.1 and 0.5% OEO concentrations, and according to their physicochemical properties, these films may have a greater possibility to have applications on food packing.

Introduction

For decades food packing materials have been developed through petroleum derivates, which despite of the adequate physical, mechanical and barrier properties, are highly contaminant and require a great number of resources for their transformation, with no other benefit than been used once, besides, in some cases, acting negatively with the food contained [1]. To mitigate this problem, materials from natural renewable sources have been obtained so as to produce bioplastics which may compete with synthetic ones, at least for some applications, such as packing material or films, without the disadvantages that synthetic plastics present [2].

Nowadays biodegradable materials are being used, mainly those obtained from natural sources, such as starches, mixed with synthetic matrices in order to reduce the biodegradability time [3], despite it has not been possible to completely solve the environmental problem derived of the excessive usage of food packing products [4], and that the resulting physical properties are still far to be equalized with those of petroleum derivates, efforts keep being focus on the development of polymeric plastic material derived of natural raw materials. Such raw materials have been successfully used at producing crafts that in the past were exclusively manufactured with synthetic supplies, so they represent an opportunity for the advance in this field of the technological development [5, 6].

Out of the supplies used to formulate plastic products or biodegradable films, starches represent the greatest sustainability, due to its abundancy (from vegetable sources), ease of obtaining and accessible cost, as well as their simplicity through the conversion process in biodegradable plastic products [7] united to that, these represent other usages or applications in different cientific and industrial fields, where applications on bioassays, at medicaments extended realease, in the stem cell and tissue engineering, in food safety and in food packing, besides beeing used as combustion retardants, bio-filters and membrane filters and even in the construction materials enginering [8, 9]. Despite all the advantages it present, the main sources of starch for industrial applications are crops that represent the main caloric

apport for human and animals, as corn, rice, starch, potato, and yucca, which reduces food availability [10].

According to the issues previously exposed, its necessary to explore and investigate starch new sources which do not represent a treat to food world supply, in the last decades a wide variety of botanic species that can be sources of this biopolymer for industrial purposes has been discovered [11]. Out of the feasible alternative sources, buffalo's gourd root (*Cucurbita foetidissima* Kunth), a weed specie that grows in semi-deserts of North America [12], may represent a potential raw material for starch obtention. In this sense and according to a current assay, buffalo's ground root provides starch yields up to a 52% (dry base) and its starch has characteristics and rheological, thermal, structural and morphological properties similar to those of other starchy sources (as yucca) which has been previously reported by this research team [13]; without previous reports of biodegradable films elaboration coming from this alternative no-conventional starch source.

On the other hand, to diversify starch film's properties, several additives from different botanic sources have been used to provide them with "new functions", so these films have advantages over synthetic plastic materials, outstanding antimicrobial and or antioxidant activities and their biodegradability [14]. These functionalities help to prolong the shelf life of the products packaged in them and to improve their acceptability by consumers as they come from natural origin [15]; despite that functionalizing materials integration in starch films usually impact on physicochemical, structural, thermal and barrier properties [16], thus a previous evaluation of the physicochemical properties (mainly mechanical, permeability and structural) is necessary, before suggesting any practical application.

Among the additives usually employed, outstands essential oils, which can be obtained from very different botanical sources, for instance thyme (*Thymus vulgaris*), clove (*Syzygum aromaticum*) and oregano (*Origanum vulgare* L.). According to scientific literature reports, these essential oils are rich in phytochemical compounds that provide antimicrobial and antioxidant properties to the matrices in which they are included [17–19]. Thymol and carvacrol are two phytochemicals, which have proven antimicrobial and antioxidant capacity [20] and that are found in Mexican oregano (*Lippia berlandieri* Schauer) which along with buffalo ground (*C. foetidissima*) grows in North America semidesert [21]. The objective of this work is to evaluate the effect or oregano's essential oil inclusion on the physicochemical, optical, mechanical, thermal, and structural properties of films elaborated with starch extracted from buffalo's ground roots.

Materials And Methods

Films Elaboration

Starch from buffalo's ground roots (BGRS) was obtained following the methodology reported by Hernández-Centeno, María, Rodríguez-González, Tirado-Gallegos, Rios-Velasco, Zamudio-Flores and Characterization [13] using *Cucurbita foetidissima* Kunth roots collected in wild areas at "Universidad

Autonoma Agraria Antonio Narro", campus located in Buenavista, Saltillo, Coahuila, Mexico. Oregano essential oil (OEO) was obtained from a foreigner laboratory, in which was also characterized by gas chromatography, according to Vazquez and Dunford [22] reports, oregano's leaves (*Lippia berlandieri* Schauer) were obtained from wild zones at the south region of Chihuahua state in Mexico. OEO main components were thyme and carvacrol 1:4 relation, respectively, phytochemicals with proven antimicrobial and antioxidant capacity [23–25]. Control film (OEO free) was produced according to casting method as Tirado-Gallegos, Zamudio-Flores, Ornelas-Paz, Rios-Velasco, Olivas Orozco, Espino-Díaz, Baeza-Jiménez, Buenrostro-Figueroa, Aguilar-González and Lardizábal-Gutiérrez [26] reported, using BGRS and glycerol at 2% and 4% as plasticizer. The OEO functionalized films were produced according to Ghasemlou, Aliheidari, Fahmi, Shojaee-Aliabadi, Keshavarz, Cran and Khaksar [14] reported methodology with modifications. Films were prepared at different OEO concentrations (0.1, 0.3, 0.5 and 1.0%, respectively) using 1 mL of absolute ethanol as OEO solubilizer and dispersant mixing in vortex at 300 rpm for 30 s at room temperature (25 ± 2°C). Ethanolic OEO sample was then added to the filmogenic solution (at the same starch-glycerol proportions of the control sample) once it reached 40°C, to void OEO volatilization, mixture was carried out in a magnetic plate at 600 rpm. Five treatments were then obtained including 0.0% OEO considered as control. Molding was carried out in acrylic plates 15 × 15 × 2 cm, with a density of 0.191 g/cm^2 of filmogenic solution. Samples were dried in a convention drying stove (model SixTrays, Ivation™, C&A IP Holdings, LLC, NJ, USA.) for 48 h at 35 ± 1°C. Later, the films were released from the acrylic plates and were conditioned at 25 ± 2°C in a 57 ± 5% RH. Films were storage in Ziploc™ bags, up to moment of the corresponding analysis.

Humidity, Solubility and Thickness

Humidity percentage (%) was carried out by gravimetric method as reported by Zamudio-Flores, Ochoa-Reyes, Ornelas-Paz, Tirado-Gallegos, Bello-Pérez, Rubio-Ríos and Cárdenas-Felix [27]. Solubility percentage was done by triplicate following Suh, Ock, Park, Lee and Park [28] reports, and films thickness analysis, was conducted through a spiral pattern, using a digital micrometer Mitutoyo™ (model H-2780, Mitutoyo Co., Kanagawa, Japan) as reported by Tirado-Gallegos, Zamudio-Flores, Ornelas-Paz, Rios-Velasco, Olivas Orozco, Espino-Díaz, Baeza-Jiménez, Buenrostro-Figueroa, Aguilar-González and Lardizábal-Gutiérrez [26].

Color, Yellowness Index and Opacity

Color measurement was carried out in a Minolta™ colorimeter (model CR-300, Minolta Co., Osaka, Japan) following the reported methodology of [29]. Color difference (ΔE) was determined by the Ec. (1), referred by Kwon, Chang, and Han (2017) and yellowness index (YI_{FC}) was determined through the color variables previously obtained according to Ec. (2) reported by Kaushik, Kaur, Rao and Mishra [30]. At the same time, films opacity was determined at by absorbance at 600 nm in a spectrophotometer UV/Vis (Labomed brand, model UVS-2700 Labomed Inc., California, USA), using Ec. (3) reported by Anchundia, Santacruz and Coloma [31].

$$\Delta E = \sqrt{(L_s - L^*)^2 + (a_s - a^*)^2 + (b_s - b^*)^2}$$ Ec. (1)

$YI_{FC} = 142.86b/L$ Ec. (2)

Opacity = ABS_{600nm}/thickness(mm) Ec. (3)

Where *L* is luminosity value and *a* and *b* are the values of the chromatic coordinates in the color space CIE*Lab*.

Infrared Spectroscopy by Fourier Transform (FTIR)

The OEO spectrograms obtention was carried out in a FTIR equipment with the additament for attenuated total reflectance (ATR) Perkin Elmer™ (model Spectrum Two, Perkin Elmer Inc., Bucks, UK) according to the methodology described by Kwon, Chang and Han [20]. An OEO drop was placed in the equipment's crystal lector and an average of 5 spectrograms were registered, with 32 scans per reading with a resolution of 4 cm^{-1} in the region of 4000 a 550 cm^{-1}. For control and OEO films it was used the reported conditions of Yao, Qin, Zhang, Zhang, Qian and Liu [32], for which an average of 5 repetitions were obtained for each treatment sample, having 32 scans in each lecture in the region of 4000 a 550 cm^{-1}. The de-convolution of spectrograms of all sample films was also performed with the software SPECTRUM™ 10 version 10.6.2 (Perkin Elmer, Inc., Bucks, UK) in order to analyze the regions of interest in the wave numbers (*n* = 3600 at 3000 cm^{-1}, 280 at 3000 cm^{-1} and 1550 at 1800 cm^{-1}). To evidence OEO inclusion, spectrograms of films with higher concentrations of OEO (5 and 10%) were analyzed, under the same conditions of the films in study. Apparent crystallinity index was also determined from the quotient of the bands 1044/1016 cm^{-1} [33].

Mechanical Properties and Water Vapor Permeability (WVP)

Mechanical properties consisted in the evaluation of the variables of tensile strength (TS, in MPa), percentage of elongation at break (%EAB) and elastic module (EM, in MPa) as the methodology reported by Zamudio-Flores, Ochoa-Reyes, Ornelas-Paz, Tirado-Gallegos, Bello-Pérez, Rubio-Ríos and Cárdenas-Felix [27], which is based on the ASTM D882-10 norm with some modifications. Probes of 1 × 6 cm, previously conditioned at 55 ± 5% RH for 24 h at room temperature (25 ± 2°C), 5 assays per treatment were performed in a Brookfield™ texturometer (model CT3, Brookfield Engineering Laboratories Inc., Massachusetts, USA), using a load cell of 4 kg and with the software TexturePro CT version 1.9.35 (Brookfield Engineering Laboratories Inc., Massachusetts, USA), and for the corresponding calculations the following equations were used:

TS = W/Ac Ec. (4)

%EAB = $(\Delta L/L_0) \times 100$ Ec. (5)

Where W is the breaking strength (N), Ac is the original contact area of the film specimen (m^2), ΔL is the length obtained at the end of the test (mm) and L_0 is the probe's original length (mm). The variable EM was obtained from the resultant slope of the linear region in the deformation effort. Ten repetitions for each treatment were carried out. Water vapor permeability was obtained using the procedure described by Yao, Qin, Zhang, Zhang, Qian and Liu [32], based in the norm ASTM E96-00, with some modifications. Evaluation was conducted in triplicate. For so probes of 6 × 6 cm were used, for humidity chamber NaCl and distilled water was used, keeping a relative humidity of 55 ± 5% at a room temperature (25 ± 2°C) employing acrylic permeability cells with films exposition diameter of 50 mm. The WVP value was calculated according to Ec. (6) referred by previous authors.

$$\mathrm{WVP} = \frac{\mathrm{W} \times \mathrm{x}}{\mathrm{t} \times \mathrm{A} \times \Delta \mathrm{P}} \quad \text{Ec. (6)}$$

Where W is the silica's gel weight increment (g), x is the film's thickness (m), t is the time (s), A is the film's permeation area (m^2), y ΔP is the pressure difference between the film's surfaces in Pa.

Thermal Properties by Differential Scanning Calorimetry (DSC)

Film's thermal properties were obtained using a differential scanning calorimeter model DSC4000 (Perkin Elmer™, Perkin Elmer, Inc., Bucks, UK), as the reported methodology by Tirado-Gallegos, Zamudio-Flores, Ornelas-Paz, Rios-Velasco, Olivas Orozco, Espino-Díaz, Baeza-Jiménez, Buenrostro-Figueroa, Aguilar-González and Lardizábal-Gutiérrez [26]. Obtaining the variables of T_o (onset melting temperature) y T_p (peak melting temperature) in triplicate for each treatment.

Morphological Study by Scanning Electronic Microscopy (SEM)

Materials probes of 1 × 2 cm for each treatment were used, surface micrographs were obtained by direct observation, without samples treatment, in a scanning electronic microscope Jeol™ (model JMS-70000S, Jeol Ltd., Tokio, Japón) at a magnification of 500X and 15 kV intensity.

Statistical Analysis

A completely random design was carried out, contrasting films elaborated with BGRS and the mixtures with different concentrations of OEO. All analysis were subject to One-way variance analysis (ANOVA), using LSD de Fisher test ($p \leq 0.05$) to discriminate among means. For statistical evaluation it was used the software Statgraphics™ Centurion version XVI (Statgraphics Technologies, Inc., Virginia, USA).

Results & Discussions

Humidity, Solubility and Thickness

Regarding to the film's humidity percentage (Table 1), an increase on the water retention was observed at using OEO concentrations of 0.3 and 0.5%, which directly impacted in the films thickness values (Table 1), which indicates these materials retained more humidity. These results agree with the ones obtained by Suput, Lazic, Pezo, Markov, Vastag, Popovic, Rudulovic, Ostojic, Zlatanovic, Popovic and Sciences [34] in corn starch films added with oregano essential oil (of a non-specified specie), in which a similar behavior was observed at increasing the essential oil concentration, since widespread thickness values were reported at concentrations of 0.5, 1 and 2% of essential oil, which was regarded to the integration an heterogeneous distribution of the hydrophobic film's fractions. Despite that, in the parameter films solubility, these showed a non-humidity related behavior (Table 1), since higher solubility values were observed in the control films and with OEO 1.0%, which allows infer that between concentrations of OEO at 0.1 and 0.5% a better integration of the materials take place and a consequent lower susceptibility to solve in water media, and that did not presented a hydrophilic structural arrangement or discontinuous as reported by Li, Ye, Lei and Zhao [35]. This behavior was presented in those materials with higher solubility, since essential oils can cause an increment on the solubility of the films in which are included [36]. This property is important in edible films while cooking food; on the other side, it is not convenient for food packing or long-term transport materials (mainly if a great amount of humidity is contained).

Table 1
Humidity variables, solubility and thickness evaluated on control and with different concentrations of OEO.

%OEO	%Humidity	Solubility (%)	Thickness (mm)
Control	15.50 ± 1.50^{b}	27.618 ± 1.100^{ab}	0.101 ± 0.003^{a}
0.1	17.30 ± 0.30^{b}	24.144 ± 0.672^{bc}	0.072 ± 0.003^{b}
0.3	20.90 ± 1.30^{a}	22.283 ± 2.424^{c}	0.096 ± 0.003^{a}
0.5	21.00 ± 0.80^{a}	23.904 ± 1.754^{bc}	0.108 ± 0.007^{a}
1.0	16.40 ± 0.40^{b}	31.482 ± 0.126^{a}	0.082 ± 0.004^{b}

Average ± standard error. Different letters indicate significant differences among variable values in each column, according to LSD Fisher's test ($p \leq 0.05$).

Color, Yellowness Index and Opacity

According to color variables, in general luminosity values (*L*) directly reduce according to the OEO concentration (Table 2), which produced as consequence a higher opacity on those materials in which OEO was added. Color variable *a* (tendency to green) directly increased with the OEO %; while *b* variable decreased its value up to the concentration of OEO 1.0%, presenting a straw-yellow like color the material, which tonality (°hue) was accentuated as OEO% increased, as shown on tone behavior and the decreasing of the color saturation (Chroma).

Table 2
Color variables evaluated on control films and with OEO.

% OEO	L	a	b	°hue	Chroma	ΔE*	YI_{FC}	Opacity (U.A.)
Control	95.277 ± 0.062^{ab}	-0.926 ± 0.009^{c}	3.991 ± 0.050^{ab}	4.043 ± 0.052^{ab}	102.852 ± 0.100^{a}	3.129 ± 0.071^{ab}	5.984 ± 0.079^{ab}	1.808 ± 0.120^{b}
0.1	95.501 ± 0.084^{a}	-1.042 ± 0.008^{c}	4.188 ± 0.069^{a}	4.256 ± 0.071^{a}	103.780 ± 0.160^{a}	3.006 ± 0.433^{b}	6.266 ± 0.108^{a}	3.185 ± 0.527^{a}
0.3	95.045 ± 0.085^{b}	-1.019 ± 0.007^{c}	4.162 ± 0.049^{a}	4.236 ± 0.051^{a}	103.543 ± 0.122^{a}	3.420 ± 0.417^{a}	6.257 ± 0.079^{a}	2.499 ± 0.053^{ab}
0.5	95.056 ± 0.111^{b}	-0.725 ± 0.073^{b}	4.110 ± 0.065^{a}	4.133 ± 0.072^{a}	99.706 ± 0.120^{b}	3.400 ± 0.532^{a}	6.179 ± 0.013^{a}	2.492 ± 0.213^{ab}
1.0	95.337 ± 0.074^{ab}	-0.452 ± 0.009^{a}	3.856 ± 0.067^{b}	3.829 ± 0.068^{b}	96.542 ± 0.032^{c}	3.064 ± 0.379^{ab}	5.779 ± 0.104^{b}	2.257 ± 0.174^{b}

Averages ± standard error. Different letters indicate significant differences among variable values in each column according to LSD Fisher test ($p \leq 0.05$).

Color difference (ΔE) was higher as OEO% was increased, despite that the yellowness index (YI_{FC}) was different until 1.0% of OEO was added (Table 2). This behavior has previously being reported in scientific literature, for example, Atarés and Chiralt [17] reported that the addition of essential oils in films showed an important impact in the optical and chromatic characteristics of these materials, which are important for their consumers acceptability, depending on the type of additive of botanic source, since the composition is different in each case. In relation with the opacity, oregano essential oil integration gave higher values for treatments with OEO intermediate concentrations, mainly for 0.1% OEO formulation, which indicates a gradual integration between the components, up to obtaining a favorable cohesion for this variable, indicated by the non-difference between the control and the treatment with OEO 1.0%, [37] described in a study that the coalescence, the light dispersion and the creamy effect during film's dehydration affects these materials. Also [36] indicated in a study that the adition of essential oils generally presente an inversly proportional impact with the concentration employed, mainly in the luminosity variables, opacity and yellowness index of the films.

FTIR/ATR Analysis

OEO IR spectrogram showed wave bands, which latter were used as reference to identify the mixed complex in the films in which essential oil was added. Bands between 3600 and 3100 cm^{-1} wave numbers ($n = 1/\lambda$), are observed as wide bands with medium intensity corresponding to OH^{-} groups, and between 3000 and 2800 cm^{-1} outstands three narrow bands of medium intensity characteristics of the -

CH_3 radicals, and, between 1700 y 1500 cm^{-1} three narrow bands of low intensity were found which are related to the presence of the aromatic ring, all these elements presented in the chemical structure of the OEO main compounds, thymol and carvacrol, as reported by Souza, dos Santos, da Silva Torin and dos Santos Rosa [38], despite that the OEOE composition depends on different biotic and abiotic (light hours, pluvial precipitation, type of soil, variety genotype, among others) as reported by Luo [39].

With the finality of evidence, the presence and integration of OEO in each formulation's resulting material, a mayor representation band analysis was carried out in the infrared spectrograms obtained. For the region related to the OH^- (Fig. 1a), it was observed and stretching of the band between 3700 and 3000 cm^{-1} with a slight shift of the peak for this bad for the OEO concentrations of 0.1% (3296 cm^{-1}) and 0.3% (3297 cm^{-1}) from 3303 corresponding to the rest of the treatments. This conduct would be caused by the formation of hydrogen bonds between the molecules of starch and glycerol [40]; as well as with this of OEO, united to that the OH^- functional group seems to be a strong electron donor in the formation of this type of links [41]. The presence of hydrogen bonds increased along with the higher concentration of OEO, a similar behavior to the one reported by Cai, Ma, Duan, Deng, Liu and Lu [42] in a study about corn starch films and thyme essential oil (which main component is thymol). Hydrogen bond formed may be responsible for the physical characteristics (Table 1), as well as some films color properties elaborated with OEO concentrations, since these presented lower luminosity and mayor opacity at interacting in greater degree with water, making it difficult for light to pass through them (Table 2), consequently impacting on the values of the mechanical and thermal properties and on the WVP of these films (Table 3). On the other hand, in the characteristic bands of the groups -CH_3 (Fig. 1b), it was detected a slight shift and progressive stretching of the wave band number (*n*) from 2930 to 2925 cm^{-1} and from 2883 to 2878 cm^{-1}, regarding to the increment of the presence of -CH_3 radicals, characteristics of the main components of OEO (thymol and carvacrol), and evidencing for the presence of a low intensity band at $n = 2850$ cm^{-1} starting on the concentration of 1.0% of essential oil. Currently, de Souza, dos Santos, da Silva Torin and dos Santos Rosa [38] indicated in their study that the movement of these bands is due to the molecular interaction between the OEO and the starch.

Besides it was detected the presence of a low intensity band in the wave number $n = 1735$ cm^{-1} (Fig. 1c), which is related with the presence of phenolic compounds [43], as thymol and carvacrol and that, besides weakly, it was possible to observe their presence starting with the concentration of 0.1% of OEO, increasing this band in direct proportion with the concentration of this compound in the films. The presence of this last band can be explained through the interactions generated between the starch's carbonyl groups and the OH^- groups coming from the OEO phenolic compounds forming hydrogen bonds, phenomenon that is evidenced with an increment of this band as the phenolic compound in the mixture [44] as can be seen in Fig. 1c. This information helped to confirm the interaction that the OEO established with the biopolymeric film of the prepared matrix. According to the prepared films apparent crystallinity index (Fig. 1d), it has been proven that the bands at 1044/1016 cm^{-1} would be related to both the starch's crystalline and amorphous proportions [33], so this quotient would indicate the change

in the formulated film's crystalline structure of this study, finding an erratic behavior in this regard, since in the control treatments and with 0.3 and 0.5% OEO the highest values were found (0.634, 0.660 and 0.644), according to the statistical test. Akhter *et al.* [37] indicated that the molecular interaction between the phenolic components of the essential oils and the starch chains favor an altered conformation and structural orientation. This behavior coincides with the data obtained for humidity and thickness (Table 1), so it could be deduced that the presence of the essential oil added at these concentrations would cause a structure with greater molecular integration between the films components, which would favor the retention of water in the polymeric matrix and a greater insolubility of the resulting material (Table 1), also causing a lower resistance to water vapor for the 0.3% OEO concentration, unlike the other treatments (Table 3).

Water Vapor Permeability, Mechanical and Thermal Properties

Regarding the mechanical properties, it was observed that in the fracture stress variable (TS), the highest behaviors were obtained when using OEO at 0.1 and 0.3%, since the rest of the treatments resulted without significant differences among the other treatments (Table 3). This may indicate a possible plasticizing effect of the added agent on the material's tensile strength when using these concentrations; an effect on the material's elasticity (%EAB) was also observed only up to the use of OEO at 1.0%; however, there was no significant impact due to the use of OEO in the elastic modulus (EM) variable. These results coincide with the use of other essential oils, as reported by Arezoo, Mohammadreza, Maryam and Abdorreza [29], who obtained similar behaviors in sago starch films when using low concentrations of cinnamon essential oil, and with the results reported by Restrepo, Rojas, García, Sánchez, Pinzón and Villa [45] when using banana starch and lemongrass essential oils, and rosemary. Even in synthetic polymeric materials such as polypropylene, OEO components impart a plasticizing effect, also modifying their mechanical properties by altering their crystalline structure [46].

Table 3
Mechanical properties variables, WVP and thermal of both control and with different concentrations of OEO films.

% EOO	TS (Mpa)	EAB (%)	EM (Mpa)	WVP ($g\ s^{-1}m^{-1}Pa^{-1}$)	T_o (°C)	T_p (°C)
Control	3.232 ± 0.128^{ab}	20.689 ± 1.538^{b}	853.337 ± 90.661^{a}	$1.276 \times 10^{-10} \pm 4.913 \times 10^{-12a}$	73.38 ± 1.20^{a}	110.05 ± 1.04^{a}
0.1	3.758 ± 0.247^{a}	21.349 ± 1.030^{b}	1015.279 ± 90.879^{a}	$9.327 \times 10^{-11} \pm 7.778 \times 10^{-12bc}$	45.20 ± 1.14^{b}	101.99 ± 1.11^{b}
0.3	2.902 ± 0.199^{b}	19.052 ± 1.132^{b}	856.644 ± 70.521^{a}	$1.022 \times 10^{-10} \pm 6.351 \times 10^{-12ab}$	45.33 ± 1.22^{b}	95.49 ± 0.92^{c}
0.5	3.233 ± 0.335^{ab}	22.334 ± 1.879^{ab}	821.715 ± 121.286^{a}	$7.717 \times 10^{-11} \pm 9.548 \times 10^{-12bc}$	40.49 ± 1.05^{c}	85.32 ± 0.37^{d}
1.0	3.449 ± 0.054^{ab}	25.462 ± 0.928^{a}	914.423 ± 41.958^{a}	$6.716 \times 10^{-11} \pm 1.165 \times 10^{-11c}$	69.35 ± 2.34^{a}	97.84 ± 1.94^{c}

Averages ± standard error. Different letters indicate significant differences among variable values in each column according to LSD Fisher test ($p \leq 0.05$).

Regarding to the water vapor permeability (WVP), a clear effect of greater resistance to the water molecules passage was observed depending on the increase in the OEO's percentage (Table 3), which agrees with that reported by Li, Ye, Lei and Zhao [35] and Aguilar-Sánchez, Munguía-Pérez, Reyes-Jurado, Navarro-Cruz, Cid-Pérez, Hernández-Carranza, Beristain-Bauza, Ochoa-Velasco and Avila-Sosa [19], remembering that polyphenols can induce alterations in the properties of the films of biomaterials such as starch, so this ingredient could have conferred a certain hydrophobicity to the films in which it was included, favored by the integration at the molecular level of the film's formulation components, which became evident when increasing the OEO amount in them, according to the behavior of the levels of significance for this test in the materials evaluated, which corresponds to apparent crystallinity index values (Fig. 1d).

In relation to the thermal properties, the results showed an important difference between T_o and T_p in all the treatments, observing a clear effect in the decrease of the values for these variables depending on the increase in the concentration of the OEO until the use of the concentrations of 0.5%, which seems to agree with the use of other essential oils, as reported by Cai, Ma, Duan, Deng, Liu and Lu [42]; however, in this study a significant increase was noted in both variables determined for OEO at 1.0%. According to Abdorreza, Cheng and Karim [47], a significant difference between T_o and T_p favors a better adhesion between polymer sheets, so that for the materials obtained, a better performance in this aspect would be obtained for the films with OEO at 0.1% and 0.3%.

Surface Morphological Analysis by SEM

According to this analysis it was observed that the inclusion of OEO in the BGRS film caused an alteration in the surface structure. The film corresponding to the control (OEO addition free) (Fig. 2a) showed a uniform continuity in its structure; however, the hydrophilicity of its components allowed a greater water vapor molecules diffusion and lower elasticity and resistance to fracture than the other films added with OEO. The inclusion of OEO at 0.1% in the film (Fig. 2b) presented a change in the structural material's continuity, which indicated an apparent loss in homogeneity similar like that reported by do Evangelho, da Silva Dannenberg, Biduski, el Halal, Kringel, Gularte, Fiorentini and da Rosa Zavareze [48] in corn starch's films with orange essential oil, so these researchers observed a change in the surface appearance attributed to the essential oil's hydrophobicity, which caused a discontinuity in the structure of the resulting materials; however, this film presented the highest resistance, which could have been caused by a more homogeneous integration between the components that make it up. For the film containing OEO at 0.3% (Fig. 2c), its structure indicated to be different from its predecessors, since it lost its "smooth" appearance and, instead, a surface with a "lumpy" appearance was observed, which could be the product of the agglomeration and attempt of structural accommodation between a greater amount of hydrophobic elements and other part of hydrophilic ones, which had a negative impact on the fracture stress value obtained in this film (Table 3).

The "lumpy" appearance decreased in the film with 0.5% OEO, increasing its value of mechanical resistance and elasticity, while that of permeability to water vapor decreased (Table 3), all of this attributed to the OEO plastifying effect, according to Li, Ye, Lei and Zhao [35] reports. Finally, when using OEO at 1.0%, a material with a structure of greater heterogeneity in the integration of its components was observed, although without a "lumpy" appearance (Fig. 2d), with greater elasticity (Table 3), this is also attributed to the OEO plasticizing property which at the same time, gave it the lowest permeability to water vapor of all the treatments evaluated, but losing fracture resistance capacity compared to the control treatment (OEO free) and to the one with the lowest OEO concentration (0.1%). Akhter *et al*. [37] studied the interaction between mint and rosemary essential oil in corn and wheat starch films, finding that the interaction between essential oil and starch chains obeys a complex mechanism of balance between the increase in hydrophobic components (in this case OEO) finally adopting this property in the functionalized films due to the effect of said components on the integrity of the films' microstructure and their possible interaction with the starches' side chains, with the consequent impact on all the other physicochemical properties of this type of biopolymeric materials.

Conclusions

The OEO addition affected the color physical property; however, it is estimated that this does not represent any problem in terms of its acceptance by potential users, due to the low impact of the additive on luminosity and yellowing index. The FTIR analysis indicated that the films with 0.1 and 0.5% OEO presented a greater molecular integration between their components, which had a positive impact on their physical, mechanical, thermal, water vapor permeability properties, as well as their morphological characteristics. Therefore, the films with 0.1 and 0.5% OEO could have greater application possibilities compared to the control treatments and with 0.3 and 1.0% OEO, due to a better balance of molecular

interaction between the hydrophilic and hydrophobic components at the referred concentrations. In general, the results indicated that the starch obtained from Buffalo's ground root represents a non-conventional alternative source for its application in films added with OEO, which led to changes in the physicochemical properties of the films obtained.

Abbreviations

BGRS
Buffalo gourd root starch
OEO
Oregano essential oil
ΔE*
Color difference
YI_{FC}
Yellowness index

Declarations

Author´s Contribution

FHC: Conceptualization; data curation; formal analysis; investigation; methodology; writing –original draft; writing –review and editing. MHG: Formal analysis; investigation; writing -review and editing. JMTG: Investigation; methodology. CRG: Investigation; methodology. CRV: Investigation; methodology; resources. AMRG: Investigation; formal analysis. HYLDP: Investigation; methodology. JMMX: Formal analysis. PBZF: Conceptualization; formal analysis; investigation; methodology; writing –original draft; project administration; resources; writing –review and editing. All authors read and approved the final manuscript.

Acknowledgements

Authors would like to thank Ing. Arturo Ramos Martínez for the technical support provided in this work. The present study is a product of the Research Group in Carbohydrates, Packaging and Functional Foods (CEAF-Laboratory) of the CIAD-Cuauhtemoc, Chihuahua, Mexico, led by Dr. Paul Baruk Zamudio Flores.

Conflict of Interest: The authors declare that they have no conflicts of interest with respect to the work described in this manuscript.

Ethical Approval: Not applicable, because this manuscript does not contain any studies with human or animal subjects.

Informed Consent: Not applicable.

Funding: No funding was obtained for this study.

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Figures

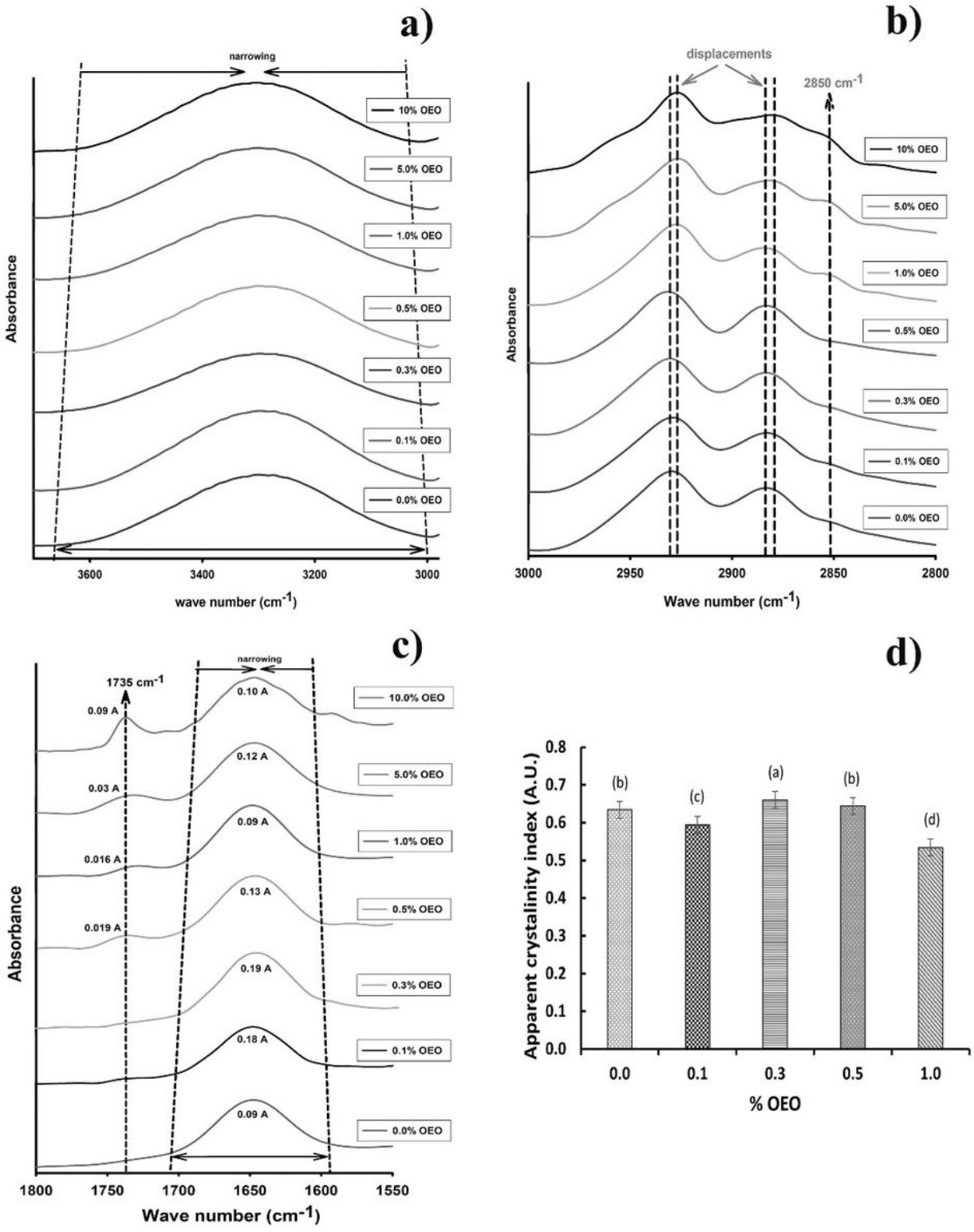

Figure 1

Regions of interest in the IR spectrum of the control film and with different concentrations of OEO: a) Region of OH^- groups, b) Region of groups -CH_3, c) Region of linked water and presence of aromatic rings, d) Apparent crystallinity index (A.U.= Arbitrary Units).

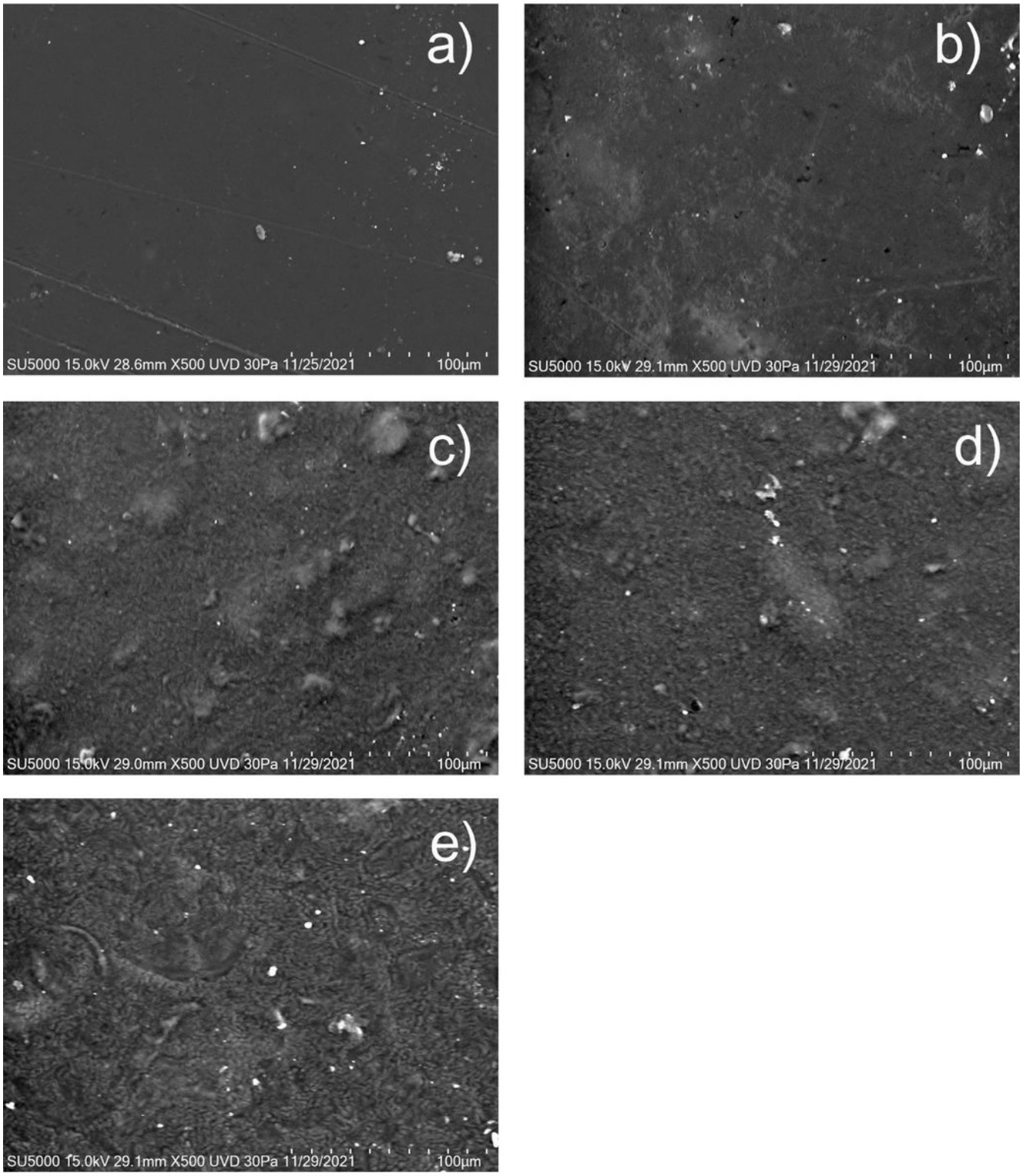

Figure 2

Micrographs obtained by SEM of the surface of the control film and with different concentrations of OEO: a) Control (0%), b) 0.1%, c) 0.3%, d) 0.5%, e) 1.0%.