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Optimization of antioxidant, mechanical and chemical physical properties of chitosan-sorbitol-gallic acid films by response surface methodology

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ABSTRACT

The interest in the development of biodegradable films is increasing, which is related to the wide availability of resources and methods of synthesis to generate them. For this reason, in this study, the preparation of films containing chitosan, sorbitol and gallic acid this study was proposed. The incorporation of natural antioxidants into films modifies their structure and provides new functionality to them.

In order to determine the optimal content of sorbitol and gallic acid in the formulation of the chitosan films to obtain the highest antioxidant capacity and the best mechanical properties, an experimental design based on a two-factor Doehlert model was used. The optimum condition of film synthesis was obtained when a mixture of 1 wt chitosan, 3.62 wt% of sorbitol and 1 wt% of gallic acid was performed. The properties studied were experimentally evaluated at this optimal point and compared with the model predictions, showing good results. It proved to be a promising material to be successfully used as packaging material.

1. Introduction

Actually, it is possible to find numerous works focused on the development of new technologies of packaging, which have emerged through different investigations and innovation, as a response to the industries demand. An active packaging aims to improve food safety from preservation food quality and the extension of its shelf life [1]. At present, two types of active packaging are used. One of them acts as a barrier against undesirable substances such as O₂, H₂O, CO₂; the other ones have active components, which are able to give several properties such as antioxidant or antimicrobial activity [2,3]. The use of biopolymers as packaging precursors is one of the alternatives to the use of conventional polymers, because they are biocompatible and biodegradable. Chitosan is a linear polysaccharide, obtained by deacetylation of chitin present in the shell of insects and crustaceans, and is composed of randomly distributed β-1,4-D-glucosamine and N-acetyl-D-glucosamine chains [4]. Although chitosan is an insoluble compound in water, it can be solubilized without altering its molecular weight under the presence of organic acids such as acetic acid and lactic acid [5]. This polysaccharide has been widely used as packaging precursors due to its good film-forming activity [6]. Several studies has shown that chitosan-based films have a high potential to extend the shelf life of fruits and

vegetables, inhibiting the growth of microorganisms [7,8]. The preparation of chitosan films without the use of plasticizer results in obtaining brittle films with poor mechanical properties [9]. It is known that sorbitol is miscible with chitosan and can act as a good cross-linking agent. Therefore, those films prepared together with sorbitol have good physical properties such as transparency and mechanical properties [10]. Sorbitol is an organic compound, a polyol with six hydroxyl groups, which can be obtained from hydrogenation of glucose. Most sorbitol is attained from corn syrup, but it is also found in nature in apples, plums, pears, cherries, dates, peaches and apricots [11].

On the other hand, the addition of phenolic compounds or natural extracts with antimicrobial and antioxidant activity in a chitosan matrix enhances its physical properties from non-covalent interactions such as hydrogen bonds. Zhang et al. [12] have demonstrated that vanillin acts as a cross-linking agent in the formation of chitosan matrix. In addition, the presence of phenolic compound such as green tea extract improves mechanical and vapor barrier properties as well as antioxidant properties of the resulting chitosan based films [13]. In this sense, gallic acid is a secondary metabolite present in various plant species such as blueberries, apples, flax and tea, and presents antioxidant and antimicrobial activity [14]. In this work, the preparation of films with antioxidant activity based on chitosan, gallic acid and sorbitol is

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proposed. Several authors have previously studied the system chitosan-gallic acid. For example, Sun et al. [15] have suggested that the incorporation of gallic acid into chitosan films significantly improves the antimicrobial properties of the film. In addition, gallic acid also improves certain mechanical properties such as tensile strength or water vapor permeability. In a similar way, the grafting of gallic acid onto chitosan backbones implies the combination of the properties of both reagents, resulting a film with antioxidant activity, which becomes a suitable material for the food industry [16,17]. Liu et al. [18] have studied the preparation of chitosan films grafted with different hydroxybenzoic acids and have demonstrated that gallic acid-grafted-chitosan film had the best physical, mechanical and antioxidant properties, using glycerol as plasticizer. However, according to our knowledge, there is no information regarding chitosan-sorbitol-gallic acid films. In this sense, the aim of the present work is to evaluate the formation of chitosan-based films containing gallic acid and sorbitol, where the latter can act as a good non-covalent cross-linking agent. In order to obtain films with a suitable antioxidant activity and mechanical properties, the composition of the mixture was optimized by using response surface methodology (RSM) to model the experimental responses.

2. Materials & methods

2.1. Reagents

Low molecular weight chitosan (molecular weight > 50000 Da, viscosity: 0.04 Pa s, Parafarm), glacial acetic acid (99.5%, Cicarelli), gallic acid anhydrous (98%, Biopack), sorbitol solution (70% w/w, Ingredion), Folin-Ciocalteu's phenol reagent (Biopack), sodium carbonate (99.5%, Cicarelli), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich Co.), neocuproine (98%, Sigma-Aldrich Co.), copper (II) chloride (99%, Cicarelli), ammonium acetate (97%, Cicarelli) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97%, Sigma-Aldrich Co.) were the reagents used in the experimental work. No further purification of the reagents was carried out.

The deacetylation degree percent (DD%) of chitosan was quantified to verify the content of amine groups per unit mass of the samples by using a potentiometric titration. In this quantification, 0.5 g of chitosan was dissolved in 50 mL of 0.30 M HCl under stirring at 25 °C. This solution was titrated with a solution of 0.10 M NaOH using an Altronix TPX-I pH meter. The DD% was calculated using the following equation [19]:

$$DD\% = \frac{2.03 \times (V_2 - V_1)}{m + 0.0042 \times (V_2 - V_1)} \quad (1)$$

where m is the weight of sample, and V_1 and V_2 corresponds to the volumes of NaOH employed at the first and second inflexion points respectively in a pH vs volume function in the titration process. Applying Eq. (1), DD% was 86.64% obtained by duplicate.

2.2. Film preparation

The mixture (100 g) was prepared dissolving 1.0 g of chitosan powder into of acetic acid buffer solution pH 4 under stirring at room temperature until chitosan is solubilized. Following, gallic acid as active compound and sorbitol solution as plasticizer were added under stirring at different concentrations. An aliquot of 10 mL of mixture was spread on Petri dishes and the chitosan film was obtained by casting after drying at 20 °C and 50% of relative humidity using a refrigerated incubator VELP-FTC 90 for 48 h. The films were done by triplicate at each formulation.

From now on, whenever different formulations are discussed, reference will be made to their original composition in the mixture, which differs from the final composition of the film, due to the evaporation of the solvent.

A preliminary study was performed in 25 films synthesized from

1 wt% of chitosan aqueous solution with the combination of (0, 2.5, 5.0, 7.5, 10.0) wt% of sorbitol and (0.125, 0.25, 0.50, 0.75, 1.00) wt% of gallic acid respectively. From these results, an experimental design was done as explained below in Section 2.4.

2.3. Characterization of chitosan films

Physicochemical, antioxidant and mechanical properties of mixtures and final films were analyzed as detailed below.

2.3.1. Rheological analysis

The rheological properties of aqueous solutions of the mixtures were characterized by a rheometer (Rheoplus Physica MCR 301, Anton Paar, Germany) equipped with a cone-plane geometry CP50-1 (diameter = 50 mm, gap = 50 μm) at 20 °C at a shear rate from 0.1 to 500 Hz.

2.3.2. UV-visible

The absorbance of the different mixtures was measured using an UV-vis spectrometer (Shimadzu UV1800) at room temperature. Distilled water for the mixtures was used for the base line correction.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

In order to observe the potential modifications of interaction of functional groups of chitosan after incorporation of gallic acid and sorbitol, different films were analyzed by Fourier transform infrared spectroscopy. FTIR spectra were performed using a Nicolet 5-SXC spectrophotometer (USA), recorded at 4 cm⁻¹ resolution in a (4000–400) cm⁻¹ range, using air as background.

2.3.4. Antioxidant assays

Total phenolic content (TPC) of the films was determined with the Folin-Ciocalteu reagent at basic pH resulting in a blue coloration determined spectrophotometrically according Ivanova et al. [20] with slight modifications. Here, an extract was prepared to perform this assay from a relation of 25 mg of film and 3.0 mL of distilled water, which was left at 25 °C for 2 h. Lately, an aliquot of 1.0 mL of this preparation was mixed with 5 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent in a test tube and left standing for 3 min. After that, 1.5 mL of sodium carbonate solution was added and it was brought to a final volume of 10.0 mL with distilled water. The mixture was carried to an ultrasonic bath at 50 °C for 20 min. The absorbance of this mixture was measured at 765 nm using the spectrophotometer. The total phenolic compounds present in the films were expressed as mg of gallic acid per mg of dry weight of the film.

DPPH assay was determined according to Siripatrawan & Harte [21]. In this sense, 3 mL of each extract obtained following the same procedure as TPC, were mixed with 1 mL of methanolic solution of DPPH (20 W/V %). The mixture was mixed using a vortex and was incubated in the dark at room temperature for 30 min. With the presence of antioxidant activity, a change of color was observed from violet to pale yellow and the absorbance of the mixture was measured at 517 nm. The DPPH antioxidant capacity of the films has been quantified as Trolox equivalent antioxidant capacity (TEAC) called as TEAC_{DPPH}, implying that the antioxidant capacity of the sample was measured and compared to Trolox standard.

The CUPRAC method was determined according to Apak et al. [22] with slight modifications. This method is based on the measurement of the absorbance at 450 nm of the chromophore CUPRAC [Cu(I)-Nc chelate] formed as a result of the redox reaction of antioxidants with the reagent CUPRAC [Cu(II)-Nc]. To a test tube, 1.0 mL of copper (II) chloride solution (0.01 M), 1.0 mL of neocuproine (0.0075 M) and 1.0 mL of ammonium acetate buffer pH 7 were added. To obtain a final volume of 4.10 mL, dilutions of the extract of the films were added. The tubes were closed and incubated at room temperature for 1 h. An evidence of change of color was obtained from pale blue to orange. As the

DPPH method, the CUPRAC method was quantified as TEAC and represented as TEAC_{CUPRAC}.

TEAC_{DPPH} and TEAC_{CUPRAC} values for films were expressed as mmol of Trolox per milligram of dry weight of the film.

2.3.5. Mechanical assays

The tensile strength at the moment at which a test specimen tears (TS) was performed by using texturometer, where the elongation at break (EB) was determined from the ratio between the elongation at rupture and initial length of the test specimen. In addition, Young's modulus (E) of films indicates the relationship between stress and strain in the deformation of a solid body and is considered to be another valuable parameter of the mechanical properties of films. These tests were carried out at least three times for a rectangular sample (25 × 100 mm) according to standard method ASTM D882-12 [23] using an Instron Texturometer (model 3342, Norwood, MA, USA), which was equipped with a 500 N cell and a 0.5 mm/s speed at 25 °C. TS, EB and E value of the film were calculated using the followings equations respectively:

$$TS = F_{max}/S \quad (2)$$

$$EB = (\Delta L/L) \quad (3)$$

$$E = (F/S)/(\Delta L/L) \quad (4)$$

where F_{max} is the maximum load at the break point; S is the original cross-sectional area; $\Delta L/L$ is the ratio of the film extension where L is the initial length of film; and F is the load of a point in the initial linear portion of the stress-strain curve.

2.3.6. Opacity

The film opacity (O) was calculated according to Park & Zhao [24] by triplicate with the following equation:

$$O = A_{600}/x \quad (5)$$

where A_{600} is the film absorbance at 600 nm and x is the average film thickness.

2.3.7. Water vapor transmission

Water vapor transmission rate (WVT) and water vapor transmission permeability (P) of the films were determined according to Water Method Procedure explained in the standard ASTM E96/E96M – 16 [25]. The values were calculated as follows:

$$WVT = G/(t \times A) \quad (6)$$

$$P = (WVT/\Delta p) \times x \quad (7)$$

where G is the slope corresponding to the weight vs time curve in gram; t is the time in hours; A is the test area cup mouth area in m²; Δp is the vapor pressure difference; and x is the average thickness of the film.

2.4. Experimental design and response surface methodology

The experimental design for the development of the present work was carried out using the Response Surface Methodology from a two-factor model according to Doehlert model [26]. This mathematical model was selected to identify the interaction between the response variables studied and the independent variables. The two factors, i.e. the independent variables used in the formulation of films were sorbitol and gallic acid concentration, where the range of these variables was based on previous studies of antioxidant properties and solubilities. Sorbitol concentration was defined as a factor with five levels (X_1 : 1, 3, 5, 7, 9 wt%) according to previous works reported negative effect on final properties in films without plasticizers [10,12]. On the other hand, gallic acid composition is a factor whose three levels (X_2 : 0.50, 0.75, 1 wt%) represent the best antioxidant activity according to preliminary assays. The response variables selected were: TPC (Y_1), TEAC_{DPPH} (Y_2),

TEAC_{CUPRAC} (Y_3), TS (Y_4), EB (Y_5), E (Y_6), opacity (Y_7), WVT (Y_8) and P (Y_9). The response variables were evaluated after performing 9 assays, where six of them were the films obtained from different formulations of mixture. Remaining tests corresponds to central point according to the Doehlert model for two factors.

Quadratic polynomials were fitted to express each response variable defined in this study as a function of the independent variables. In these equations, Y_n is the response variable where b_0 is a constant term; b_1 and b_2 are the coefficients of the linear parameters such as sorbitol (X_1) and gallic acid (X_2) compositions respectively. In addition, b_{11} and b_{22} correspond to the coefficients of the quadratic parameter, while b_{12} is the coefficient of the interaction parameters. In this way, the response variables can be calculated from equation (8):

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (8)$$

The regression models of each response variable studied and the statistical analysis were performed using Statgraphics Centurion XVI software (v16.1, USA). The response surface was estimated from experimental data with a confidence interval of 95%. In order to obtain films with a good resistant mechanical and the best antioxidant properties, the response surface was optimized. After that, films based on chitosan were prepared using those optimum conditions predicted by response surfaces, and the experimental data were contrasted against theoretical values, which allowed to validate the performance of the model. Furthermore, the response variables (TPC, TEAC_{DPPH}, TEAC_{CUPRAC}, TS, EB, E, opacity, WVT and P) were subjected to analysis of variance (ANOVA) and the results were compared by Fisher's test at a significance level of 0.05. These analyses were performed using the INFOSTAT statistical software (Student version, 2017).

3. Results and discussion

3.1. Characterization of mixtures and final films

Preliminary studies in the rheological analysis, UV-visible and Fourier transform infrared spectroscopy have been performed for films synthesized from 25 combinations obtained with a 1 wt% of chitosan aqueous solution, and a varied composition of sorbitol (0, 2.5, 5.0, 7.5, 10.0 wt%) as well as gallic acid (0.125, 0.25, 0.50, 0.75, 1.00 wt%). The films obtained from these mixtures showed good appearance, taking into account their homogeneity, transparency and brightness (Fig. S1). The mixtures used to synthesize the final films were subjected to rheological assays where a rotational method was performed at 20 °C. Fig. 1 shows the curve profile of shear viscosity as a function of the

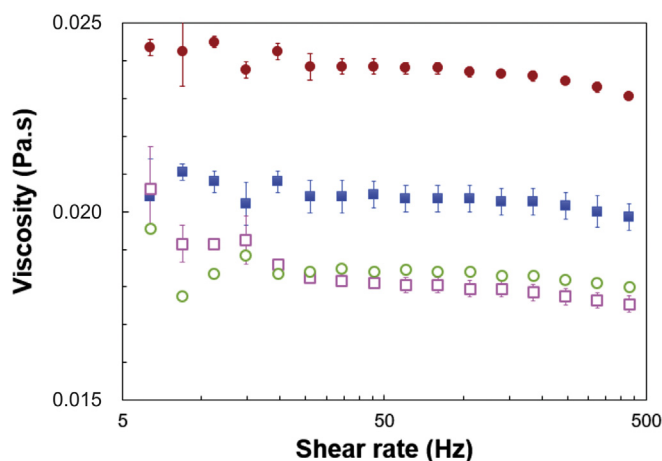


Fig. 1. Dynamic (shear) viscosity of 1 wt% chitosan (■), 1 wt% chitosan and 5 wt% of sorbitol (●), 1 wt% chitosan and 1 wt% of gallic acid (□), and the mixture with a composition of 1 wt% chitosan, 5 wt% of sorbitol and 1 wt% of gallic acid (○) were determined at 20 °C.

shear rate with 1 wt% chitosan, the latter and 5 wt% of sorbitol or 1 wt% of gallic acid, and for a mixture containing all components. It is found that in the range of shear rate between 20 and 350 Hz the profile of curves looks like an ideal viscous liquid where the presence of sorbitol or gallic acid slightly changes the chitosan viscosity in those conditions. This behavior supports the fact that these molecules interact with chitosan backbones by hydrogen bond or electrostatic interaction. In addition, the mixture chitosan/sorbitol exhibits a viscosity (near 24 mPa s) higher than that found for chitosan aqueous solution (close to 20 mPa s). Taking into account that sorbitol has six hydroxyl groups; it can form hydrogen bonds with chitosan chains and act as a non-covalent agent from cross-linking of polysaccharide backbones. This phenomenon yields an agglomeration of chitosan coils, which leads to an increase of the system viscosity. On the other hand, when gallic acid is incorporated into chitosan aqueous solution a decreasing of the system viscosity takes place (~ 18 mPa s). Probably the anion acetate against ammonium group of chitosan is substituted by the anion of gallic acid from electrostatic interaction, yielding a smaller hydrodynamic volume of chitosan coils. Moreover, the chitosan mixture containing gallic acid and sorbitol has a viscosity value (~ 19 mPa s) lower than chitosan aqueous solution but higher than that found for chitosan/gallic acid mixture. This tendency supports the fact that under these conditions gallic acid interacts with chitosan backbone by electrostatic interaction decreasing the size of chitosan coil while sorbitol is a cross-linker of chitosan chains from hydrogen bonds.

In addition, mixtures were characterized by UV–visible spectrometry to find same differences in the profile of their spectral curves. In this sense, Fig. 2 shows the UV–visible spectral curves of aqueous solutions of pure chitosan and chitosan mixtures, one of them containing also a 1 wt% of gallic acid, another with a 5.0 wt% of sorbitol, and that with a 1 wt% of gallic acid and a 5 wt% of sorbitol. It is found that the spectral curve for pure chitosan and that for chitosan-sorbitol mixture show a similar profile, where a band around 270 nm and a shoulder near 300 nm can be observed. This behavior is probably related to carbonyl group of chitosan. On the other hand, the mixture of chitosan-gallic acid and chitosan-sorbitol-gallic acid show spectral curves with similar profiles, where an oversaturation of absorption is found in the range of 200–350 nm. This performance is attributed to the transition of pi electrons of double bond of the aromatic carbonyl compound with OH substituents. This phenomenon supports the presence of gallic acid into mixture, which is a substance with a high molar absorptivity coefficient in that wavelength range.

Moreover, FTIR spectrometry was used to confirm the potential modification of chitosan backbones interaction after the incorporation of gallic acid and sorbitol. Fig. 3 shows the FTIR spectra of the films obtained from pure chitosan and its mixtures with 1 wt% gallic acid;

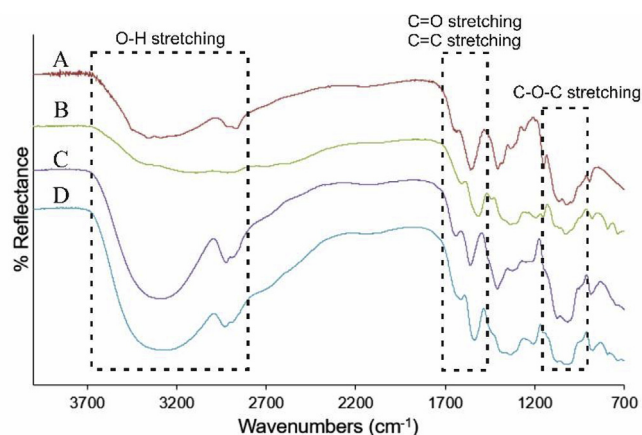


Fig. 3. FTIR-ATR spectra of films with 1 wt% chitosan (A) and its mixture with 1 wt% gallic acid (B); 5 wt% sorbitol (C), and 5 wt% sorbitol-1wt% gallic acid (D).

5 wt% sorbitol or 5 wt% sorbitol-1wt% gallic acid. Gallic acid spectrum has been shown in Fig. S2. The broad band between 3700 and 2600 cm^{-1} corresponds to O-H stretching of associated carboxyl groups present in gallic acid. In addition, sorbitol and chitosan have a strong signal (3700-3080 cm^{-1}) of hydroxyl group, which overlaps the N-H stretching of the polysaccharide. This band of OH stretching is more accentuated in the spectra chitosan-sorbitol and chitosan-gallic acid-sorbitol due to the presence of sorbitol in those mixtures. The signal at 2920 cm^{-1} is associated to $\text{C}_{\text{sp}^3}\text{-H}$ stretching, which can be found for sorbitol and chitosan. The band at 1640 cm^{-1} corresponds to C=O stretching vibration of amide group, which could be seen for chitosan and its mixture with sorbitol (Fig. 3). In addition, this signal shifts toward 1610 cm^{-1} when gallic acid is incorporated into final composition of the product. The latter is assigned to the C=C stretching corresponding to the aromatic ring of gallic acid, which almost completely overlaps the amide's band at 1640 cm^{-1} . Chitosan has a characteristic band at 1560 cm^{-1} , which corresponds to the N-H bending; the C-N stretching vibration at 1330 cm^{-1} , the C-O-C stretching as a strong peak at 1020 cm^{-1} appear at all the mixtures due to the presence of chitosan. Finally sorbitol shows a characteristic broad band in the range 1400–1250 cm^{-1} , which is associated to C-O-H bending vibration.

Fig. 3 also shows from D curve profile, that the bands of O-H and the $\text{C}_{\text{sp}^3}\text{-H}$ stretching vibration can be clearly observed. This phenomenon suggests that sorbitol interacts by hydrogen bond with the carboxyl groups of gallic acid since the broad band of O-H stretching associated to carboxyl groups is not seen. In addition, taking into account the sorbitol size is smaller than other components of the mixture, the sorbitol molecule can be homogeneously distributed in liquid bulk and gets hydrogen bonding interaction with chitosan backbones as well as gallic acid. Therefore, it is expected that sorbitol performs a relevant role as a cross-linking agent and as a plasticizer from these non-covalent interactions during formation of chitosan films by casting.

Antioxidant activity assay of films from TPC, $\text{TEAC}_{\text{DPPH}}$ and $\text{TEAC}_{\text{CUPRAC}}$ were analyzed. Fig. 4 shows the influence of sorbitol and gallic acid concentration on the films performance.

In general, it is seemed that these parameters increase with the content of gallic acid in the film. However, it is observed that the antioxidant activity of the films decreases when a higher sorbitol concentration is used during their formation. This behavior is related to the fact that sorbitol increases the final mass of the films and dilutes the gallic acid concentration. In addition, the decrease in reagent diffusion caused by the non-covalent cross-linking of chitosan chains should not be ruled out, which increases when a higher sorbitol content is used in the mixture. It should be noted that no antioxidant activity is found in films without gallic acid. Fig. 4 shows that the best antioxidant activity

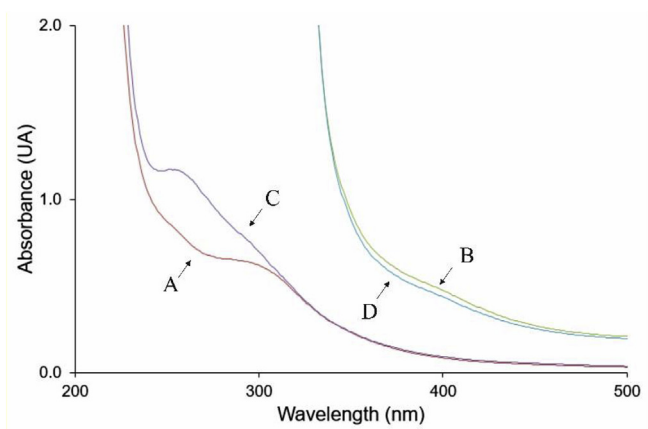


Fig. 2. UV–visible spectral curves for an aqueous solution of 1 wt% chitosan (A) and its mixture with 1 wt% gallic acid (B); the chitosan mixtures with a 5 wt% of sorbitol (C), and a 1 wt% gallic acid 5 wt% of sorbitol (D).

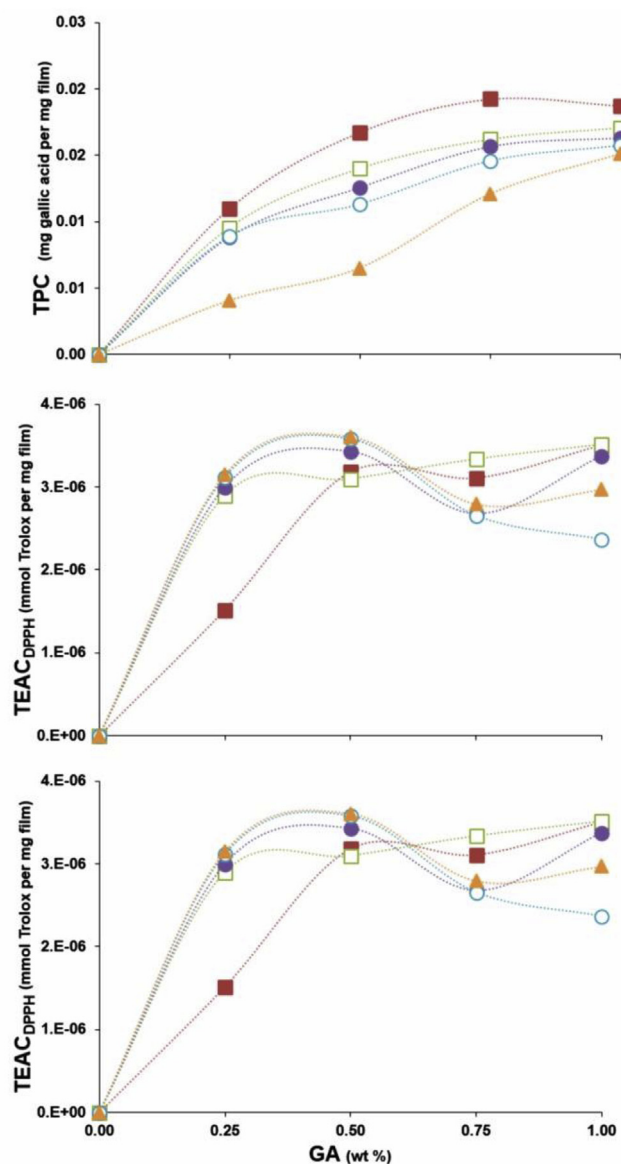


Fig. 4. Influence of gallic acid (GA) concentration and sorbitol on TPC (A), TEAC_{DPPH} (B) and TEAC_{CUPRAC} (C) values in films with 1 wt% chitosan and 0 (■), 2.5 (□), 5 (●), 7.5 (○) and 10 (▲) wt% of sorbitol (SB).

corresponds to those films with gallic acid content higher than a 0.5 wt %.

3.2. Fitting the response surfaces

The design arrangement for the compositions of the mixtures, the experimental results of dependent variables in the different films and the analysis of variance are reported in Table 1. Values followed by the same letter within a response show no significant difference ($p > 0.05$). Equation (8) was used to fit response surface from experimental data for each sample investigated as shown in Table 2. The responses variables were correctly fitted when a determination coefficient (R^2) between 0.80 and 0.99 was reached. In this way, all the investigated responses variables were included in the optimization process. The nature of the relationship between independent variables and investigated responses was represented by a three-dimensional response surface, which was generated from the regression equation as can be seen in Fig. 5.

3.3. Influence of independent variables on the investigated responses

The effect of sorbitol and gallic acid concentration was significant ($p < 0.05$) in the antioxidant activity responses of TPC, TEAC_{DPPH} and TEAC_{CUPRAC} (Y_1 , Y_2 , Y_3) in the first linear order effect (X_1 and X_2) and the combined effect (X_1X_2). It is found that experimental TPC value oscillates from 0.0217 to 0.0437 mg gallic acid/mg film, TEAC_{DPPH} varies between 4.26×10^{-6} and 5.8×10^{-6} mmol Trolox/mg film, while TEAC_{CUPRAC} changes from 0.00081 to 0.0057 mmol Trolox/mg film (Table 1).

Fig. 5(a, b and c) shows that the responses surfaces of TPC and TEAC_{CUPRAC} have a similar behavior where both parameters increase together with the content of gallic acid or a lower sorbitol content in the mixture is used. However, the response surface for TEAC_{DPPH} presents a different performance, showing a minimum value at 5 wt% and 0.75 wt % of sorbitol and gallic acid concentration, respectively. In this way, this surface presents its maximum TEAC_{DPPH} value at the highest concentration of sorbitol and a 0.5 wt of GA content.

Regarding the mechanical properties from TS, EB and E (Y_4 , Y_5 , Y_6), in general it is found that only the first linear order effect of sorbitol (X_1) was significant ($p < 0.05$). This behavior agrees with the fact that sorbitol works as a non-covalent cross-linking agent, which directly affects the mechanical properties of the films from formation of hydrogen bonds. Depending on gallic acid and sorbitol concentration in the films, the mechanical properties show a large variation (Table 1). Here, tensile strength fluctuates from 0.90 to 8.46 MPa, the EB value varies from 0.03 to 0.54, and Young's Modulus from 1.73 to 614.29 MPa.

The response surface of TS exhibits that its value increases when the sorbitol concentration decreases. On the other hand, the response surface of E module shows a marked change for a sorbitol concentration upper than a 5 wt%. Under this condition, an increase of the content of sorbitol and gallic acid leads to an increase in the percentage of elongation of the film. In addition, it is observed that the response surface of Young's modulus decreases against the sorbitol concentration until reaching a value near 7 wt%. After that, the Young's Modulus increases together with the sorbitol concentration. This phenomenon can be explained from the predominance of hydrogen bond interactions between chitosan, sorbitol and gallic acid. When the sorbitol content is low, the electrostatic interaction and hydrogen bond between chitosan and gallic acid predominate. The latter acts as a cross-linker through non-covalent bonds, which gives a higher E value due to the fact that gallic acid is a rigid molecule. After a 7 wt% of sorbitol content its hydrogen bond interactions with other reagents increase, and acts as a successful cross-linking agent. In this way, the E module grows again, but with a lower value due to the fact sorbitol is a molecule more flexible than gallic acid (Fig. 5f). Fig. S3, shows the stress-strain curves of a 1 wt% chitosan and 0.75 wt% gallic acid film with and 1 wt%, 5 wt% and 9 wt % sorbitol respectively. A higher strain can be observed at the higher sorbitol composition.

In addition, it is found that the sorbitol content (X_1) has a significant effect on the optical properties ($p < 0.05$) of obtained films. Opacity value varies in a range 0.56–3.24 UA/mm for these films, with a water vapor transmission rate of 37.22–62.56 g/m²h, and a permeability value of 0.24–0.62 (g mm/mmHg.m². h) is exhibited by the films (Table 1). Fig. 5g shows that a higher sorbitol concentration in the mixture leads to a decrease the film opacity since sorbitol disorders chitosan chains obtaining a greater degree of amorphous regions. The highest value of water vapor transmission rate is reached at the lowest sorbitol content together with a 0.75 wt% of gallic acid concentration (Fig. 5h). However, a high permeability value could be observed for films containing the highest concentration of sorbitol where no dependence on the content of gallic acid is evidenced (Fig. 5i). This is important to highlight, because if the intention of the film is to be used as packaging, both WVT and P must be taken into account.

Table 1

Experimentally average values of responses obtained in the Doehlert model experimental design with the analysis of variance*.

Run	Variables		Responses								
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9
SB	GA	TPC	TEAC _{DPPH}	TEAC _{CUPRAC}	TS	EB	E	O	WVT	P	
wt%	wt%	$\frac{mg\ GA}{mg\ film}$	$\frac{mmol\ trolox}{mg\ film}$	$\frac{mmol\ trolox}{mg\ film}$	MPa		MPa	$\frac{UA}{mm}$	$\frac{g}{m^2 \cdot h}$	$\frac{g \cdot mm}{mm\ Hg \cdot m^2 \cdot h}$	
1	5	0.75	$3.3 \times 10^{-2} \pm 3 \times 10^{-3}b$	$4.3 \times 10^{-6} \pm 2. \times 10^{-7}a$	$2.4 \times 10^{-3} \pm 1 \times 10^{-4}a$	$1.9 \pm 0.3a$	$0.26 \pm 0.1 b$	$15.8 \pm 0.9a$	$0.56 \pm 0.1a$	$59 \pm 4 b$	$0.34 \pm 0.03 b$
2	5	0.75	$3.3 \times 10^{-2} \pm 8 \times 10^{-4}b$	$4.7 \times 10^{-6} \pm 4 \times 10^{-7}a$	$2.3 \times 10^{-3} \pm 2 \times 10^{-5}a$	$2.7 \pm 0.5a$	$0.43 \pm 0.1 b$	$14.4 \pm 0.9a$	$0.62 \pm 0.2a$	$55 \pm 3 b$	$0.38 \pm 0.09 b$
3	5	0.75	$3.3 \times 10^{-2} \pm 6 \times 10^{-4}b$	$4.7 \times 10^{-6} \pm 2 \times 10^{-7}a$	$2.5 \times 10^{-3} \pm 1 \times 10^{-3}a$	$1.9 \pm 0.7a$	$0.32 \pm 0.1 b$	$13.0 \pm 3.3a$	$1.13 \pm 0.3a$	$54 \pm 4 b$	$0.39 \pm 0.05 b$
4	1	0.75	$3.7 \times 10^{-2} \pm 2 \times 10^{-3}b$	$4.4 \times 10^{-6} \pm 4 \times 10^{-7}a$	$5 \times 10^{-3} \pm 6 \times 10^{-4}b$	$8.5 \pm 1.4c$	$0.03 \pm 0.0a$	$614 \pm 141 b$	$3.24 \pm 1.4 b$	$63 \pm 15 b$	$0.24 \pm 0.04a$
5	9	0.75	$3.0 \times 10^{-2} \pm 7 \times 10^{-4}b$	$5.8 \times 10^{-6} \pm 2 \times 10^{-7}b$	$1.4 \times 10^{-3} \pm 3 \times 10^{-4}a$	$0.9 \pm 0.3a$	$0.44 \pm 0.1 b$	$1.7 \pm 0.2a$	$0.65 \pm 0.1a$	$57 \pm 9 b$	$0.62 \pm 0.10c$
6	3	1	$4.4 \times 10^{-2} \pm 2 \times 10^{-3}b$	$4.7 \times 10^{-6} \pm 2 \times 10^{-7}a$	$5.7 \times 10^{-3} \pm 1 \times 10^{-3}b$	$4.1 \pm 1.9 b$	$0.23 \pm 0.2 b$	$98.2 \pm 6.4a$	$0.88 \pm 0.3a$	$53 \pm 6 b$	$0.37 \pm 0.04 b$
7	3	0.5	$2.2 \times 10^{-2} \pm 7 \times 10^{-3}a$	$5.6 \times 10^{-6} \pm 3 \times 10^{-7}b$	$1.5 \times 10^{-3} \pm 4 \times 10^{-4}a$	$4.5 \pm 0.8 b$	$0.40 \pm 0.1 b$	$27.2 \pm 8.3a$	$1.69 \pm 0.1a$	$57 \pm 5 b$	$0.32 \pm 0.01 b$
8	7	1	$2.3 \times 10^{-2} \pm 2 \times 10^{-2}a$	$5 \times 10^{-6} \pm 3 \times 10^{-7}a$	$2.6 \times 10^{-3} \pm 7 \times 10^{-4}a$	$1.8 \pm 0.1a$	$0.54 \pm 0.1 b$	$4.1 \pm 0.4a$	$0.58 \pm 0.2a$	$46 \pm 5 b$	$0.49 \pm 0.09 b$
9	7	0.5	$2.2 \times 10^{-2} \pm 2 \times 10^{-3}a$	$5.8 \times 10^{-6} \pm 1 \times 10^{-7}b$	$8.1 \times 10^{-4} \pm 3 \times 10^{-4}a$	$1.0 \pm 0.1a$	$0.35 \pm 0.1 b$	$2.8 \pm 0.4a$	$0.98 \pm 0.4a$	$37 \pm 7 b$	$0.39 \pm 0.06 b$

*Values followed by the same letter within a response show no significant difference ($p > 0.05$).SB: Sorbitol, GA: gallic acid, TPC: total phenolic content, TEAC_{DPPH}: trolox equivalent assay capacity by DPPH method, TEAC_{CUPRAC}: trolox equivalent assay capacity by CUPRAC method, TS: tensile strength, EB: elongation at break, E: Young's modulus, O: Opacity, WVT: water vapor transmission rate, P: permeability.

3.4. Optimization and verification of mathematical models

With the aim of obtain films with a good enough resistant mechanical and also the best antioxidant property, many numerical optimizations were performed in order to determine the optimum values for the independent variables in the desired response variables.

The best antioxidant capacities were obtained for films with the highest value of TPC, TEAC_{DPPH} and TEAC_{CUPRAC}. In this sense, these variables were maximized from method of response surface used. Regard mechanical properties, a film must have a good tensile strength and an acceptable elasticity when its use is focused as packaging. Therefore, the value of tensile strength and elongation at break for these films were maximized, while Young's modulus, which indicates the longitudinal elasticity of the material, was minimized. Furthermore, taking into account this kind of film must have a good control of water transfer as well as a good appearance are topics that must be reached, the value of opacity, water vapor transmission rate and permeability were minimized. In brief, the response variables of TPC, TEAC_{DPPH}, TEAC_{CUPRAC}, EB and TS (Y_1 to Y_5) were maximized, while E, O, WVT and P (Y_6 to Y_9) were minimized.

Considering these assumptions for mathematical model proposed, a mixture with a 3.62 wt% of sorbitol, a 1.00 wt% of gallic acid content and 1 wt% chitosan is found to be the optimal composition to build the

desirable film. After that, the films were prepared by triplicate under these optimized conditions. Table 3 reports a comparative analysis between predicted and experimental values with their corresponding standard deviation for each response variable. It was found a good agreement between experimental and predicted values for TPC, TEAC_{DPPH}, EB, E, O and P. The highest difference between the predicted and the experimental value was found for TS and WVT parameter, which is related to their natural high standard deviation. Maybe the boundary conditions set for these parameters in the development of the model were not the most adequate, which magnifies errors.

The determination coefficient of the complete model is 0.98, which can be seen in Fig. S4. This determination coefficient supports the fact that the proposed model exhibits a good fit against the experimental data. This mathematical model allowed determining the optimal conditions of the system to obtain films of chitosan with previously assigned properties. These results suggest that the use of these models in the study of variables is promising since they improve significantly the performance of chemical engineering to solve a particular technological challenge.

4. Conclusions

Preliminary studies in the antioxidant properties allowed

Table 2

Polynomial equations and determination coefficient for investigated responses variables regards the independent variables used in the formulation of films.

2nd order polynomial equation	R ²
$Y_1 = -6.6497 \times 10^{-2} + 6.2753 \times 10^{-3} X_1 + 0.2107 X_2 + 1.5732 \times 10^{-5} X_1^2 - 1.0466 \times 10^{-2} X_1 X_2 - 9.0217 \times 10^{-2} X_2^2$	0.95
$Y_2 = 1.1250 \times 10^{-5} - 2.5638 \times 10^{-7} X_1 - 1.5362 \times 10^{-5} X_2 + 3.6146 \times 10^{-8} X_1^2 + 3.6 \times 10^{-8} X_1 X_2 + 8.996 \times 10^{-6} X_2^2$	0.91
$Y_3 = 2.3732 \times 10^{-3} - 6.0961 \times 10^{-5} X_1 + 1.0126 \times 10^{-2} X_2 + 4.9208 \times 10^{-5} X_1^2 - 1.1886 \times 10^{-3} X_1 X_2 + 1.2556 \times 10^{-3} X_2^2$	0.99
$Y_4 = 12.7519 - 2.86792 X_1 - 3.755 X_2 + 0.158542 X_1^2 + 0.545 X_1 X_2 + 0.96 X_2^2$	0.98
$Y_5 = 123.156 - 2.64583 X_1 - 253.0 X_2 - 0.635417 X_1^2 + 18.0 X_1 X_2 + 110.0 X_2^2$	0.89
$Y_6 = 76.4047 - 213.425 X_1 + 1558.25 X_2 + 18.3568 X_1^2 - 34.835 X_1 X_2 - 874.58 X_2^2$	0.87
$Y_7 = 5.34135 - 1.15021 X_1 - 1.58 X_2 + 0.0734375 X_1^2 + 0.21 X_1 X_2 - 0.46 X_2^2$	0.90
$Y_8 = 9.86549 - 8.67522 X_1 + 188.235 X_2 + 0.222473 X_1^2 + 6.51585 X_1 X_2 - 144.555 X_2^2$	0.80
$Y_9 = 0.283219 - 0.0106232 X_1 - 0.0997015 X_2 + 0.00332764 X_1^2 + 0.0224055 X_1 X_2 + 0.089398 X_2^2$	0.92

Y_1 : TPC, Y_2 : TEAC_{DPPH}, Y_3 : TEAC_{CUPRAC}, Y_4 : TS, Y_5 : EB, Y_6 : E, Y_7 : opacity, Y_8 : WVT, Y_9 : P, X_1 : sorbitol composition, X_2 : gallic acid composition.

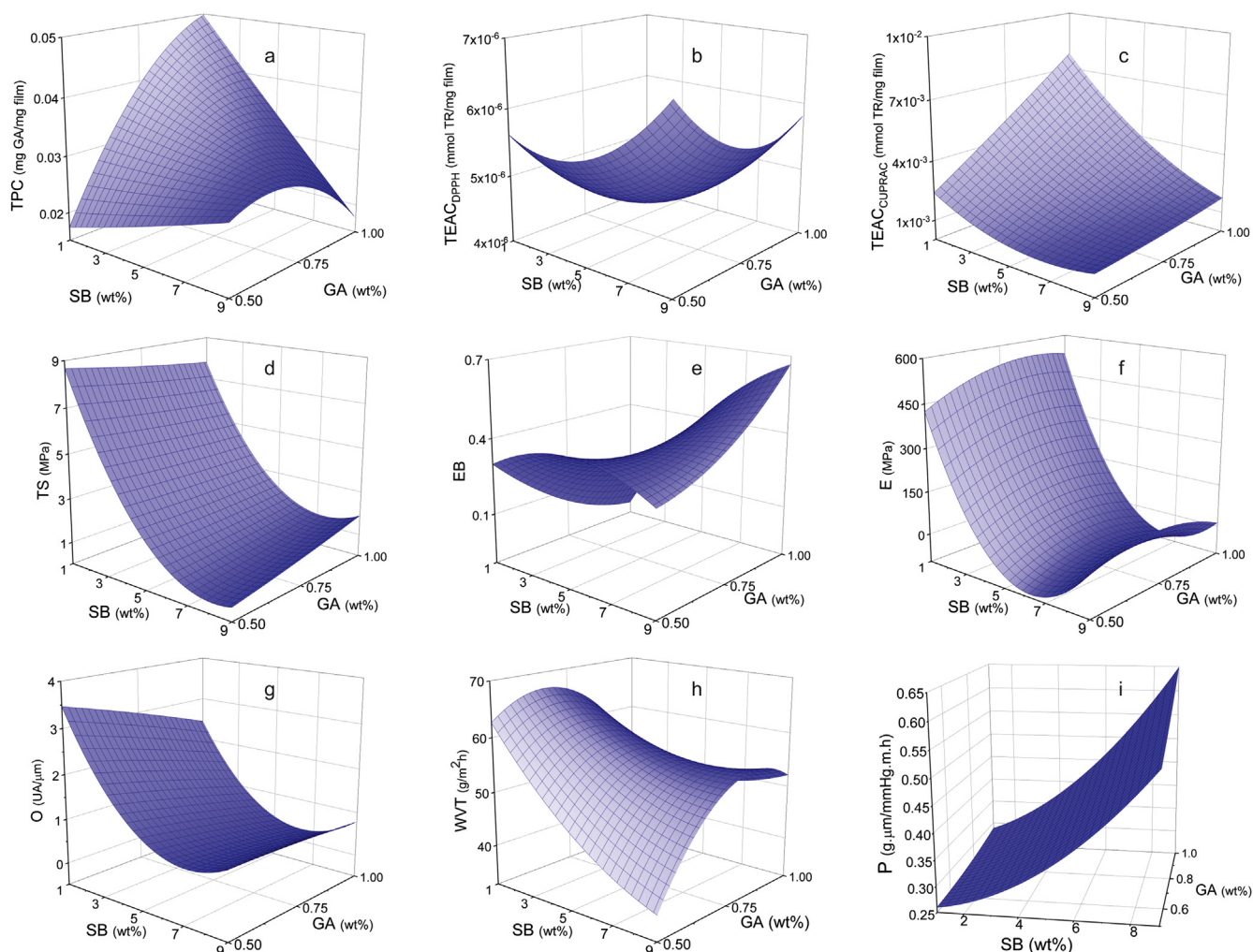


Fig. 5. Response surface plots of TPC (a), TEAC_{DPPH} (b), TEAC_{CUPRAC} (c), tensile strength (d), elongation at break (e), Young's modulus (f), opacity (g), water vapor transmission rate (h) and permeability (i) showing combined effect of sorbitol (SB) and gallic acid (GA) in a chitosan film.

Table 3

Predicted and experimental values response variable at the optimal film composition of 1 wt% chitosan, 3.62 wt% sorbitol and 1.00 wt% gallic acid.

		Predicted	Experimental
Y_1	TPC (mg gallic acid/mg film)	3.90×10^{-2}	$3.7 \times 10^{-2} \pm 4 \times 10^{-3}$
Y_2	TEAC _{DPPH} (mg Trolox/mg film)	4.56×10^{-6}	$4.3 \times 10^{-6} \pm 8 \times 10^{-7}$
Y_3	TEAC _{CUPRAC} (mg Trolox/mg film)	5.13×10^{-3}	$4.3 \times 10^{-3} \pm 6 \times 10^{-5}$
Y_4	TS (MPa)	3.63	7.7 ± 0.4
Y_5	EB	0.27	0.16 ± 0.1
Y_6	E (MPa)	102	107 ± 30
Y_7	O (UA/mm)	0.859	1.0 ± 0.1
Y_8	WVT (g/m ² .h)	48.6	67 ± 6
Y_9	P (g.mm/mmHg.m ² .h)	0.359	0.33 ± 0.02

TPC: total phenolic content, TEAC_{DPPH}: trolox equivalent assay capacity by DPPH method, TEAC_{CUPRAC}: trolox equivalent assay capacity by CUPRAC method, TS: tensile strength, EB: elongation at break, E: Young's modulus, O: Opacity, WVT: water vapor transmission rate, P: permeability.

determine the range of the independent variables of sorbitol as plasticizer and gallic acid as antioxidant for the Response Surface Methodology in a two-factor Doehlert model.

The experimental values of each response variable investigated using the Doehlert model, were fitted with a quadratic polynomials equation with a regression coefficient obtained in the range 0.80–0.99. In order to obtain a resistant mechanical film with the best antioxidant and physical properties, the response variables of the model of TPC,

TEAC_{DPPH}, TEAC_{CUPRAC}, EB, TS were maximized and E, opacity, WVT rate and permeability were minimized. Considering these assumptions, the program identified the mixture with 3.62 wt% of sorbitol and 1.00 wt% of gallic acid added to the 1.00 wt% chitosan solution as the optimal composition to perform a film with physicochemical characteristics sought. At this optimal point, the response variables were obtained experimentally and were compared with the model predictions as validation test with close agreement. To date, the parameters studied have been promising to achieve films with desirable properties for future application as packaging material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.polymeresting.2018.07.003>.

References

- [1] B.A. Morris, 1 – Introduction, *Sci. Technol. Flex. Packag*, first ed., William Andrew, 2017, pp. 3–21, <https://doi.org/10.1016/B978-0-323-24273-8.00001-0>.
- [2] B.P.F. Day, L. Potter, *Active Packaging*, in: R. Coles, M.J. Kirwan (Eds.), *Food Beverage Packag. Technol*, Second, Wiley-Blackwell, 2011, pp. 251–262.
- [3] L. Vermeiren, F. Devlieghere, M. VanBeen, N. de Kruijf, J. Debevere, *Developments in the active food packaging of foods*, *J. Food Technol. Afr.* 5 (2000) 6–13.
- [4] M. Xie, B. Hu, Y. Wang, X. Zeng, Grafting of gallic acid onto chitosan enhances antioxidant activities and alters rheological properties of the copolymer, *J. Agric. Food Chem.* 62 (2014) 9128–9136, <https://doi.org/10.1021/jf503207s>.
- [5] C.K.S. Pillai, W. Paul, C.P. Sharma, Chitin and chitosan polymers: chemistry, solubility and fiber formation, *Prog. Polym. Sci.* 34 (2009) 641–678, <https://doi.org/10.1016/j.progpolymsci.2009.04.001>.
- [6] L.A.M. Van Den Broek, R.J.I. Knoop, F.H.J. Kappen, C.G. Boeriu, Chitosan films and blends for packaging material, *Carbohydr. Polym.* 116 (2015) 237–242, <https://doi.org/10.1016/j.carbpol.2014.07.039>.
- [7] S.P. Miranda-Castro, *Application of Chitosan in Fresh and Minimally Processed Fruits and Vegetables*, Elsevier Inc., 2016, <https://doi.org/10.1016/B978-0-12-802735-6.00003-3>.
- [8] Y. Wang, B. Li, X. Zhang, N. Peng, Y. Mei, Y. Liang, Low molecular weight chitosan is an effective antifungal agent against *Botryosphaeria* sp. and preservative agent for pear (*Pyrus*) fruits, *Int. J. Biol. Macromol.* 95 (2017) 1135–1143, <https://doi.org/10.1016/j.ijbiomac.2016.10.105>.
- [9] V. Epure, M. Griffon, E. Pollet, L. Avérous, Structure and properties of glycerol-plasticized chitosan obtained by mechanical kneading, *Carbohydr. Polym.* 83 (2011) 947–952, <https://doi.org/10.1016/j.carbpol.2010.09.003>.
- [10] F.M. Fakhoury, S. Maria Martelli, L. Canhadadas Bertan, F. Yamashita, L.H. Innocentini Mei, F.P. Collares Queiroz, Edible films made from blends of manioc starch and gelatin - influence of different types of plasticizer and different levels of macromolecules on their properties, *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 49 (2012) 149–154, <https://doi.org/10.1016/j.lwt.2012.04.017>.
- [11] M.E. Lawson, Sugar alcohols, *Kirk-Othmer Encycl. Chem. Technol*, 2000 <http://www.diabetes.org/food-and-fitness/food/what-can-i-eat/sugar-alcohols.html>.
- [12] Z.H. Zhang, Z. Han, X.A. Zeng, X.Y. Xiong, Y.J. Liu, Enhancing mechanical properties of chitosan films via modification with vanillin, *Int. J. Biol. Macromol.* 81 (2015) 638–643, <https://doi.org/10.1016/j.ijbiomac.2015.08.042>.
- [13] U. Siripatrawan, S. Noipha, Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages, *Food Hydrocolloids* 27 (2012) 102–108, <https://doi.org/10.1016/j.foodhyd.2011.08.011>.
- [14] O.I. Aruoma, A. Murcia, J. Butler, B. Halliwell, Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives, *J. Agric. Food Chem.* 41 (1993) 1880–1885, <https://doi.org/10.1021/jf00035a014>.
- [15] X. Sun, Z. Wang, H. Kadouh, K. Zhou, The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films, *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 57 (2014) 83–89, <https://doi.org/10.1016/j.lwt.2013.11.037>.
- [16] Y.S. Cho, S.K. Kim, C.B. Ahn, J.Y. Je, Preparation, characterization, and antioxidant properties of gallic acid-grafted-chitosans, *Carbohydr. Polym.* 83 (2011) 1617–1622, <https://doi.org/10.1016/j.carbpol.2010.10.019>.
- [17] M. Xie, B. Hu, Y. Yan, L. Zhou, S. Ou, X. Zeng, Rheological properties of gallic acid-grafted-chitosans with different substitution degrees, *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 74 (2016) 472–479, <https://doi.org/10.1016/j.lwt.2016.08.018>.
- [18] J. Liu, S. Liu, Y. Chen, L. Zhang, J. Kan, C. Jin, Physical, mechanical and antioxidant properties of chitosan films grafted with different hydroxybenzoic acids, *Food Hydrocolloids* 71 (2017) 176–186, <https://doi.org/10.1016/j.foodhyd.2017.05.019>.
- [19] R. Czechowska-biskup, D. Jarosińska, B. Rokita, P. Ulański, J.M. Rosiak, Determination of degree of deacetylation of chitosan - comparison of methods, *Prog. Chem. Appl. Chitin Its Deriv* 17 (2012) 5–20.
- [20] V. Ivanova, Á. Dörnyei, L. Márk, B. Vojnoski, T. Stafilov, M. Stefova, F. Kilár, Polyphenolic content of Vranec wines produced by different vinification conditions, *Food Chem.* 124 (2011) 316–325, <https://doi.org/10.1016/j.foodchem.2010.06.039>.
- [21] U. Siripatrawan, B.R. Harte, Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract, *Food Hydrocolloids* 24 (2010) 770–775, <https://doi.org/10.1016/j.foodhyd.2010.04.003>.
- [22] R. Apak, K. Güçlü, M. Ozyürek, S.E. Karademir, Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *J. Agric. Food Chem.* 52 (2004) 7970–7981, <https://doi.org/10.1021/jf048741x>.
- [23] ASTM, standard test method for tensile properties of thin plastic sheeting, www.astm.org, (2012).
- [24] S. Il Park, Y. Zhao, Incorporation of a high concentration of mineral or vitamin into chitosan-based films, *J. Agric. Food Chem.* 52 (2004) 1933–1939, <https://doi.org/10.1021/jf034612p>.
- [25] ASTM, standard test methods for water vapor transmission of materials, www.astm.org, (2016).
- [26] D.H. Doehlert, Uniform shell designs, *J. Roy. Stat. Soc.* 19 (1970) 231–239, <https://doi.org/10.2307/2346327>.