Optimization of the moisture content, thickness, water solubility and water vapor permeability of sodium alginate edible films

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Abstract - The present study focuses on the effects of concentrations of sodium alginate (0.5-2.5% w/v) and glycerol (0.5-1.5% w/v) and the film forming method (FFM) (dry and wet) on the moisture content, thickness, water solubility, and water vapor permeability (WVP) of edible films. The effects were analyzed using a two-level factorial design. Prediction equations were obtained and optimized for each response variable. To generate insoluble polymers 2% of $CaCl_2$ was added directly to the film emulsion (wet method). For the dry method, the emulsions were dried at 60 ºC for 6 hours, and then $CaCl_2$ was added. The moisture content, thickness, and water solubility were significantly affected ($p < 0.05$) by principal effects. Furthermore the glycerol did not affect the water vapor permeability. The films obtained by wet method, showed the highest water solubility values 66.06% (0.5% alginate-glycerol) and WVP 579.74 g mm/kPa h m^2 (2.5 - 0.5% glycerol alginate). Unlike, the films formed by the dry method, the water solubility was 44.66% and WVP was 13.66 g mm/kPa h m^2 for the 0.5 - 1.5 and 2.5 - 0.5% alginate-glycerol concentrations respectively. Predictive equations for each response variable showed a good fit of the experimental data ($R^2 > 0.999$).

Key-Words: - sodium alginate, calcium chloride, glycerol, films, physical properties, experimental design.

1 Introduction

The films and coatings have been used to reduce the minimum deterioration during processing and storage of fresh-cut fruits. Edible films provide a semi permeable barrier that helps to extend shelf life by reducing the migration of moisture, loss of solutes from the fruit respiration and oxidation reactions [1, 2, 3]. Among the main biopolymers used in the preparation of edible films were found proteins, derivatives of cellulose, alginates, pectin, and starches [4].

The alginate is a salt of alginic acid, which is a mixture of polyuronic acids, isolated from the cell walls of a number of species from brown seaweeds (*Phaeophyceae*). This polysaccharide is formed by acid monomers β-D-mannuronic acid and α-L-guluronic, being a non-toxic polysaccharide [5,6]. This biopolymer has interesting functional properties as its colloidal properties and thickeners, helps to stabilize suspensions and emulsions; and has the ability to form films and gels [7]. Alginate is capable of forming an insoluble gel or polymer resistant in the presence from polyvalent metal cations such as calcium. The gelling mechanism is based on interactions between the calcium ions and carboxylic groups, forming a three-dimensional crosslinked network. This interaction occurs when mixing components (alginate-calcium) to form a film or by pouring a solution from calcium on alginate layer previously dried [8].

Pavlath et al. [9] reported based edible films alginate slowly dissolves when they are immersed in a solution of multivalent ions (calcium and zinc). Moreover, edible films containing hydrocolloids such as alginate do not perform as well as a moisture barrier for hydrophilic in nature, since the flow from water vapor through the film is not linear with gradient the partial pressure of water vapor [10].

Plasticizers such as glycerol are necessary to form the basis from edible polysaccharide films, since they can increase the flexibility of the film due to the decrease of hydrogen bonds between polymer chains, resulting increased of intermolecular space. Therefore, it can increase the permeability of the film to oxygen and moisture [11]. Several studies [1,2,8,12,13] had been focused primarily on film formation of alginate based edible by pouring the mixture (alginate-glycerol) obtaining the polymer

by adding calcium chloride, and subsequently the emulsion subjected to a relative humidity generated pre-set (generally low), to remove the solvent (water) and obtaining the edible film. However, there is little information on the effect of dewatering the mixture (alginate-glycerol) and then adding calcium chloride to the film formation.

The factorial design is a method that is often used to speed up the task´s solution. It has been applied in various branches of science and industry [14]. Furthermore, the optimization has been used in food engineering and an efficient operating system and unitary process in order to obtain a highly acceptable solution [15].

The objective of this study was 1) to determine the effect of concentrations of sodium alginate (0.5-2.5% w/v), glycerol (0.5-1.5% w/v) and the film forming method (dry or wet) on the moisture content, thickness, water solubility and water vapor permeability to make of edible films, 2) to obtain prediction equations and response variables.

2 Materials and Methods

2.1 Materials

Alginate (Biopolymer Mexico), glycerol used as a plasticizer, was from Merck (Inc. Corp. Whitehouse Station, NJ, USA) and calcium chloride (PRM, Mexico).

2.2 Film Preparation

The films forming solutions were prepared by dissolving sodium alginate (0.5 or 2.5% w/v) in distilled water with stirring and heating (80 ºC) to obtain a clear solution. Glycerol was added at concentrations of 0.5 or 1.5% w/v, the mixed emulsions were formed by using an Ultra Turrax T18 Basic (IKA Works, Inc., USA) at 9000 rpm for 3 min, and immediately degassed to eliminate the presence of air bubbles. The obtained film forming solutions were divided into two lots to add calcium chloride (2% w/v) films (wet or dry).

2.3 Formation and conditioning of films

The edible films were prepared by adding 12 grams of film forming solution in Petri boxes of 10 cm in diameter previously identified. The first batch was incubated in an oven at 60 ºC for 6 h, at the end, calcium chloride were added (15mL) 2% (w/v) (dry film formulation), while in the second is immediately added calcium chloride (wet formulation). Then, they were stored at a relative humidity of 62% at 25 ºC for 3 hours, and conditioned at 33% relative humidity for analyzing physical properties.

2.4 Physical Properties

2.4.1 Moisture content

Moisture content of the films was determined by the loss of weight of the film after drying at 105 ºC for 24 h [16].

2.4.2 Water solubility (WS)

Film solubility was determined as follows: three randomly selected samples (boxes 2 x 2 cm^2) from each type of film were first dried at 105ºC for 24 h. Then, samples were immersed in 50 mL of distilled water and kept in a bath with constant shaking (Yamato Scientific model BT 25, Tokyo, Japan) at 25 ºC for 24 h, after the samples were filtered and dried at 105 ºC for 24 h to determine dry matter not dissolved in water [16]. The rate of solubility was obtained by the following expression:

$$\% WS = \left(\frac{W_{msi} - W_{msf}}{Wmsi}\right) x\ 100 \qquad (1)$$

Where: W_{msi} is the initial weight of the dry matter and W_{msf} the dry matter weight of the dispersion process after 24 h.

2.4.3 Thickness

A micrometer (Starrett No. 208, USA) was used to determine the thickness of films formed. Measuring was realized at four different points from film (micron).

2.4.4. Water vapor permeability (WVP)

The permeability of the films was determined gravimetrically using a modified version of standard method ASTM -E96-93 for alginate films [17,18]. Edible films were cut into 5 cm diameter, then, were placed on glass jar being adjusted on the circumference with parafilm.

The glass jar containing 5 mL of distilled water, leaving an inch air space between the water surface and the film. The above system was stored in dessicator containing a saturated solution of $MgCl_2.6H_20$ at 33% relative humidity at 25ºC. Measurements were recorded over an 8 hours period with intervals of 60 min. First transfer was obtained of water vapor transmission rate (WVTR) through the slope of the regression analysis of weight loss as a function of time (g/s) and related to the water vapor permeability (WVP) [19] according to the following expressions:

$$WVTR = m_1/A = g/m^2s \quad (2)$$

$$WVP = L \times WVTR/(p_1 - p_a) \quad (3)$$

Where m_1 is the slope of the weight loss versus time (g/s), A is the exposed area; p_i and p_a are respectively the partial pressures of water vapor in the air and air saturated to 33% relative humidity at 25 ºC. L is the average thickness (mm).

2.5 Experimental design

To evaluate the effect of alginate concentrations (0.5 or 2.5% w/v), glycerol (0.5 or 1.5% w/v) and the method of forming the edible film (dry or wet) on the response variables (thickness, moisture content, water solubility and water vapor permeability), a two level factorial design (2^3), with three replicates generated by Minitab 16.0 statistical software (Minitab, Inc., State College, PA, USA) in a random order, was used (Table 1). The significance of the factors evaluated on each physical property is selected according to $p < 0.05$.

Table 1 Formulations of edible films

Treatment	Alginate (%)	Glycerol (%)	FFM[1]
1	0.5	0.5	W
2	0.5	0.5	D
3	2.5	0.5	W
4	2.5	0.5	D
5	0.5	1.5	W
6	0.5	1.5	D
7	2.5	1.5	W
8	2.5	1.5	D

[1] FFM: film forming method, W: weat, D: dry

2.6 Response model and optimization

The experiments described in Table 1 were conducted at random to obtain the coefficients and the interactions of the evaluated effects. The following expression, contain the factors used to predict and optimize the response variables studied.

$$y = \beta_0 + \beta_1 * X_1 + \beta_2 * X_2 + \beta_3 * X_3 + \beta_{12} * X_1 * X_2 + \beta_{13} * X_1 * X_3 + \beta_{23} * X_2 * X_3 + \beta_{123} * X_1 * X_2 * X_3 \quad (4)$$

Where y is the predicted response, β_0 is the estimated regression coefficient, β_1, β_2, β_3 are the coefficients of alginate, glycerol, and FFM, β_{12}, β_{13}, β_{23} and β_{123} are double and triple interactions of the main effects.

For the prediction of responses studied was used Microsoft Office Excel 2010. The predictive equations were evaluated in terms of the statistical error by the percentage of the root mean square (% RMS) differences between the predicted values and the predicted experimental thickness, moisture content, water solubility, and water vapor permeability, root mean square must be less than 10% in order to be considered acceptable and the model is expressed as:

$$\%RMS = \left(\sqrt{\frac{1}{n} \sum_{i=1} \left(\frac{M_{\exp} - M_{pre}}{M_{\exp}} \right)^2} \right) x100 \quad (5)$$

Where n is equal to the number of observations, the experimental value is M_{exp} responses studied M_{pre} represents the predicted responses [20].

3 Results and discussion

3.1 Moisture content

It was found that the moisture content decreases in the wet method, when the alginate and glycerol concentration increases. Furthermore, in the dry method when alginate and glycerol decreases, the moisture content increases. Because, the mechanism for forming the polymer-polysaccharide interactions glycerol and glycerol-water, change the physical properties of the film [21] as demonstrated by statistical analysis (Table 3) where the main effects, double interactions and triple interactions significantly affect the moisture content of the film ($p < 0.05$).

Fig 1 shows that the edible films obtained with the wet method (treatment 1), 0.5% alginate and 0.5% glycerol showed the highest moisture content (97.20 ± 0.33%) compared with the other formulations; this is due to low concentrations of polysaccharide which allow greater availability of free water to participate in the polymerization reactions. Unlike the dry method where an inverse behavior was observed at the same concentrations of alginate and glycerol, obtaining the lowest moisture content (33.48 ± 2.62%). This behavior can be attributed the fact when preparing edible films with the dry method at concentrations of 2.5% alginate and 1.5% glycerol (treatment 8), the calcium chloride promotes the interaction of the carboxyl groups of the alginate to form a polymeric structure more easily retain the water.

When increasing glycerol concentrations, it increases the moisture content because of its water

holding capacity. Ahmadi et al. [22] and Godbillot et al. [23] found similar results based edible films obtained from hydrocolloids psyllium (Plantago seeds of the plant) and potato starch, where they observed that when glycerol concentrations increase significantly increases the moisture content of the films elaborate.

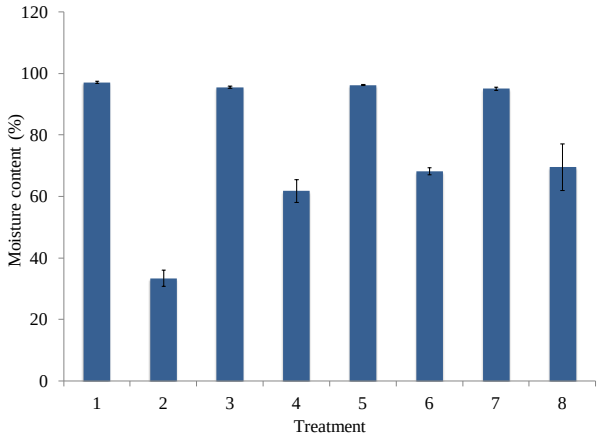

Fig. 1 Effects of alginate-glycerol concentrations and film forming method (Table 1) on edible films moisture content.

3.2 Water solubility

The method for the wet treatment had a higher solubility of edible films regardless of the alginate and glycerol concentrations (Fig 2). With respect to the dry method, the solubility of edible films increases as the concentration of glycerol is added, by increasing the concentration of the plasticizer it decreases intermolecular forces by interacting with the functional groups of the polysaccharide, causing an increase in solubility. It was also noted that higher concentrations of alginate (2.5%) and glycerol (1.5%) (treatment 8) favor the stability of the film formed.

Mwesigwa et al. [24] attributed to the increased solubility of edible films based on polysaccharides, due to the water affinity of the polar groups, so cohesiveness generated is greater than the attractive forces present water-water. The main effects, double interaction (glycerol-forming method of the film) and the triple interaction are shown to be significant ($p < 0.05$) in the solubility of edible film (Table 3).

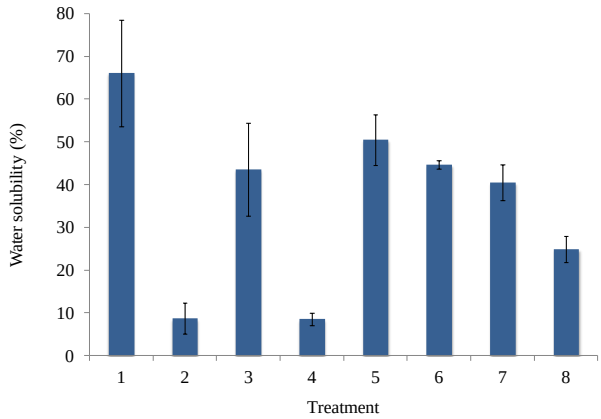

Fig. 2 Effects of alginate-glycerol concentrations, and film forming method (Table 1) on edible film water solubility.

3.3 Thickness

Table 2 shows the mean of triplicates of thickness obtained for each of the 8 treatments evaluated according to the experimental design (factor 2^3). It was observed that less thickness was obtained (0.02 mm) of edible films, when using the dry method and low concentrations (0.5%) and glycerol alginate, unlike the wet method to the same concentrations of polysaccharide and plasticizer, where was obtained a thickness of 0.69 ± 0.13mm (Table 2). While the maximum thickness achieved corresponded to the wet method (2.5% alginate and 1.5% glycerol) obtaining 3.7 ± 1.15 mm. Similar results were obtained by Pavltah et al. [9] who assume that when submerged based films alginate calcium chloride occurring two competing reactions occur during the formation of the polymer: 1) dissolution of the alginate in solution and 2) the insolubilization of the film by crosslinking entity Ca^{2+} and carboxylic groups, generating an increase in the film thickness.

The main effects and interactions double (alginate-FFM) and (glycerol-FFM) significantly affect the edible film thickness regardless to the concentrations used (Table 3).

Table 2 Experimental values of the response variables evaluated in edible films

Treatment	Thickness (mm)	WVP[1] g mm/kPa h m^2
1	0.69 ± 0.13	57.95 ± 16.41
2	0.02 ± 0.00	0.08 ± 0.01
3	2.04 ± 0.46	579.74 ± 182.89
4	0.24 ± 0.12	13.66 ± 2.50
5	1.36 ± 0.03	30.99 ± 2.94
6	0.04 ± 0.03	0.52 ± 0.60
7	3.07 ± 1.15	313.75 ± 163.09
8	0.18 ± 0.02	6.07 ± 0.69

[1]WVP: water vapor permeability

3.4 Water vapor permeability

Table 2 shows that a higher concentration of alginate (2.5%) and glycerol (0.5%) with edible films obtained by the dry method had a higher water vapor permeability (13.66 mm ± 2.50 g / h m^2 kPa). In this case the alginate being of hydrophilic nature helps to increase the permeability of the edible film being ineffective as moisture barrier. Statistical analysis showed that the main effects (alginate and glycerol) and double interaction (alginate-FFM) are shown to be significant ($p < 0.05$) in the water vapor permeability (Table 3).

Edible films, produced with the wet method had the highest water vapor permeability (30.99 - 579.74 g mm / kPa h m^2) since during the generation of three-dimensional polymer network formed by calcium chloride trapping water molecules, which act as a plasticizer in the crystal lattice, reducing the number of intermolecular bonds in the polymer chain by facilitating the transfer of water vapor through the film [25]. A similar effect was reported by Benavides et al. [26] in the study of sodium alginate films with oregano essential oil.

Table 3 Estimated probabilities for the response variables

Term	Moisture content	Water solubility	Thickness	WVP[1]
Constant	0.000	0.000	0.000	0.000
Alginate (%)	0.000	0.000	0.000	0.000
Glycerol (%)	0.000	0.007	0.036	0.043
FFM[2]	0.000	0.000	0.000	0.000
Alginate (%) * Glycerol (%)	0.000	0.528*	0.706*	0.087*
Alginate (%) * FFM	0.000	0.263*	0.002	0.000
Glycerol (%) * FFM	0.000	0.000	0.029	0.073*
Alginate (%) * Glycerol (%) * FFM	0.000	0.009	0.55*	0.144*

[1]WVP: water vapor permeability, [2] FFM: film forming method

* Not significant at $p>0.05$

3.5 Response modeling and optimization

Table 4 shows the ratios obtained with the experimental design (2^3) of the response variables studied: thickness, moisture content, solubility and WVP used in the prediction equation (4).

Table 4 Coefficients estimated uncoded response variables

Term	Moisture content	Water solubility	Thickness	WVP[1]
Constant	51.873	34.689	0.002	-46.717
Alginate (%)	10.007	-4.786	0.358	164.724
Glycerol (%)	20.254	11.031	0.310	17.622
FFM[2]	-46.245	-46.405	-0.062	42.192
Alginate (%)*Glycerol (%)	-6.636	-1.747*	0.070*	-61.764*
Alginate (%)*FFM	10.926	9.6293*	-0.226	-155.936
Glycerol (%)*FFM	21.274	29.840	-0.269	-15.185*
Alginate (%)*Glycerol (%)*FFM	-6.860	-8.060	-0.110*	57.756*

[1]WVP: water vapor permeability, [2] FFM: film forming method

* Not significant at $p>0.05$

Prediction equations obtained for each response showed correlation coefficients (R^2) greater than 0.999. The percentage of the root mean square (% RMS) between predicted and experimental values

for the thickness, moisture content, solubility and permeability to water vapor is less than 1%, indicating that the proposed models are suitable to describe and predict the effects of the factors on the evaluated variables.

It was determined that the concentrations of sodium alginate (0.5-2.5% w / v) glycerol (0.5-1.5% w / v) and the FFM (dry or wet) had a significant effect ($p < 0.05$) on the content moisture, thickness, and water solubility of edible films made. The double interaction (alginate-glycerol) was only significant ($p < 0.05$) for moisture content. Alginate-FFM interaction did not affect the solubility of the film, but neither on the other physical properties evaluated. The glycerol- FFM ratio did not influence the water vapor permeability, while alginate-glycerol-FFM had an effect in the moisture content and water solubility.

In foods with high moisture content, such as fruits and vegetables, moisture loss brings weight loss and firmness, so to minimize these effects and according to the results found in this study films could be made with the dry method, 0.5% alginate and glycerol, which showed less solubility and water vapor permeability (8.69% and 0.08g mm / kPa h m^2, respectively).

4 Conclusions

Prediction equations for responses studied are proved to be adequate to describe the experimental data on the thickness, moisture content, water solubility and water vapor permeability ($R^2 > 0.999$).

Edible films should be chosen based on their suitability for the desired and application. Therefore, it is important to consider the components used in the formulation as well as the training in obtaining films as a decisive influence on their physical properties, directly affecting the organoleptic quality of coated food.

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