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Optimization of physical properties of xanthan gum/tapioca starch edible matrices containing potassium sorbate and evaluation of its antimicrobial effectiveness

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ABSTRACT

Response surface methodology was applied to study the effect of different levels of tapioca starch (TS) and xanthan gum (XG) on physical properties of edible films supporting potassium sorbate (KS) with the goal of contributing to the development of edible matrices with controlled release of the antimicrobial. Mechanical properties, water vapor permeability (WVP), solubility in water (SW) and color attributes were evaluated on TS–XG based films. XG addition produced an increase of Young modulus (YM), stress at break (σ_b) and SW. It also raised the yellow index (YI) values and decreased the strain at break (ε_b). Edible film formulation was optimized with the goal of maximizing YM and ε_b and minimizing SW and YI. The film with the selected formulation resulted an effective antimicrobial barrier against *Zygosaccharomyces bailii* external contamination and its sorptional behavior was highly influenced by XG presence. It can be concluded that developed matrices could act as an effective active film with potential applications for food preservation.

1. Introduction

Edible films and coatings are one of the emerging strategies for food quality optimization. Their usefulness is based on the capacity of optimizing global quality, extending shelf life and, possibly, improving the economic efficiency of packaging materials (Rojas-Graü, Soliva-Fortuny, & Martin-Belloso, 2009).

These matrices can be used to cover food surfaces, compartmentalize ingredients, form a gas barrier or constitute wraps. They can be used as carriers of additives or to improve appearance and handling (Campos, Gerschenson, & Flores, 2011). In general, these films have high water solubility and poor WVP. To solve this shortcoming, the blending of different biopolymers (Xu, Kim, Hanna, & Nag, 2005) or the addition of hydrophobic materials such as oils or waxes (Ayranci & Tunc, 2003) has been proposed.

Tapioca starch ability as a former of edible film matrices has been reported (Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007).

Xanthan gum (XG) is a high molecular weight extracellular polysaccharide produced by the bacterium *Xanthomonas campestris*. Flores, Costa, Yamashita, Gerschenson, and Grossmann (2010) informed that its incorporation to starch-based films obtained through extrusion, affected mechanical properties and water sorption of the films.

The KS is usually employed in food preservation because of its GRAS (Generally Recognized as Safe) status and high solubility in water. Films and coatings containing sorbates (KS and sorbic acid) have been developed to inhibit the growth of yeasts in foods systems (Campos et al., 2011). Previous research of the authors showed that sorbates changed the physical properties of films based on slurries containing 5 g/100 g of tapioca starch (Flores, Famá, et al., 2007) or a blend of starch and chitosan (Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Cagri, Ustunol, and Ryser (2001) and Shen, Wu, Chen, and Zhao (2010) informed the same trend for films based on whey proteins and sweet potato starch, respectively.

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The application of antimicrobial edible films might help to obtain a localized functional effect at the surface of the food product (Flores et al., 2010) or to produce the gradual release of the antimicrobial through the control of its diffusion to the food or to protect the additive from the interaction with other food components or from ambient conditions which could promote its destruction or inactivation (Rojas-Graü et al., 2009). It is important to remark that film forming conditions and film composition affect additive migration and, as a consequence, its effectiveness (Flores, Conte, Campos, Gerschenson, & Del Nobile, 2007).

Response surface methodology (RSM) is a useful collection of mathematical and statistical techniques for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery, 2005).

The objectives of this study were:

- to evaluate through an RSM, the influence of TS and XG levels on the physical properties of TS-based edible films, with the purpose of optimizing the formulation.
- to determine the antimicrobial behavior and water vapor sorption of the optimum film,

for contributing to the knowledge of biopolymer matrices behavior as carriers of additives in order to control the release of antimicrobials on the surface of foods.

2. Material and methods

2.1. Preparation of edible films

XG (Degussa S.A., Argentina) was dissolved in water under stirring at room temperature for 30 min. A suspension of TS (Bernesa, Argentina) in a glycerol (Sintorgan, Argentina) – KS (Sigma, USA) aqueous solution was added to the gum, under stirring, until total homogenization. Heating of hydrocolloid systems was performed (1.5 °C/min) on a hotplate under stirring to completely gelatinize the starch (final temperature: 80–82 °C). The XG and TS final contents varied according to Table 1. Glycerol and KS final concentrations were maintained at 2.5 and 0.3 g/100 g slurry respectively for all systems through the addition of 7.5 g of glycerol and 0.9 g of KS into a system of 300 g final weight. Vacuum was applied to remove air from the solutions.

Casting was used for edible film obtention: 20 g of film forming solutions were dispensed on Petri dishes (9 cm diameter) and dried in a controlled temperature chamber (25 °C) for 48 h. Film was separated and equilibrated to a final water activity (a_w) of 0.57 (25 °C) over NaBr saturated solution.

2.2. Mechanical properties of films

Traction tests were performed according to ASTM D882-10 (2010) with slight modifications. Tested filmstrips (6 mm × 60 mm) were cut and mounted between pneumatic grips. Initial grip separation and crosshead speed were 20 mm and 0.8 mm/s respectively. The stress σ (F/A, being F the force and A the area of the specimen; MPa) and the strain ε (H/L_0, being H the crosshead displacement occurred and L_0 the initial effective length of the sample; %) were recorded using a universal testing machine (Instron 3345, USA). The stress and the strain at break (σ_b, ε_b) were evaluated. For strains lower than 10%, the stress–strain curves were fitted to a linear model and the YM (MPa) was evaluated from the slope. Nine specimens were tested for each sample.

Table 1
Independent variables tapioca starch (TS), xanthan gum (XG) and their levels for the Central Composite Design.

Variable levels	Independent variables	
	TS (g/100 g slurry)	XG (g/100 g slurry)
−2	4.00	0.00
−1	4.25	0.25
0	4.50	0.50
+1	4.75	0.75
+2	5.00	1.00

2.3. Solubility in water

Solubility is defined (Gontard, Guilbert, & Cuq, 1992) as the percentage of dry matter solubilized after 24 h of film immersion in distilled water with respect to initial dry matter. The initial dry matter was determined by drying 2 cm diameter disks in a vacuum oven at 100 °C during 24 h. Other disks were cut, weighed and immersed in 50 mL of distilled water, with stirring, during 24 h at 25 °C. Not solubilized films were taken out and dried (100 °C, 24 h) to determine the final weight of dry matter. Three specimens were tested for each sample.

2.4. Color evaluation

Film disks were rested on white background standard. Measurements were performed in a Minolta colorimeter (Minolta CM-508d, Japan) using an aperture of 1.5 cm-diameter. The color Hunter Lab parameters and the yellow index, YI (ASTM E1925, 1988), were determined in at least five positions randomly selected for each sample. Calculations were made for D65 illuminant and 2° observer. Three specimens were tested for each sample.

2.5. Water vapor permeability

WVP was determined gravimetrically at 25 °C according to ASTM E96-00 (2000) procedure with modifications. The acrylic permeation cells contained $CaCl_2$ (0% RH). Film was located between the cell and its ring cover. The covered cell was placed in a temperature and RH controlled chamber (Ibertest, España) at 25 °C and an RH of 70%. Changes in weight of the cell were recorded and used for WVP calculation. The film thickness used for calculations was measured using a thickness gauge (Mitutoyo, Japan) with a precision of 0.001 mm. All tests were conducted in duplicate.

2.6. Moisture sorption isotherm

Aliquots of 0.3–0.4 g of films based on slurries containing TS 4.75 g and XG 0.25 g/100 g slurry or TS 5 g/100 g slurry and both with 2.5 g of glycerol and 0.3 g of KS per 100 g of slurry, were equilibrated at 25 °C in desiccators containing $CaCl_2$ (0% RH). The moisture adsorption isotherms of the films were determined in triplicate, at 25 °C, with the gravimetric method (Mathlouthi, 2001). Ten different relative humidity conditions (11.3, 32.8, 43.1, 57.6, 75.3, 84.3, 90.1 and 97.3% R.H.) obtained by using saturated salt solutions (Greenspan, 1977) were evaluated. The sample weights were measured to the nearest 0.0001 g at 25 °C, until the films reached equilibrium. The equilibrium moisture content was determined by drying samples in a vacuum oven at 70 °C until reaching a constant weight. The experiment was performed in triplicate.

The experimental moisture sorption values were fitted to the Oswin empirical model:

$$m = A\left(\frac{a_w}{1-a_w}\right)^N \quad (1)$$

where m is the moisture content of the film (g/100 g dry basis, d.b.), a_w is the water activity (RH/100), A and N are the fitting parameters (Chen, 1990).

2.7. Effectiveness of potassium sorbate containing films as barriers to yeast contamination

Antimicrobial activity of the films was evaluated using as indicator *Zygosaccharomyces bailii*, spoilage yeast known due to its resistance to several stress factors (i.e.: pH decrease, depression of a_w) according to Sofos (2000).

A *Z. bailii* NRRL 7256 inoculum was prepared in Saboureaud broth at 25 °C until early stationary phase was achieved. Saboureaud agar with a_w depressed to 0.980 with glucose and pH adjusted to 4.5 with citric acid was formulated to resemble a food product. Discs of 1 cm diameter were cut from films of the selected formulation and with or without KS. The discs were applied on the surface of the agar. Then, 10 μL of a culture of *Z. bailii* containing approximately 3–5 × 10^6 CFU/mL were seeded on the film discs. Samples were incubated at 25 °C for 48 h. Sampling was performed at selected times by taking two discs, each one being suspended in 1 mL of peptone water (Biokar Diagnostics, France). Samples were shaken for 2 min at 2500 rpm with a vortex (IKA, USA), prior to enumerating *Z. bailii* population by surface plating on Saboureaud agar and incubation at 25 °C for 5 days prior to counting (Flores, Haedo, Campos, & Gerschenson, 2007).

2.8. Experimental design and statistical analysis

The influence of TS and XG on film properties was studied by modulating the two selected variables according to a two factors, five levels, Central Composite Design (CCD), as reported in Table 1. The lowest and the highest levels of the independent variables studied as well as the glycerol and KS concentrations were chosen from preliminary laboratory tests. Table 2 lists all experimental runs.

Statistical software (Statistica V 6.0, StatSoft, USA) was used to fit experimental data and estimate the coefficients of a second-degree polynomial function:

$$\psi = B_0 + B_1x_1 + B_2x_2 + B_{11}x_1^2 + B_{22}x_2^2 + B_{12}x_1x_2 \quad (2)$$

where, ψ is the generic dependent variable examined; B_0 is the value of fitted response at the central point of the design (0, 0); B_1 and B_2 are the linear regression coefficients; B_{11} and B_{22} are the quadratic regression coefficients and B_{12} is the cross-product regression coefficient; x_1 and x_2 are the independent variables (TS and XG). An analysis of variance (ANOVA) was performed in order to assess the adequacy of the Eq. (2), through the F test value for the regression (F_{reg}), lack of fit value (P) and the determination coefficient (R^2), and also to evaluate the significance of the equation parameters. The response surface analysis was performed by using the fitted surface, generating two-dimensional response counter plots and the optimal composition of TS–XG based edible films was selected using the desirability function procedure (Barros Neto, Spacino Scarminio, & Bruns, 2003). This procedure was successfully used, as a tool to investigate optimum component concentration or process conditions, in several researches (Puttongsiri & Haruenkit, 2010; Rahman, Kamaruddin, & Uzir, 2010).

3. Results and discussion

The responses obtained for mechanical properties (YM, σ_b and ε_b), polymeric matrix color, WVP and SW can be observed in Table 2.

It was not possible to describe satisfactorily the responses of WVP and *a* and *b* parameters using the quadratic model. Probably, this fact could be attributed, in the case of WVP, to the very similar values ($P > 0.05$) obtained for different formulations considered. On the contrary, Hunter a data could be adjusted to a quadratic model with appropriate F_{reg} and R^2, but the lack of fit was significant ($P < 0.01$). Hunter *b* data presented a low R^2 (<0.8).

For the other parameters evaluated, the results of the ANOVA are indicated in Table 3. The proposed model had no significant lack of fit ($p > 0.05$) and the R^2 values ranged from 0.8884 to 0.9693, which suggests that the model adequately predicted the responses obtained.

3.1. Mechanical properties

Fig. 1 shows the stress–strain curves for the film samples. In general, stress showed an important increase with strain for values of strain lower than 10%. After that, the stress increased at a lower rate, supporting high deformations until the break point, especially for samples containing XG amounts lower than 0.75 g/100 g slurry which presented a tendency to develop a plastic or fluency zone. Such pattern revealed a ductile behavior which is being modulated by the film formulation. Chillo et al. (2008) reported that the mechanical response of starch edible films can be modulated by the mixture of starch with different hydrocolloids like gums, cellulose derivatives or chitosan.

Table 2
Measured responses for Central Composite Design.

Run	TS (g/100 g slurry)	XG (g/100 g slurry)	YM (MPa)	σ_b (MPa)	ε_b (%)	SW (%)	WVP (g/m s Pa)	YI	Hunter scale		
									L	*a*	*b*
1	4.75	0.75	2.93	1.61	99.6	36.1	2.2E−09	10.5	90.8	−0.67	5.0
2	4.25	0.25	0.29	0.38	213.5	35.5	1.7E−09	6.4	94.1	−0.44	3.5
3	4.00	0.50	1.58	0.99	109.9	35.9	2.1E−09	12.1	87.6	−1.63	6.3
4	5.00	0.50	1.81	0.78	134.5	30.9	2.1E−09	13.8	85.7	−1.59	6.9
5	4.50	0.00	0.19	0.38	280.6	21.9	2.0E−09	4.1	94.4	−0.22	2.1
6	4.50	1.00	4.33	2.00	71.6	38.7	2.0E−09	17.5	85.0	−1.68	8.5
7	4.50	0.50	1.12	0.69	100.5	40.7	1.9E−09	8.8	92.6	−0.45	3.9
8	4.50	0.50	1.03	0.85	120.0	42.0	1.8E−09	8.2	95.9	−0.47	4.3
9	4.50	0.50	1.28	0.71	164.5	37.2	2.3E−09	8.7	91.2	−0.47	6.4
10	4.25	0.75	2.73	1.46	100.3	41.0	1.9E−09	14.1	85.6	−1.49	6.9
11	4.75	0.25	1.57	1.12	208.3	32.6	1.7E−09	8.9	89.0	−1.30	4.7

TS: tapioca starch. XG: xanthan gum. YM: Young modulus. σ_b: stress at break. ε_b: strain at break. SW: solubility in water. WVP: water vapor permeability. YI: yellow index. Hunter parameters *L*, *a* and *b*.

Table 3
Coefficients of second-degree polynomial function for different variables and ANOVA analysis.

Coefficient	YM (MPa)	σ_b (MPa)	ε_b (%)	SW (%)	YI	L
B_o	29.64*	2.9	−376.00	−499.72	304.27***	−336.17
Linear						
B_1	−15.80*	−2.43	289.57	234.37*	−146.44***	212.70*
B_2	18.97*	10.64*	−477.87**	88.89	115.48***	−178.98
Square						
B_{11}	2.06**	0.42	−31.56	−26.25*	17.75***	−26.04*
B_{22}	4.34**	1.64**	183.87	−38.74**	9.11**	−13.73
Interaction						
B_{12}	−4.29*	−2.37*	17.94	−7.98	−25.02***	40.95
Lack of fit (P)	0.0853	0.1178	0.8424	0.6643	0.0643	0.8810
F_{reg}	24.6	5.96	12.43	12.43	31.57	8.66
R^2	0.9609	0.8884	0.9256	0.9255	0.9693	0.8965

1: Starch, 2: xanthan gum.
*Significant at $P < 0.1$.
**Significant at $P < 0.05$.
***Significant at $P < 0.01$.
YM: Young modulus. σ_b: stress at break. ε_b: strain at break. SW: solubility in water. YI: yellow index. L: Hunter L parameter.

It could be seen (Table 3) that YM coefficients were all significant. Linear coefficients were the highest ones and were positive for XG, demonstrating that an increase in gum content raised the YM value while TS linear coefficient was negative. However, quadratic terms were positive and more significant than linear ones, showing an important curvature on the observed response. The interaction factor was negative suggesting that biopolymers in combination had an antagonistic effect. The statistically generated counter plot of YM response is shown in Fig. 2A. It can be seen that the lowest YM values were obtained at the experimental zone where biopolymer concentrations were lower ($4.0 < TS < 4.5$ g/100 g slurry and $0.0 < XG < 0.5$ g/100 g slurry). The YM response increased with the TS level, when the XG concentration was ≤0.25%. However, for a medium gum level (0.5 g/100 g slurry) it can be observed a significant reduction of YM as TS was increased from 4.0 to 4.5 g/100 g slurry. After that, for TS levels between 4.5 and 5.0 g/100 g slurry and for the same XG content, YM values raised up again. It is important to remark that when XG concentration was between 0.75 and 1 g/100 g slurry, this hydrocolloid clearly governed the YM response and TS had little influence. Flores et al. (2010) extruded edible films based on XG, TS and added with KS and observed that XG content increased elastic modulus values, suggesting that the gum imparted a more solid character to the film.

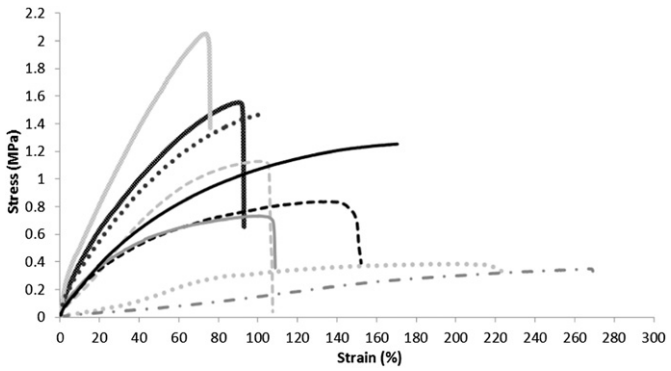


Fig. 1. Typical stress–strain curves for tapioca starch (TS)–xanthan gum (XG) based edible films of different formulations according to Central Composite Design. Composition expressed in g/100 g slurry: TS 4.75–XG 0.75; TS 4.25–XG 0.25; TS 4.00–XG 0.50; TS 5.00–XG 0.50; TS 4.50–XG 0.00; TS 4.50–XG 1.00; TS 4.50–XG 0.50; TS 4.25–XG 0.75; TS 4.75–XG 0.25.

For σ_b the XG linear and quadratic coefficients were positive and significant (Table 3), showing the relevant influence of this gum on the observed behavior; the interaction term indicated an antagonism. Fig. 2B shows the counter plot generated for σ_b. In general, the trends observed were similar to those obtained for YM. It showed a sharp increase of the σ_b with the XG rise and TS had a little effect on this response, especially at high gum concentration (>0.75 g/100 g slurry). For TS levels < 4.5 g/100 g slurry and XG < 0.25 g/100 g slurry there was not an important increase of that response with biopolymer concentration and the lowest σ_b values were obtained in that zone.

Considering ε_b response, it was observed that the gum linear coefficient was negative and significant (Table 3) while quadratic and interaction terms were not significant. It can be seen, on the counter plot of Fig. 2C, the high influence of XG on the ε_b response which showed to be almost independent of TS concentration: ε_b presented a reduction due to the incorporation of XG and the films constituted when XG was absent presented the maximum deformability.

In general, XG had the greatest influence in mechanical response of studied edible films. The XG presence enhanced film traction resistance but generated a less deformable matrix. Probably, gum and starch chains interacted through hydrogen bonds developing a more resistant network but with lower deformability due to the impossibility of polymeric chains to slide for maintaining the ductile behavior observed in the absence of gum. According to Veiga-Santos, Oliveira, Cereda, Alves, and Scamparini (2005) interaction between the XG and the starch might prevent the occurrence of amylose–amylose interaction.

3.2. Solubility in water

Solubility indicates film integrity in a water environment. When film is thought as a packaging material, a high water resistance or a low solubility is required. On the other hand, for simultaneous consumption of food and coating, a fast degradation or dissolution must be achieved (Maizura, Fazilah, Norziah, & Karim, 2007).

Table 3 shows that linear coefficients were positive and quadratic terms were negative, being all of them significant. Counter

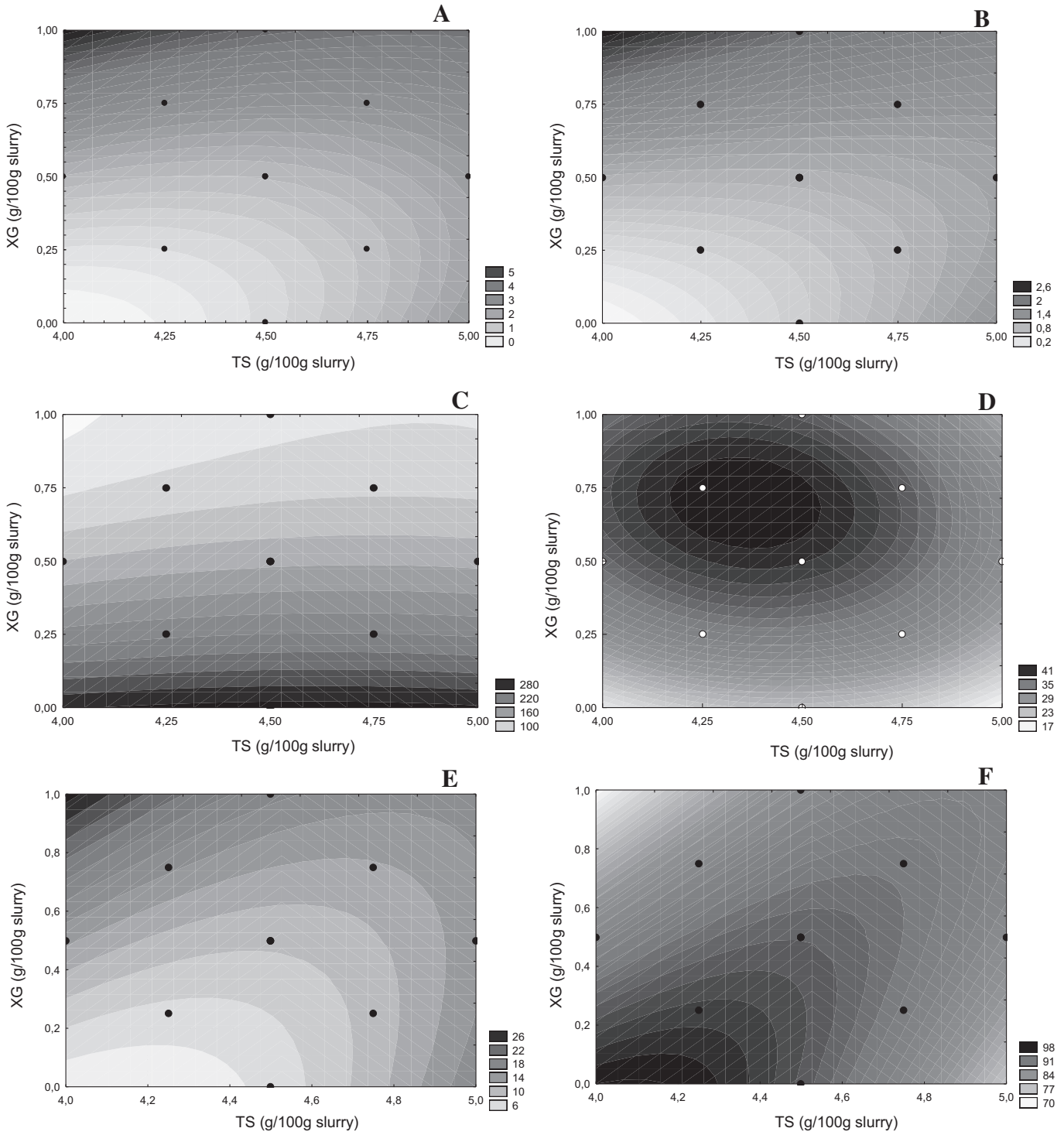


Fig. 2. Counter plot obtained for measured responses according to Central Composite Design. (A) Young modulus, (B) stress at break, (C) strain at break, (D) solubility in water, (E) yellow index, (F) *L* Hunter parameter.

plot on Fig. 2D shows a maximum solubility in the zone with 0.55–0.85 g XG per 100 g slurry and 4.10–4.60 g TS per 100 g slurry. For higher or lower levels of XG and TS it was observed a decrease in solubility value. There were obtained samples with lower SW in the experimental area with a low or null XG content. An important increase in SW was observed as XG levels were increased. Flores et al. (2010) reported that XG at levels of 4.25–10 g/100 g film contributed to raise the solubility of extruded starch films. It is important to remark that the interactions of XG, TS and glycerol might affect the formation of the starchy network determining the trends observed in the present research.

3.3. Water vapor permeability

The WVP measured values were all around 2×10^{-9} g/m s Pa (Table 2). Flores, Famá, et al. (2007a) reported WVP values of (0.6–

2.0) $\times 10^{-9}$ g/m s Pa for TS–KS films and Vásconez et al. (2009) reported values of 0.7 $\times 10^{-9}$ g/m s Pa for TS–chitosan–KS films. It was not observed a clear trend concerning the effect of XG and TS on WVP. Water vapor permeability depends on water vapor diffusivity (D) and water vapor solubility (S) in the matrices studied. Different compositions of the film forming slurries, probably affected the D and S values, modulating the WVP results.

The thickness of the studied films was ≈0.22 ± 0.04 mm.

3.4. Color evaluation

TS–XG films were slightly colored. Table 3 shows that all the coefficients for YI were highly significant. They indicated a positive influence of XG on the YI response. Quadratic terms showed an important curvature of the predictive function but they were one order lower than linear ones and, finally, the interaction factor was negative showing an antagonistic effect. Fig. 2E represents the counter plot obtained. It shows lower YI values at low TS and XG contents, and an increase of this response as XG concentration was higher, reaching a maximum value at 1 g of gum per 100 g of slurry. These results suggest that XG presence gave origin to more yellow films. TS also increased slightly the YI values when XG level was lower than 0.5 g/100 g slurry. However, for medium gum content (0.5 g/100 g slurry), it was also observed a reduction in YI when TS increased from 4.0 to 4.5 g/100 g slurry, and then an increase of this response when TS increased from 4.5 to 5.0 g/100 g slurry.

As can be observed in Table 2, a parameter was always negative (greenness range: −0.22 to −1.68). Regarding b parameter, it was highly influenced by the XG presence and it always took positive values (yellowness). In fact, in the absence of XG, b took the lowest value (2.1) and at the highest XG level, b parameter showed the highest value (8.5).

For L parameter, the linear and quadratic TS coefficients were significant (Table 3). The linear term was positive and the highest one, showing the great influence of TS on L values. As the model have a significant and negative contribution of TS quadratic term, a variation of starch content produced a response reduction with a noticeable quadrature. Fig. 2F shows the counter plot of L parameter. It could be appreciated that L showed the highest values for the lowest hydrocolloids concentration, showing an opposite trend to that observed for YI response. Moreover, for XG level of 0.5 g/100 g slurry and 4.0 < TS < 5.0 g/100 g slurry, L attained a maximum value for a starch concentration of 4.5 g/100 g slurry. According to Veiga-Santos, Suzuki, Cereda, and Scamparini (2005) the effect of xanthan gum, cassava starch and additives on Hunter L, a and b values can be attributed, in part, to the characteristic colors of such raw materials and to non-enzymatic browning reactions.

3.5. Optimal composition of tapioca starch-based edible film

In order to analyze the possibility of selecting one formulation which optimizes the desirable characteristics of studied edible films, some responses were considered: YM, ε_b, SW and YI. Table 4 summarizes the criteria employed to optimize each response individually and in an overall approach. As it can be observed, the goal was to maximize YM and ε_b and to minimize SW and YI in the range considered. The D values obtained from the desirability function procedure, were between 0.55 and 1 when each dependent variable was considered individually for optimization, indicating that it is possible to produce suitable films that reasonably satisfy the individual requirements from formulations used in the design. When desirability function was evaluated in a global way which means optimizing YM, ε_b, SW and YI simultaneously, D value was 0.81 which is highly satisfactory (Barros Neto et al., 2003). Therefore, it was obtained the composition of the most recommendable film formulation which comprises the use of 4.70 g TS per 100 g slurry and 0.25 g XG per 100 g slurry. Such formulation was very similar to run 11 (TS: 4.75 g/100 g slurry and XG: 0.25 g/100 g slurry) and, consequently, this run was selected for further characterization. No significant differences were found between actual and predicted responses ($P < 0.05$) except for YM for which the predicted value was significantly lower than the actual one (Tables 2 and 4). The other responses observed for run 11 (Table 2) showed a good σ_b, a standard WVP and an intermediate value of L, a and b.

Table 4
Optimization of tapioca starch–xanthan gum edible film formulation.

Response	Values				Predicted response	D^a
	Goal	Lower	Target	Upper		
YM (MPa)	Maximum	0.2	>1.6	3.0	0.97	0.55
ε_b (%)	Maximum	100	200	200	200.9	1
SW (%)	Minimum	30.0	<32.0	34.0	32.3	0.84
YI	Minimum	4.1	<8.2	12.0	8.2	0.93

YM: Young modulus, ε_b: strain at break, SW: solubility in water, YI: yellow index.
[a] D: Individual desirability value.

3.6. Further characterization of selected formulation

It was evaluated the efficacy of TS–XG films elaborated with selected formulation, as barriers against microbial contamination and their sorption characteristics.

Fig. 3 shows their antimicrobial response when a *Z. bailii* external contamination was simulated. Edible films containing KS reduced the yeast count in comparison with the control system (without KS). At short times (8 h), antimicrobial films presented 1 log cycle reduction in *Z. bailii* population. After that, and until 120 h of storage, yeast count showed a 2 log cycle increase. On the contrary, control films allow a fast microorganism development reaching, at the end of the storage, a 4 log cycles increase of yeast count. Therefore, for films containing sorbate, the counts remained 2 log cycles below the control system.

Moisture sorption behavior of films can be seen in Fig. 4. Gum addition to TS edible formulation, reduced the moisture uptake in all the a_w range evaluated, suggesting a lower affinity of TS–XG films for water vapor molecules. Both curves revealed a similar trend: a slight increment in the moisture value with the increase of a_w, until a value of ≈0.6; then, moisture content raised sharply between 0.75 and 0.97 a_w values. Data could be properly fitted to

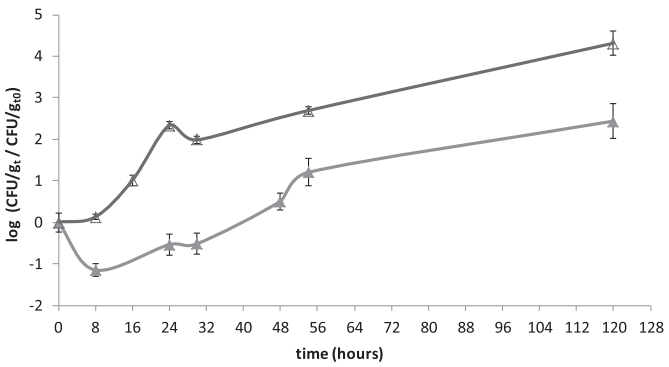
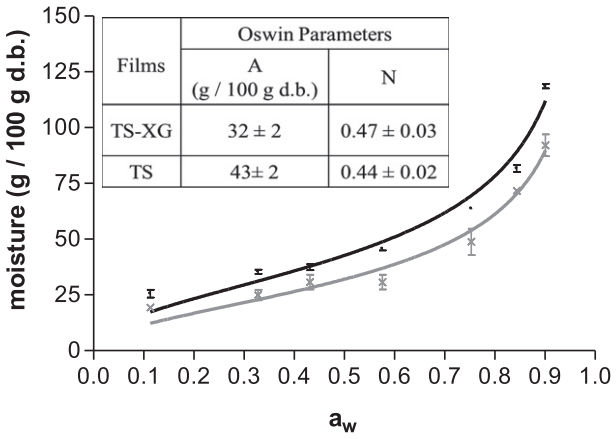


Fig. 3. *Z. bailii* growth in the surface of a film in contact with a semisolid medium of a_w 0.98 and pH 4.5. TS 4.75 g/100 g slurry–XG 0.25 g/100 g slurry: △ without KS; ▲ with KS. Vertical bars represent standard deviation of the mean ($n = 3$). Results are expressed as the log of the ratio between colony forming units per gram of film (CFU/g) at time t and at initial time t_0.



Films	Oswin Parameters	
	A (g / 100 g d.b.)	N
TS-XG	32 ± 2	0.47 ± 0.03
TS	43± 2	0.44 ± 0.02

Fig. 4. Water sorption isotherms (25 °C) of xanthan gum (XG)–tapioca starch (TS) based films: × TS 4.75 g/100 g slurry–XG 0.25 g/100 g slurry; ● TS 5.00 g/100 g slurry. Vertical bars represent standard deviation of the mean ($n = 3$). Dry basis: d.b.

the Oswin equation. The A parameter for XG free system was significantly higher ($P < 0.05$), indicating a higher tendency to water uptake for the TS films. However, *N* parameters were not different, suggesting a similar sigmoid shape. Probably, the moisture sorption behavior observed in this work is reflecting that the XG might interfere with amylose packing through the development of gum–starch hydrogen bonds which replaced interactions between starch chains (Yang & Paulson, 2000) and inhibited the formation of polymer–water hydrogen bonds in the amorphous areas.

4. Conclusions

The results revealed that XG had the most important influence on mechanical parameters, such as YM, σ_b and ε_b, producing more solid like matrices. Films with elevated XG content showed the highest SW and darkness.

Optimum formulation for edible films studied was proposed in terms of mechanical performance, solubility and color development; this formulation was very similar to one of the experimental runs. TS–XG selected film could control yeast growth when it was used to cover an acidified and a_w reduced semisolid food model, showing good antimicrobial barrier properties against a *Z. bailii* external contamination. Moisture sorption isotherm of selected film exhibited a reduced capacity for water sorption in comparison with TS films, probably due to interactions between both polysaccharides which resulted in reduced water vapor–biopolymer interactions.

The results reported in the present work, help to understand the effect of film composition on the physicochemical properties and to predict the potential usefulness of films for food preservation.

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