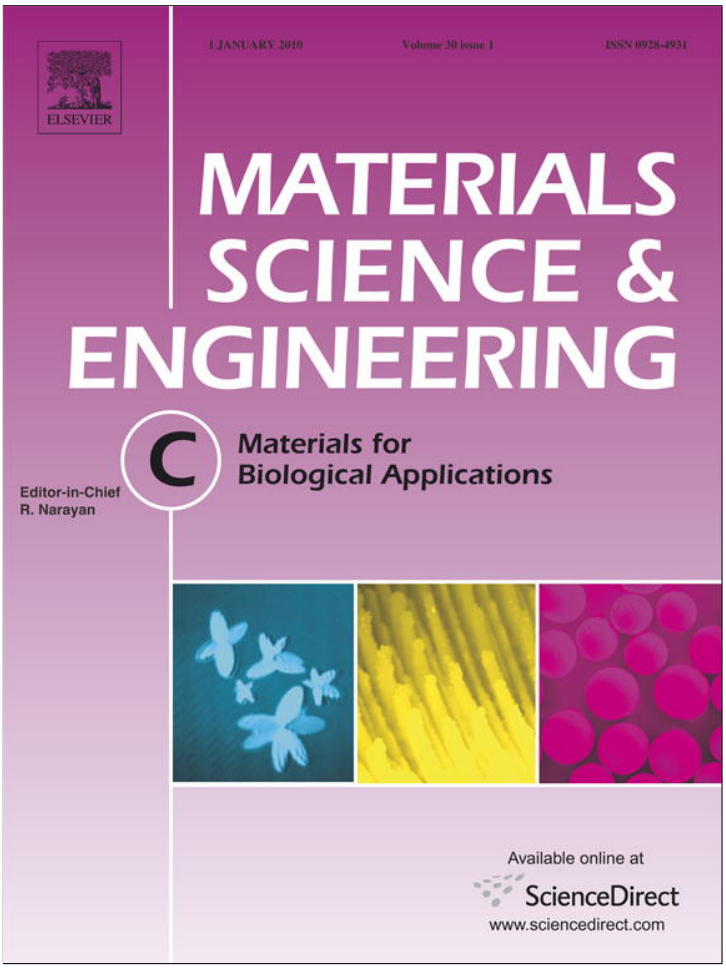

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Mixture design for evaluation of potassium sorbate and xanthan gum effect on properties of tapioca starch films obtained by extrusion

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ABSTRACT

A mixture experimental design was used to study the physical and microbiological properties of tapioca starch–glycerol based edible films added with xanthan gum (XG) and potassium sorbate (PS) and obtained by extrusion technology. The results showed that PS presence decreased the ultimate tensile strength and elastic modulus and increased strain at break. XG produced a reinforcing effect on the films and also enhanced solubility in water and decreased moisture content. The analysis revealed significant interactions between components in the mixture. The water vapor permeability values ranged from 3.72×10^{-10} to 6.4×10^{-10} g/m s Pa. The PS concentration was not affected by the extrusion process and the preservative was available to act as an antimicrobial agent. Extruded films made from biopolymers showed a potential application in food technology as an active packaging.

1. Introduction

Natural sources of biopolymers, commonly used in the food industry, have been tested as a raw material to manufacture new, environmentally-friendly and functional packaging. Extensive literature is available on renewable and biodegradable polymers useful to elaborate films and coatings with specific functionalities, such as the control of moisture, gases or lipid migration or the carrying of additives and/or nutrients [1–3]. Some of these food polymers include cellulose and derivatives [4,5], starches [6,7], gums [8], proteins [9] and more recently, chitosan [10,11].

The bibliography on filmmaking processes suggests that, probably, the casting technique is the normal procedure applied in research areas. However, taking into account the industry's need for high levels of film production, it is a must to develop other technologies that can fulfill this demand. Extrusion is one of the most successful technologies employed in food manufacturing because of its versatility to obtain different kinds of products, high levels of productivity with low costs, high speed and automation of production [12].

The extrusion processing of biopolymers (starch, gums, and proteins) involves a progressive compression of macromolecules and, because of the shearing and heating to high temperatures, a gradual loss of crystallinity is produced when it previously existed, due to the fact that the biopolymer melts to form a hot amorphous and thermoplastic mass. Thermal gelation or coagulation is essentially the approach used for film formation through the heating of the macromolecule mass which ultimately results in denaturation, gelation and precipitation [2]. Substantial damage or depolymerization of the molecules occurs during the process and it affects the original properties of the raw material [13]. Sereno et al. [14] reported that xanthan gum radically improves its dispersibility and rheological behavior in comparison with non-extruded xanthan gum, as a consequence of the melting and alignment of macromolecules. Regarding starch extrusion, the influence of extrusion processing variables on the product quality of starch based products has been studied extensively. Thymi et al. [15] reported that extruded product apparent density, porosity and expansion ratio were found to be dependent on feed moisture content, residence time and temperature while they were not affected by screw speed. In another paper, Wójtowicz and Mościcki [16] established that the different moisture content of the raw material and the different screw speed influenced the functionality, microstructure and sensory characteristic of precooked pasta-like products. In addition, Chaudhary et al. [17] reported that the maize starch structural properties (variations in the ratios of amylose and amylopectin) and starch modification (hydroxypropylated starch) had a statistically significant effect on parameters such as torque, die pressure and specific mechanical energy.

While literature concerning the production of edible films through casting is very abundant [1,3,4,6,8,9], the bibliography reporting the use of extrusion is limited and includes the study of optimal setting parameters

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to obtain suitable extruded films and to understand how the process affects the film's physical and functional properties [2,18] and, more recently, molecular structure and morphology of thermoplastic starch [19].

Tapioca starch is widely produced in Latin America, being popularly used as a meal, or cooked and eaten as a vegetable. Tapioca starch is used to a lesser extent than other starches, like corn, in the food industry [20]. At any rate, its importance as a source of starch is growing rapidly in many applications including the development of edible films [6]. Starch based films are reported to be transparent, odorless, tasteless, semi-permeable to CO_2 and resistant to O_2 passage [21]. However, the application of starch based films is limited by their solubility in water as well as their poor water vapor barrier characteristics.

In order to improve the film's physical and functional properties, biopolymer blending has been proposed [22]. Xanthan gum is largely the most important commercial microbial hydrocolloid used in the food industry as a thickening agent and stabilizer. Its incorporation into the starch based film elaboration has proven to affect some properties of the films such as elongation at break and water absorption [23].

In recent years, active packaging technologies with the main emphasis on antimicrobial applications have been especially considered [24]. Particularly, antimicrobial films and coatings have been established as an efficient alternative for controlling food contamination [25,26]. Sorbates (sorbic acid and its potassium salt) are considered GRAS (generally recognized as safe) additives, being among the most used antimicrobials, particularly because they are active against yeasts, molds and many bacteria [27]. Flores et al. [28] analyzed the performance of tapioca starch based films as carriers of sorbates and established that films were effective in controlling the growth of *Z. bailii* population, acting as a preservative release agent or as a barrier for external yeast contamination.

Mixture experimental designs are suitable for the study of those products that involve more than one ingredient, since proportions of the components in the mixture and their levels are dependent on each other [29,30]. This technique allows one to obtain a predictive mathematical representation of the relationship between mixture factors and responses. In general, a special cubic model is satisfactory to describe the additive terms and the interactions among components [31]. These models are further applied in science, engineering and industry.

The objective of the present work was to study the influence of xanthan gum and potassium sorbate incorporation on the physical and antimicrobial properties of extruded glycerol plasticized-tapioca starch films using a mixture experimental design.

2. Materials and methods

2.1. Ingredients

Edible films were prepared from mixtures of tapioca starch (Indemil Indústria e comércio Ltda., PR, Brazil), glycerol (Dinámica, Brazil), xanthan gum (Keltrol® RD, Kelco, Brazil) and potassium sorbate (Sigma, St Louis, Missouri, USA).

2.2. Experimental design

A mixture experiment is a special type of experimental design methodology in which factors are the proportions of components in a mixture and their levels are not independent of each other since the following restriction has to be verified: the sum of the proportions of the mixture components is always 1. For a mixture of q components:

$$\sum_{i=1}^{q} x_i = x_1 + x_2 + \ldots\ldots + x_q = 1 \quad (1)$$

where x_i represents the proportion of ith component in the mixture.

A three-component constrained mixture design with two overall centroid point replications (Table 1) was used to study the effects of interactions between ingredients on the physical and microbiological performance of the films. The variables studied were the concentrations of tapioca starch–glycerol blend (S–G) with a fixed proportion of 82:18 (starch:glycerol), potassium sorbate (PS) and xanthan gum (XG). The proportion of starch:glycerol used was determined by running preliminary assays and selecting that relation that allowed films to be obtained that were neither too rigid nor too sticky. The constrained limits of each component were determined in the preliminary assays taking into account the characteristics of the extruded films obtained in terms of flexibility, homogeneity and stickiness. The minimum and maximum levels of each mixture component were as follow: S–G (89.0–99.0%), PS (1.0–4.0%) and XG (0.0–10.0%). The pseudocomponents were calculated as: $P_{S-G} = C_{S-G} - 0.89/0.10$; $P_{PS} = C_{PS} - 0.01/0.10$; $P_{XG} = C_{XG} - 0.0/0.10$, where P_i = pseudocomponent and C_i = real concentration of component. The measured responses for film characterization were the ultimate tensile strength (UTS), strain at break (SB), elastic modulus (Ec), solubility in water (S), water vapor permeability (WVP), yellow index (YI) and antimicrobial activity (AA).

Table 1
Three component constrained mixture design.

Assay	Component proportion			Pseudocomponents		
	S–G	PS	XG	S–G	PS	XG
1	0.99	0.01	0.00	1	0	0
2	0.89	0.01	0.10	0	0	1
3	0.96	0.04	0.00	0.7	0.3	0
4	0.89	0.04	0.07	0	0.3	0.7
5	0.975	0.025	0.0	0.85	0.15	0
6	0.89	0.025	0.085	0	0.15	0.85
7	0.94	0.01	0.05	0.5	0	0.5
8	0.925	0.04	0.035	0.35	0.3	0.35
9	0.9325	0.025	0.0425	0.425	0.15	0.425
10	0.9325	0.025	0.0425	0.425	0.15	0.425
11	0.9325	0.025	0.0425	0.425	0.15	0.425

S–G: tapioca starch–glycerol blend; PS: potassium sorbate; XG: xanthan gum.

2.3. Extrusion process

The S–G, PS and XG mixtures were prepared, according to the experimental design (Table 1), by gently mixing the solid powders using a manual mixer device for 5 min. No extra water was added.

The preliminary extrusion assays were performed in order to select convenient operation parameters (i.e.: temperature profile, screw speed) that allowed the suitable films to be obtained, thereby minimizing bubble formation and thickness.

In the first step, pellets were produced from the dry mixtures of S–G, PS and XG in a single-screw extruder (model EL-25, BGM Industria e Comércio Ltda., SP, Brazil). The screw dimensions were 25 mm in diameter (D) and the overall active length of 700 mm (28D). The screw speed was 30 rpm and the barrel zone temperature profile was set at 120/120/120/115 °C from the feeding zone (zone 1) to the die zone (zone 4). The extruded strands were obtained using a pellet die (3 mm in diameter) and cut into pellets with a cutting device.

In the second step, a controlled temperature film blowing die (50 mm in diameter), which required two extra zone temperature settings: zone 1 (125 °C) and zone 2 (130 °C), was connected to the barrel exit. The pellets were loaded into the extruder (feed speed ≅ 2 kg/h) at a screw speed of 35 rpm. The previous barrel temperature profile was not modified. The material was extruded through a film blowing die in order to obtain stable "film balloons". The air pressure blown into the "film balloons" was adjusted to get films as thin as possible in a stationary process (Fig. 1). The blow-up ratio (diameter of blown balloon/diameter of the die) was, in general, around a value of two.

Once constituted, the films were conditioned at 25 °C, over a saturated solution of NaBr (water activity, $a_w = 0.576$) for 7 days

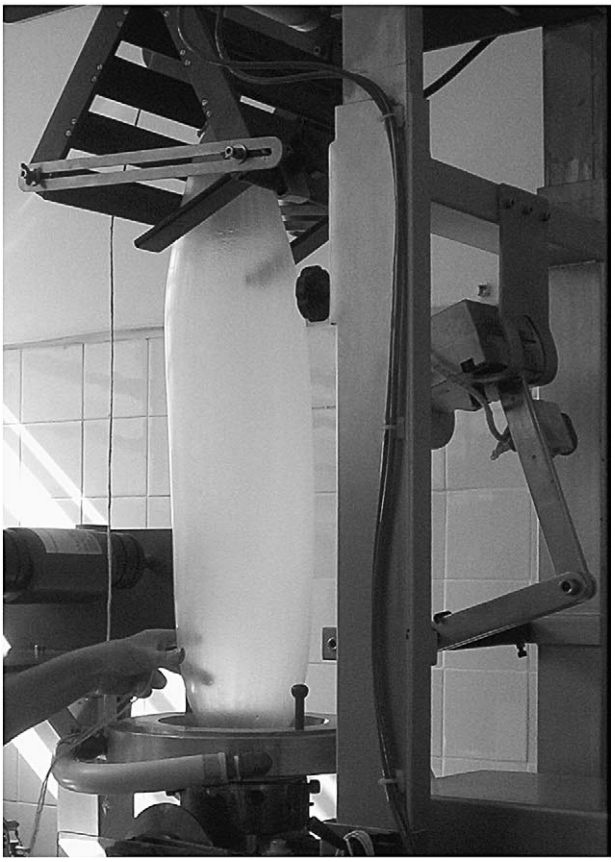

Fig. 1. Extruded tapioca starch based film production.

before testing. The film thickness was measured, at least in three different locations of each specimen, using a micrometer (Mitutoyo Corporation, Japan) with a precision of 0.001 mm.

2.4. Mechanical properties

The tensile load (N) and extension (mm) profile of the preconditioned films were measured using a TA-XT2i texture analyzer (Stable Micro Systems Ltd, UK). The tested filmstrips (6×150 mm) were cut and mounted between the grips of the machine. The initial grip separation and crosshead speed were set at 100 mm and 0.8 mm/sec, respectively. The maximum load and extension at break were recorded and used to calculate the UTS, (kPa) and the SB (%). The load and extension data were used to generate true stress (kPa) vs. true strain curves of the tested films [32]. The elastic modulus was evaluated from the initial slope of the true stress–true strain curve obtained from data fitting to the following exponential equation [33,34] which is adequate to model results from large deformation assays:

$$\sigma_T(\varepsilon_T) = Ec \cdot \varepsilon_T \cdot \exp(\varepsilon_T \cdot K) \quad (2)$$

where σ_T and ε_T are the true stress and the true strain, respectively; Ec is the elastic modulus; K is a constant value, regarded as a fitting parameter.

Nine specimens were tested for each sample, both in the flow and in the perpendicular direction.

2.5. Solubility in water

Solubility in water is defined [35] as the percentage of the dry matter in the film solubilized after 24 h of immersion in distilled water. The initial percentage of the dry matter was determined by drying 2 cm diameter disks in a vacuum oven at 100 °C for 24 h. Other disks were cut, weighed and immersed in 50 mL of distilled water, with periodic stirring, for 24 h at 25 °C. Non-solubilized films were taken out and dried at (100 °C for 24 h) to determine the final weight of the dry matter. Solubility is reported as the difference between the initial and final dry matter with respect to the initial dry matter.

2.6. Color evaluation

The film disks of an appropriate diameter were rested on a standard white background [36]. Measurements were performed with a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan) using a 1.5 cm diameter aperture. The exposed area was sufficiently great with regard to the illuminated area to avoid any light trapping effect. The yellow index (YI) was measured according to an ASTM E1925 standard test method [37], in at least five positions randomly selected for each sample. Standard values considered were those of the white background. Calculations were made for the C illuminant and 2° observer.

2.7. Water vapor permeability

Water vapor permeability (WVP) of films was determined gravimetrically at 25 °C using a modified ASTM [38] procedure. The permeation cell (acrylic cups) had an internal diameter of 4.4 cm and an external diameter of 8.4 cm. They were 3.5 cm deep and contained $CaCl_2$ (0% RH; 0 Pa water vapor partial pressure). The film was located between the cell and its acrylic ring shaped cover which was adjusted to the cup with four screws placed in the shape of a cross. A 10 mm air gap was left between the film and the $CaCl_2$ layer. Rubber o-rings and vacuum grease helped to assure a good seal. The covered cell was placed in a temperature and relative humidity (RH) controlled chamber (Ibertest, Spain) maintaining a temperature of 25 °C and a RH of 70% (≅2300 Pa water vapor partial pressure). After 20–24 h, the stationary water vapor transmission rate was attained and, from that moment on, changes in the weight of the cell (to the nearest 0.1 mg) were recorded daily over a 6-day period. All tests were conducted, at least, in duplicate and the WVP values were calculated using the WVP correction method [39].

2.8. Moisture determination

The samples were dried in a vacuum oven at 70 °C until reaching a constant weight. The determination was performed on three film specimens of each formulation. The results were expressed on a dry basis (db) and the average is reported.

2.9. Potassium sorbate dosage

The PS content was measured according to the AOAC [40] oxidation method which includes steam distillation followed by oxidation to malonaldehyde and measurement at 532 nm of the pigment formed between malonaldehyde and thiobarbituric acid. Four determinations for each specimen were performed.

2.10. Antimicrobial activity

The effectiveness of the films acting as a barrier to yeast contamination of high a_w products was studied. The antimicrobial activity of the films was evaluated using as an indicator *Zygosaccharomyces bailii*, a well-known spoilage yeast due to its resistance to preservatives [41].

2.10.1. Inoculum preparation

A *Zygosaccharomyces bailii* NRRL 7256 inoculum was prepared in Sabouraud broth at 25 °C until an early stationary phase was achieved (24 h).

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

In order to study the performance of the films to prevent the microbial contamination of a high water activity (a_w) product, Sabouraud agar with a_w depressed to 0.980 by the addition of glucose and pH adjusted to 4.5 with citric acid (5.2 mol/L) was formulated to resemble that kind of products. Disks of 1 cm diameter were aseptically cut from films and applied on the surface of the agar. Then, 10 μL of a culture of *Z. bailii* containing approximately 7×10^6CFU/mL (colony forming units per milliliter) were seeded onto the film disks. The samples were incubated at 25 °C for 48 h.

At selected times, two disks were sampled and suspended, each one, in 1 mL of peptone water (Biokar Diagnostics, Beauvais, France) placed into a short glass tube (16×100 mm) and shaken for 2 min at 2500 rpm with a vortex, previous to enumerating *Z. bailii* populations. Results were expressed as antimicrobial activity which is defined as the log of the ratio between (CFUt/g film) and (CFUt_0/g film), t being the time at which the disks were sampled and t_0 being the initial time.

2.10.3. Enumeration of Z. bailii

For all assays performed, the *Z. bailii* population was enumerated by surface plating on Sabouraud agar and incubation at 25 °C for 5 days prior to counting.

2.11. Data analysis

Data from the physical and microbiological assays were used to calculate the special cubic equation for three components:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3$$

where Y is the measured response, βs are parameters estimated for each linear and cross-product term of the model and X_1, X_2 and X_3 represent levels (expressed as pseudocomponents) of S–G, PS and XG, respectively. The positive values for the binary coefficients β_{ij}, indicate synergistic effects while the negative values represent antagonism. The analyses of variance to examine the significance of the data fitting to the model and triangular contour plots generated from polynomial equation for each response were accomplished using the software Statistica 6.0 for Windows (StatSoft Inc., USA, 2001).

3. Results and discussion

The selected conditions for the extrusion process allowed the films to be obtained based on tapioca starch–glycerol, potassium sorbate and xanthan gum (Fig. 1). The films were flexible and even homogeneous with a slightly varied light brown tone depending on formulation. It must be mentioned that the initial stickiness of the extruded films did not make it possible to roll because of the films' high temperature after emerging from the die. This problem was overcome by maintaining the film balloon blown until it cooled to room temperature. After this, films could be easily manipulated to obtain even sheets. Thunwall et al. [18] also reported a sticky appearance for the extruded films based on the potato starch containing glycerol. This was, in part, attributed to the hydrophilic character of the starch material and to the possible migration of the plasticizer to the film surface.

The moisture content of the conditioned extruded films ranged from 13.4 to 15.5 % (w/w, db) and, in general, there were no significant differences between formulations ($p>0.05$). This moisture range was slightly greater than native tapioca starch moisture (13% w/w, db). Highest moisture values (15.4 and 15.5% w/w, db) were observed for samples with high proportion of PS and without or with a low quantity of XG (assays 3 and 8, Table 1). It has been reported [42] that PS increases the moisture content of tapioca starch based films obtained by using the casting technique in relation to a control system without PS. It could be proposed that a PS modified starch network structure allows more interactions between water molecules and HO– positions in starch chains. On the other hand, mixtures 2, 4 and 6 (Table 1) which had the highest XG proportions, presented lower moisture values (14.6, 13.8 and 13.6% b.s. respectively) suggesting that XG might interact with starch by means of hydrogen type bonds, blocking hydroxyl positions capable of association with water. This kind of interaction between XG and starches was previously mentioned by other authors [43,44].

Table 2 shows measured responses obtained throughout the mixture experiment. Table 3 summarizes the best adjusted models ($p<0.01$) for measured responses of the UTS, SB, Ec (both in the flow and perpendicular directions), S and YI. It can be seen that all these developed models had no significant lack of fit ($P>0.01$) and that the adjusted regression coefficient (R^2_{adj}) ranged from 0.8074 to 0.9892, which suggests that the models adequately predicted the responses that were studied.

For WVP and AA it was not possible to adjust a model that satisfactorily described the responses. Probably, this trend could be attributed, in the case of WVP, to the very similar values ($p>0.05$) obtained for different mixtures studied. On the contrary, AA data developed a quadratic model with p and R^2_{ad} values (0.0019 and 0.8085, respectively) but the lack of fit was significant ($P>0.000025$).

3.1. Mechanical properties

Film mechanical characterization by traction assays gives valuable information about its flexibility and strength. This information is useful to predict potential applications of the films. Table 3 shows the

Table 2
Measured responses for mixture experimental design.

Assay	Measured responses									
	UTS×10^{-3} (kPa)		SB (%)		Ec×10^{-3} (kPa)[a]		S (%)	WVP×10^{10} (g/m s Pa)	YI	AA log (UFC$_t$/UFC$_{t0}$)
	Flow	Perpend.	Flow	Perpend.	Flow	Perpend.				
1	1.4	1.1	71	68	13	14	48	4.9	23	1.53
2	3.0	3.0	19	20	113	137	60	6.1	19	2.20
3	1.0	0.8	78	96	2	2	54	6.4	23	0.26
4	1.4	1.1	55	64	15	11	64	6.2	25	−0.43
5	1.4	1.0	85	97	7	4	50	4.8	27	−0.06
6	2.4	2.0	41	33	53	57	64	4.0	27	2.03
7	1.8	1.9	46	78	23	32	47	3.7	21	1.58
8	1.2	1.0	73	70	6	6	59	6.0	25	−0.37
9	1.3	1.5	52	67	22	25	45	6.7	28	0.10
10	1.2	1.1	67	101	1	6	50	4.5	26	0.15
11	1.7	1.3	51	65	24	19	49	5.9	24	0.19

UTS: ultimate tensile strength; SB: strain at break; Ec: elastic modulus; S: solubility in water; WVP: water vapor permeability; YI: yellow index and AA: antimicrobial activity.
[a] The goodness of the fit (R^2) to eq. (2) was always higher than 0.97.

Table 3
Best adjusted models and goodness of fit obtained for mixture design.

Response	Model	*p*	Lack of fit (*P*)	R^2_{adj}
UTS flow dir.	Y = 1401 S–G − 2026 PS + 3009 XS + 3488 S–G*PS − 1924 S–G*XG	0.0004	0.8672	0.9208
UTS perp. dir.	Y = 1007 S–G − 3227 PS + 2870 XG + 4800 S–G*PS	0.00008	0.6350	0.9243
SB flow dir.	Y = 69 S–G + 123 PS + 21 XG	0.00007	0.0777	0.9168
SB perp. dir.	Y = 73 S–G + 164 PS + 17 XG + 130 S–G*XG − 746 S–G*PS*XG	0.0012	0.1824	0.9258
Ec flow dir.	Y = 13138 S–G − 25684 PS + 111456 XG − 149532 S–G*XG − 274375 PS*XG + 580788 S–G*PS*XG	0.00015	0.3054	0.9868
Ec perp. dir.	Y = 12051 S–G − 25997 PS + 134773 XG − 164686 S–G*XG − 374859 PS*XG + 609559 S–G*PS*XG	0.0001	0.5478	0.9892
S	Y = 47 S–G + 74 PS + 60 XG − 38 S–G*XG	0.0008	0.4860	0.8491
YI	Y = 23 S–G − 85 PS + 19 XG + 155 S–G*PS + 181 PS*XG	0.0056	0.9672	0.8074

S–G (tapioca starch–glycerol blend), PS (potassium sorbate), XG (xanthan gum) expressed as pseudocomponents.
UTS: ultimate tensile strength; SB: strain at break; Ec: elastic modulus; *S*: solubility in water; YI: yellow index.

best adjusted models for the UTS in the flow and perpendicular direction. For these responses S–G and XG presented positive linear coefficients while PS coefficients were negative, indicating a matrix reinforcing effect of the biopolymers and a plasticizing effect of the preservative. A similar plasticizing behavior of the antimicrobial was reported by Flores et al. [6] who observed that the PS presence reduced the storage and loss modulus of starch based films using the casting technique in comparison with films without a preservative. A synergistic effect of S–G*PS affected the two responses while the antagonist interaction S–G*XG influenced only UTS in the flow direction. Fig. 2 A illustrates the contour plot for the UTS in the flow direction. It can be seen that high PS proportions contributed to decrease the UTS value, while a high XG proportion increased it. A similar trend was observed for the UTS in the perpendicular direction but generally the values were lower (Table 2).

SB in the flow direction model was the simplest equation developed since only linear terms were significant. The coefficients of S–G, PS and XG were positive (Table 3). PS showed the highest coefficient and, consequently, had a more pronounced influence on SB responses. It was observed that SB increased when the PS concentration was higher and also for lower XG proportions (Fig. 2B), giving more evidence of the preservative plasticizing effect and of the gum reinforcing character. Table 3 also shows that the model for SB, in the perpendicular direction, resulted in being even more complex. One positive term of binary (S–G*XG) interaction, and a negative term of ternary interaction became significant. To better understand the response predicted by the model, the contour plot should be analyzed (Fig. 2C). It can be observed that in a similar manner to the one shown for SB in the flow direction, the SB value was lower whereas the XG concentration increased. A similar trend was reported by Veiga-Santos et al. [23], who observed a significant and negative effect of the XG on elongation at break of cassava starch based edible films. Such effect could be explained through the proposal of an interaction between the gum and the starch, which prevented the occurrence of amylose–amylose interaction. Fig. 2C also shows that PS contributed to an SB increase.

The Ec in a flow and perpendicular directions showed similar predictive models (Table 3). The S–G and XG linear terms were positive and the PS term was negative. XG presented the highest coefficient for the linear ones and therefore had a greater impact on the Ec results. The coefficient of interaction terms of S–G*XG and PS*XG were negative (antagonist effect). Finally, a positive triple interaction coefficient was significant for both models. Fig. 2D shows the contour plot of the Ec in the flow direction. The XG content increased the Ec values, suggesting that the gum imparted more a solid character to the film. Chaisawang et al. [44] concluded, from dynamic rheological measurements, that interactions occurred in the tapioca starch–XG mixture resulting in a decrease in the loss tangent as compared with starch alone.

According to the results there were differences in the mechanical behavior observed parallel to and perpendicular to the flow direction of the extruded films. It could be seen that the UTS was, in general, lower in the perpendicular direction and the SB, contrarily, was higher. On the other hand, the Ec did not show a clear tendency (Table 2). This anisotropy has been widely reported for synthetic polymers and also, recently, for films based on modified potato starch–glycerol using the air blown technique [18]. During the extrusion process a laminar flow was generated through the die and the biopolymer molecules flowed in straight lines with variations in flow velocity between the adjacent layers of the fluid (shear rate). This is a significant factor considering the large and asymmetric molecular shapes of amylose, amylopectin and xanthan gum. Under such process conditions, big molecules suffer alignment in the laminar flow field and it can result in strain and mechanical depolymerization or fracturing [13].

3.2. Solubility in water

Solubility in water (*S*) is of major importance since it could condition the actual uses of the films in some technological situations. The mixture experiment procedure indicated that *S* followed a quadratic predictive model (Table 3); all linear coefficients were positive. The unique significant interaction term was S–G*XG (antagonism). Probably gum–starch associations [45] interfered in the formation of the starchy network; due to the fact that the resulting structure was more sensitive to water. The samples with lower *S* were obtained in the experimental area with a low PS proportion and high ratios of S–G/XG (Fig. 2 E).

3.3. Color evaluation

The YI parameter is a measurement of the color of the film that is extremely significant when considering consumer acceptance. Therefore, it would be desirable for the YI value not rise too much in order to maintain the product organoleptically acceptable. It is also important to remark that the extrusion process rendered brown pellets as well as films from white starch and slightly yellow XG powder. Evidently, cooking film components within the extruder impaired color attributes. The adjusted model presented one negative independent term (PS) and two positive interaction terms (S–G*PS and PS*XG) with the double interaction coefficients presenting the highest values. These results stated that PS, in combination with biopolymers exerted a synergistic, and one of the most significant effects on the YI parameter. The contour plot for the YI (Fig. 2F) shows that higher responses occurred in the experimental zone where biopolymer proportions were predominant.

3.4. Water vapor permeability

Unfortunately, it was not possible to obtain a model capable of predicting this response. The WVP measured values ranged from 3.72 to 6.40×10^{-10} g/m s Pa (Table 2) and were similar to those reported for tapioca starch edible films (6.1×10^{-10} g/m s Pa) [6] using a similar

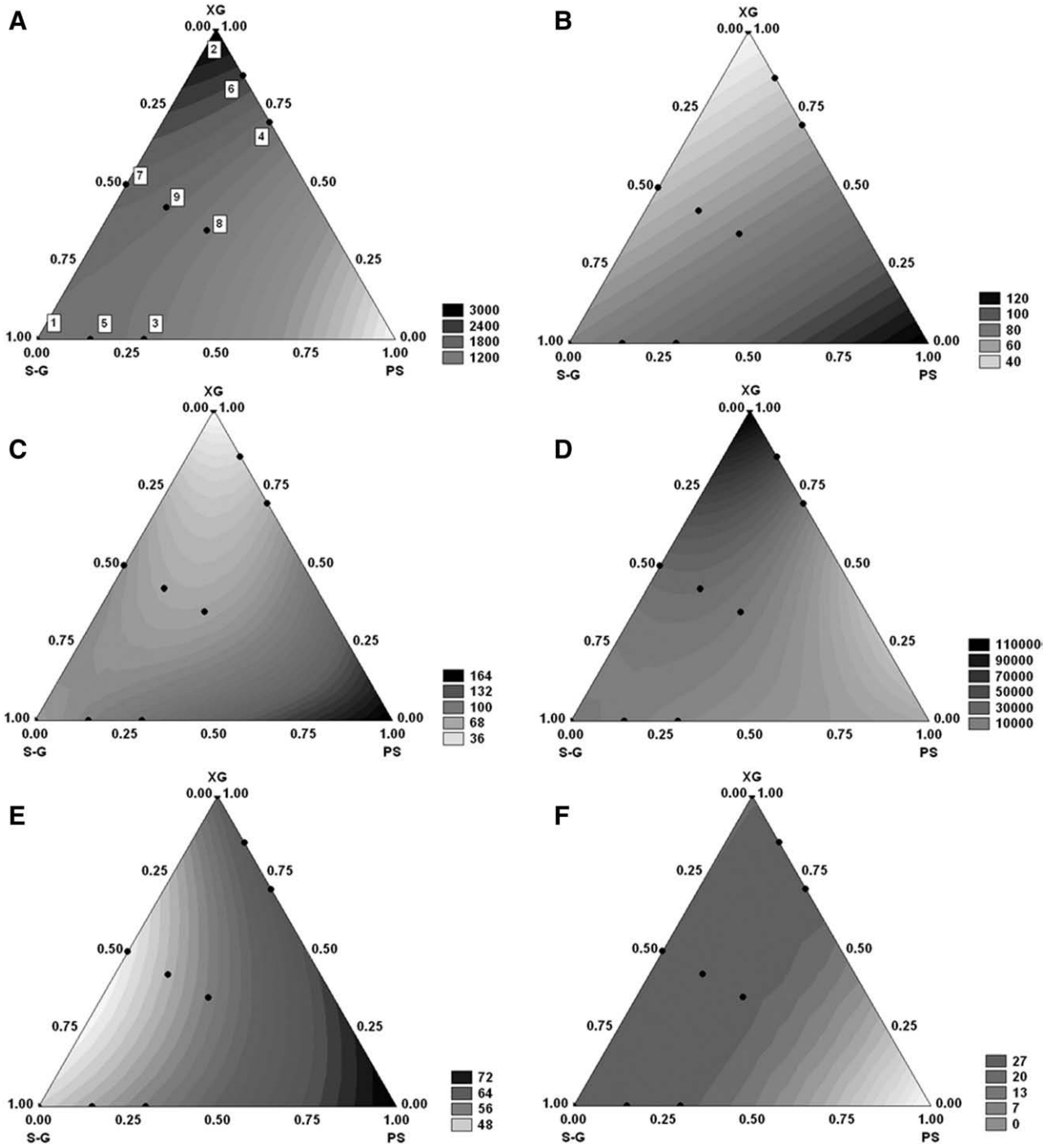

Fig. 2. Mixture contour plots for: A: ultimate tensile strength, kPa (measured in flow direction); B: strain at break, % (measured in flow direction); C: strain at break, % (measured in perpendicular direction); D: elastic modulus, kPa (measured in flow direction); E: solubility in water, %; F: yellow index. The points on the plots are the component proportions in the mixtures expressed as pseudocomponents. The numbers inside rectangles are the mixture label according to Table 1.

technique of measurement. The thickness of the extruded films was around 0.49 ± 0.11 mm.

3.5. Antimicrobial activity

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

As previously stated, the extrusion did not affect the PS content. Fig. 3 shows the effect of PS incorporated in some films on the inhibition of *Z. bailii* inoculated at the surface of the film disks. A semisolid food model covered with the films from mixtures with a lower PS proportion, assays 1, 2 and 6 (Table 1) suffered a 1.5, 2.2 and 2.0 log cycle increase of yeast counts after 48 h of storage. On the contrary, the films containing higher levels of PS, like those of assays 8 and 4 (Table 1), exerted the most pronounced antimicrobial action since yeast population remained in the lag phase. This assay demonstrated that sorbates were bioavailable to act as a barrier for external yeast contamination, when the film was in contact with an acidified high water activity (a_w) product (Sabouraud agar with a_w depressed to 0.98 with the addition of glucose and a pH adjusted to 4.5 with citric acid). It can be seen in Table 2 that XG had a negative effect on the PS antimicrobial action since; in general, cell counts were higher when xanthan gum was present for mixtures with a similar PS content. This effect was more evident for low preservative proportions. According to results obtained, the films studied could have

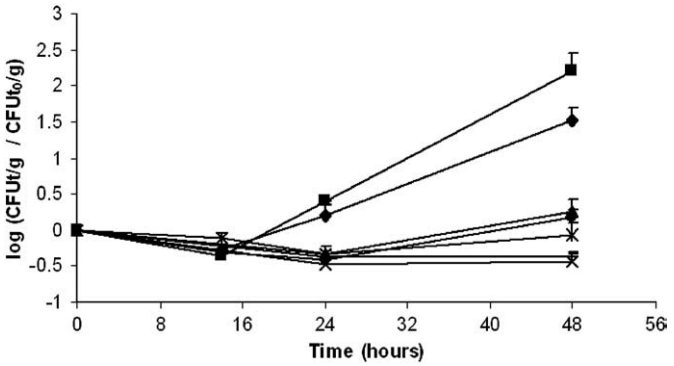

Fig. 3. *Z. bailii* growth in the surface of a film in contact with a semisolid medium of a_w 0.98 and pH 4.5. ♦ mixture 1; ■ mixture 2; ▲ mixture 3; ×mixture 4; ∗ mixture 5; | mixture 8; ● mixture 9. Vertical bars represent standard deviation of the mean ($n = 3$).

a potential application as an active packaging in relation with their protective action to prevent microbial spoilage.

4. Conclusions

The extrusion technology was applied to produce tapioca starch based edible films using glycerol as a plasticizing agent and adding xanthan gum and potassium sorbate. The obtained films were flexible, homogeneous and with potential antimicrobial action. The results are an important achievement in relation to the potential production of biodegradable packaging using biopolymers as a raw material.

The mixture experimental design was effective in obtaining mathematical models to predict response values and, as a consequence, it was a helpful tool to better understand the influence of different variables on properties of extruded films. Changes in the proportions of film components affected the physical and antimicrobial properties of the films. The PS presence was important for the decrease of the UTS and Ec and for the increase of SB. XG produced a reinforcing effect in the films raising the UTS and Ec and lowering the SB values. The gum (xanthan gum) also increased *S*, decreased moisture content and showed significant interactions with other components in the mixture. It was not possible to find a suitable model to predict the WVP responses, which measured values ranged from 3.72 to 6.4×10^{-10} g/m s Pa. The PS concentration was not affected by the extrusion process and the preservative was available to act as an antimicrobial while contained in the film. XG seems to have a negative effect on antimicrobial activity of the films.

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