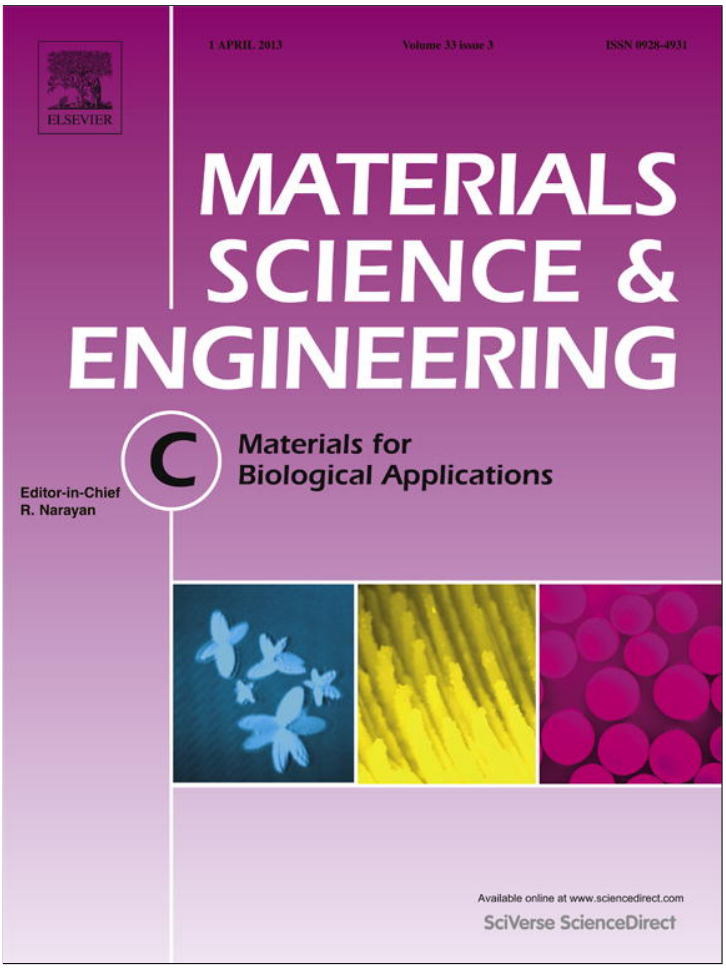


Materials Science and Engineering C 33 (2013) 1583–1591

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Potassium sorbate controlled release from corn starch films

Olivia V. López [a,*], Leda Giannuzzi [a], Noemí E. Zaritzky [a,b], M. Alejandra García [a]

[a] CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CONICET, 47 y 116 S/N°, La Plata (B1900AJJ), Buenos Aires, Argentina
[b] Depto de Ingeniería Química, Facultad de Ingeniería, Universidad Nacional de La Plata, La Plata (B1900AJJ), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 1 October 2012
Received in revised form 20 November 2012
Accepted 17 December 2012
Available online 23 December 2012

Keywords:
Active corn starch films
Potassium sorbate
Controlled release
Mathematical modeled
Antimicrobial capacity

ABSTRACT

Active starch films with glycerol and potassium sorbate were obtained by casting. Native and acetylated corn starches, as well as the mixture of them in equal proportions were used and filmogenic suspensions with pH 4.5 were also prepared. Sorbate concentration decreased during film storage due to its oxidative degradation. Active films resulted more yellow and less transparent than films without sorbate. The minimum inhibitory concentration of sorbate resulted 0.3%, regardless of the starch type and the formulation pH. The use of antimicrobial package was more effective to prevent microbial growth on food surfaces than the use of conventional methods. Additive kinetic release was neither affected by the starch type nor by the formulation pH. Sorbate diffusion process was mathematically modeled satisfactorily. Active films were able to inhibit *Candida spp.*, *Penicillium spp.*, *S. aureus* and *Salmonella spp.* growth. Active films extended 21% the shelf life of refrigerated cheese, regardless of the formulation pH.

1. Introduction

Food industry is focusing their efforts towards offering to the consumers safer, more nutritious and high quality products. Microbial contamination of food products causes serious diseases and consequent economic losses [1]. Thus, the addition of bactericidal agents or growth inhibitors into food formulation by spraying or immersion methods had been employed to overcome food contamination. However, direct application of the antimicrobial agents has some limitations since they could be neutralized, evaporated or diffused rapidly and inadequately into the food bulk, reducing their effective concentration on the surface [2,3]. In this context, current trend with good perspectives consists in the incorporation of the additives to the food packages, improving their functionality [4,5]. The active agents are added to the film matrix and slowly released to food surface, where they remain at high concentration for a long time [6].

Although synthetic polymers could be employed to develop these active packages, the actual tendency is targeted to the use of natural and biodegradable polymers due to environmental concerns. Within natural polymers, starch is one of the most promising candidates due its abundance, availability, low cost and biodegradability [7,8]. In previous works corn starch based films had been developed and characterized, resulting homogeneous and transparent [8–10]. The addition of glycerol as plasticizer at 1.5% p/p improved the material flexibility and decreased significantly their water vapor permeability. Besides, the use of acetylated corn starch into formulations led to develop films more resistant and less permeable to the water vapor [8,9]. Moreover, films based on native and acetylated corn starch presented a good heat sealing capacity making them appropriate materials to develop food packages [10].

Sorbic acid and its mineral salts are among the safest food preservatives; they are efficient and versatile due to their highly effective inhibition of most common microorganisms (fungi, molds and yeasts) which can attack foods causing their deterioration [11].

The use of potassium sorbate as antimicrobial agent in film formulation was studied by several authors and its antimicrobial capacity was also demonstrated. Flores and coworkers [12] studied the performance of tapioca starch based films as carriers of sorbate and established that films were effective in controlling the growth of *Z. bacilli* population, acting as a preservative release agent or as a barrier for external yeast contamination. On the other hand, Türe and coworkers [1] developed films based on wheat gluten containing potassium sorbate and they demonstrated the antifungal properties of these active materials. It was also reported the antifungal effectiveness of sorbate potassium incorporated in guar gum and pea starch coatings [13]. Pranoto and coworkers [2] informed that chitosan films with potassium sorbate presented antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. It was also demonstrated that the use of an antimicrobial coating based on CMC with potassium sorbate controlled the molds growth on pistachios, inhibiting substantially the development of some mycotoxigenic *Aspergillus* species [14].

Despite many works present in the literature concerning active films with potassium sorbate, it is still relevant to study the diffusion kinetic of the additive from the polymeric matrixes to the packaged product and its migration profile inside the food bulk. Besides, a mathematical

* Corresponding author. Tel.: +54 221 4254853; fax: +54 221 4249287.
E-mail address: ovlopez75@yahoo.com.ar (O.V. López).

0928-4931/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2012.12.064

model of this phenomenon could allow to determine active agent diffusion coefficients in both, the film and the product, hence it would be feasible to estimate concentration profiles and to predict the time period in which the antimicrobial concentration will be maintained above the critical inhibitory concentration in the packaged food.

The aim of this work was to demonstrate the effectiveness of the addition of potassium sorbate to corn plasticized starch films to prevent microbial growth on a food product surface extending its shelf life. For this purpose, the sorbate controlled release from the polymeric matrix to a semisolid medium was evaluated and a mathematical model of the diffusion process was proposed.

2. Material and methods

2.1. Starch samples

Native and acetylated corn starches were provided by Arcor (Tucumán, Argentina); modified starch presented an acetylation degree of 2.2% [9].

2.2. Filmogenic suspensions

Aqueous suspensions (5% w/w) of native and acetylated corn starch, as well as a mixture of them in equal proportions were prepared. These suspensions were gelatinized at 90 °C during 20 min. Then, glycerol as plasticizer (1.5% w/w) and potassium sorbate (PS) as antimicrobial agent (0.1–0.5% w/w) were added. Filmogenic suspensions with pH adjusted at 4.5 were also prepared by adding 5 M citric acid (Parafarm, Argentina) in order to increase preservative effect of PS, since the undissociated form is the effective one. For further analysis and discussions, plasticized films without active agent were used as control ones.

2.3. Active starch films

Films were obtained by casting method, employing a relation of 2 g of filmogenic suspension per cm^2 of casting area. They were dried at 50 °C during 2 h and 7 days at 20 °C. They were removed from casting plates and stored at 20 °C and 65% HR during a week.

2.4. PS dosage

Official method AOAC [15] to determine sorbic acid with some modifications was employed. Film disks of 1 cm diameter were weighed and dissolved in 50 mL of distilled water, heating softly to facilitate their dissolution. From this solution 5 mL was taken and transferred to a volumetric flask, and 625 μL of 0.1 N HCl was added and diluted to 100 mL with distilled water. Absorbance solution was measured at 260 nm using distilled water as reference and quartz cells. Reported values corresponded at least to five determinations.

2.4.1. Calibration curve

PS standard solution at 1 mg/mL was prepared; 0, 10, 20, 30 and 40 mL of this standard solution were pipetted into separate 100 mL volumetric flasks and diluted with distilled water. 2.5 mL of each solution was pipetted into 250 mL volumetric flasks, and 625 μL of 0.1 N HCl was added and diluted with distilled water. Absorbance was read at 260 nm using distilled water as blank solution. Calibration curve corresponded to the solution absorbance as a function of PS concentration (mg/mL) and experimental data were linearly regressed.

2.5. PS minimum inhibitory concentration

The method proposed by Fajardo and coworkers [16] was employed. Commercial cheese with high humidity content (55%), elaborated with full cream milk acidified with lactic acid bacteria, was used. Approximately 25 g of cheese was deposited onto starch films containing different PS concentrations (0.1–0.5% w/w). Samples were stored at 20 °C during 6 days; visual observation and photographic record were done daily. Lowest additive concentration which did not allow visual observation of fungi growth on cheese surface was regarded as PS minimum inhibitory concentration (MIC). A cheese sample deposited on starch films without PS stored under the same described conditions was used as control.

2.6. Surface color evaluation

Measurements were performed employing a colorimeter Minolta (CR 400, Osaka, Japan). The Hunter parameters: L^*, a^* and b^* were recorded according to CIE scale, in at least 10 randomly selected positions for each film sample. Color parameters range from L = 0 (black) to L = 100 (white), −a (greenness) to +a (redness) and −b (blueness) to +b (yellowness). From these values, ΔL, Δa and Δb were calculated, taking into account the standard values of the white background. Besides, the color difference parameter ($\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$) was also determined.

2.7. PS diffusion

Polymeric matrix effectiveness to retain PS was evaluated. Diffusion assays of this active agent from starch films to a semisolid medium (agar gel), simulating a food product, were carried out. Besides, experiments analyzing PS diffusion from a potassium sorbate solution to the same semisolid medium were also performed. All diffusion tests were performed at least in duplicate.

These two assays were performed in order to compare the use of an active package based on starch and PS with the traditional application techniques (immersion or spray) of this antimicrobial agent to food products.

2.7.1. Agar gels

An aqueous agar (Parafarm, Argentina) solution at 2% w/w was prepared and heated softly to facilitate its dissolution. The warm solution was molded in cylinders of 2 cm height and 2.5 cm diameter (volume = 9.80×10^{-6} m^3) and solidified by cooling.

2.7.2. PS solution

An aqueous PS solution at 1.31% w/v (87.2 mol/m^3) was prepared; this concentration was used in order to maintain the same preservative amount in both the aqueous solution and in the active film.

2.7.3. Samples preparation

Three different experiments were carried out:

a) Gels in contact with active films: film disks of 2.5 cm diameter containing PS were weighted and deposited onto the top surface of the agar gel cylinders. This sample contained 3.27×10^{-3} g (2.18×10^{-5} mol) of PS.
b) Gels in contact with PS solution: 250 μL of the PS solution (1.31% w/v) was placed on the agar gel cylinders. This solution volume contained the same preservative content than that of the active film disks (3.27×10^{-3} g or 2.18×10^{-5} mol).
c) Control gels: agar gel cylinders without film or solution were used as controls (blanks) for the sorbate spectrophotometric determination. They were submitted under the same treatment than samples to consider matrix agar gel absorption.

Gels were stored at 4 °C and at different times, samples were taken to evaluate sorbate release. Tested times were 0.17, 0.33, 0.5, 0.83, 1, 1.16, 1.5, 2, 2.42, 4.32, 6, 12, 24, 36, 48 and 60 h.

2.7.4. Released PS determination

To determine PS concentration in agar gels, official method for sorbic acid [15] with some modifications was used. In the case of assays in

which the sorbate diffused from starch films to agar gels, the polymeric matrix swelling was calculated. Films were weighted before being placed on the agar gel and after storage. The swelling was calculated from the difference between both weights and it was expressed as percentage. Agar gel cylinders were cut in four slices of 0.5 cm thickness and 2.45×10^{-6} m^3 volume. Each slice was weighted and dissolved in 40 mL distilled water by mild heating to enhance its dissolution. 2.5 mL of the resulting solution was put into a 100 mL volumetric flask, and 625 μL of 1 N HCl was added and diluted with distilled water. Absorbance solution was measured at 260 nm using distilled water as reference and quartz cells. The calibration curve was the same used to determine the PS dosage.

2.7.5. Mathematical modeling of the PS diffusion process

PS diffusion occurred from a medium M_1 to an agar gel medium M_2 representing the semisolid food system (Fig. 1). Concerning M_1, two cases were analyzed: (a) a PS solution; (b) a corn starch film containing PS.

A given mass of sorbate was included initially in M_1; during the experiments sorbate diffused from M_1 to M_2 and sorbate concentration decreased along time in M_1 increasing in the adjacent medium M_2. Concentration profiles were simulated in both media as a function of time.

It was applied 2° Fick's law to mathematically model the diffusion process; Eq. (1) corresponds to the unidirectional diffusion under non-stationary state.

$$\frac{\partial C}{\partial t}=\frac{\partial}{\partial x}\left(\frac{D\partial C}{\partial x}\right) \tag{1}$$

where C corresponds to PS concentration in the medium, t is the time, x is the position and D is the PS diffusion coefficient in the medium.

Diffusion process considering constant diffusion coefficient D in each medium is represented by Eqs. (2) and (3) as follows:

- For M_1 medium:

$$\frac{\partial C_1}{\partial t}=D_1\frac{\partial^2 C_1}{\partial x^2} \tag{2}$$

  where C_1 is the PS concentration in the solution or in the corn starch film and x varies between 0 and L_1.
- For M_2 medium:

$$\frac{\partial C_2}{\partial t}=D_2\frac{\partial^2 C_2}{\partial x^2} \tag{3}$$

  where C_2 is the PS concentration in the agar gel and x varies between L_1 and L_1+L_2, being L_2 the length of the gel.

The initial conditions ($t=0$) are described by:

$$C_1(x,0)=C_0 \; in \; 0\le x\le L_1 \tag{4}$$

$$C_2(x,0)=0 \; in \; L_1\le x\le L_2 \tag{5}$$

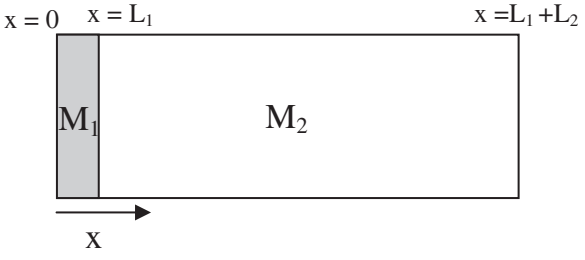


Fig. 1. Scheme of potassium sorbate diffusion process from M_1 (solution or active film) to M_2 (agar gel).

Boundary conditions at $t>0$ are represented by:

$$D_1\frac{\partial C_1}{\partial x}=D_2\frac{\partial C_2}{\partial x} \quad \text{(Continuity of the material flux at } x=L_1) \tag{6}$$

$$C_2(L_1,t)=L_{12}C_1(L_1,t) \quad \text{(Equilibrium at the interface at } x=L_1) \tag{7}$$

$$\frac{\partial C_2}{\partial x}=0 \quad \text{(Null flux in } x=L_1+L_2) \tag{8}$$

The software COMSOL Multiphysics Finite Element Analysis Simulation Software (USA, 2007) was used to model mathematically the experimental data.

2.8. Active films antimicrobial activity

2.8.1. Preparation of inocula

Tested microorganisms were *S. aureus*, *Candida spp.*, *Salmonella spp.* and *Penicillium spp*, since they are mainly responsible of foodborne diseases. They were cultivated from stock vials in Tryptic Soy Broth (TSB) and incubated at 37 °C during 12 h to reach a concentration of 10^8–10^9 CFU/mL, determined by the solutions optical density (OD). Besides, diluted inocula (1:10) were prepared using 1% sterile peptone water (Oxoid) to reach a concentration of 10^7–10^8 CFU/mL.

2.8.2. Antimicrobial capacity

Agar diffusion method described by Pranoto and coworkers [2] was followed using the previously described inocula. Starch film disks of 1 and 3 cm diameter were cut for 10^8–10^9 and 10^7–10^8 CFU/mL inoculums, respectively. Film samples were deposited onto Petri dishes with PCA (Plate Count Agar, Merck) for bacteria testing and nutrient agar for yeast and molds, which were previously inoculated with 100 μL of the corresponding inoculum. Petri dishes were incubated at 37 °C. For *S. aureus*, *Candida spp.* and *Salmonella spp.* visual observations and film inhibition zone measurements were done at different incubation times: 24, 48 and 94 h. For *Penicilium spp.* the observations, as well as, the film inhibition zone measurements were carried out at 48 h and at 8 days of storage. To determine the inhibition zones, sample photographs were taken at different storage times and they were processed with the software GIMP 2.2 (GNU Image Manipulation Program). Assays were done at least by triplicate.

2.8.3. Antimicrobial capacity on a dairy product

Plasticized starch films with PS at minimum inhibitory concentration were obtained from filmogenic suspensions with and without adjusting pH to 4.5.

Approximately 25 g of the commercial cheese described previously was deposited onto active starch films and samples were placed into bags of thermo-sealable synthetic films. The synthetic film used PD 141 (CRYOVAC®) is a polyethylene multilayer materials with a thickness of 75 μm, which is specially designed for fresh refrigerated food packaging since this material presents low oxygen and dioxide permeabilities leading to control the product respiration. Another important characteristic of these synthetic films is the high thermo-sealable strength which leads to maintain the package integrity. Cheese portions deposited onto Petri dishes and stored under the same described conditions were used as controls.

Samples and controls were stored at 4 °C; at 7, 15 and 22 days storage time samples were taken and yeasts and molds were counted. Cheese samples (10 g) were homogenized in a Stomacher Seward Model 400 (United Kingdom) with 90 mL of sterile peptone water (1%). 100 μL of the homogenate was seeded in YGC medium (Yeast extract Glucose Cloramphenicol, Merck) and samples were incubated 8 days at 30 °C. Viable microorganisms were determined by counting the number of formed colonies, expressing the results in CFU/g

cheese. All tests were done in triplicate and the reported results correspond to the average of them.

2.9. Statistical analysis

A completely randomized experimental design was used to evaluate the properties and the antimicrobial capacity of active starch films, as well as the obtained results corresponding to the diffusion assays. All experiments were performed at least in duplicates, with individually prepared and casted films as replicated experimental units as described previously in each determination. Systat-software (SYSTAT, Inc., Evanston, IL, USA) version 10.0 was used for multifactor analysis of variance. Differences in the properties of the films were determined by Fisher's least significant difference (LSD) mean discrimination test, using a significance level of $\alpha = 0.05$.

3. Results and discussion

PS incorporation did not modify the filmogenic capacity of the studied formulations since the obtained films resulted homogeneous and they could be easily removed from the casting plates.

The pH values of the filmogenic suspensions were 5.55, 6.96 and 5.98 for formulations based on native corn starch, acetylated starch and the 50% mixture of both starches, respectively. In all cases, pH of the suspensions was adjusted to 4.5 by adding 5 M citric acid and it was observed that this treatment did not interfere with their filmogenic capacity.

3.1. PS dosage

Table 1 shows the PS concentration in the active starch films developed in the present work. The spectrophotometric method used was efficient and adequate since the measured values were close to the theoretical ones (Table 1). The adjustment of the pH did not modify the film PS content (Table 1). Regardless of the corn starch type, similar results were obtained for sorbate concentrations.

To analyze the stability of the employed additive in the films during storage, and the effect of pH on these results, PS concentration in the active starch films containing 0.3% w/w of the additive (initial and stored during 60 days under controlled conditions) was determined.

The antimicrobial agent concentration in the stored film was $4.16 \pm 0.08\%$ for unadjusted pH conditions and $4.32 \pm 0.23\%$ when the filmogenic suspension with pH = 4.5.

These results indicate that the additive concentration diminished approximately 21% during film storage due to sorbate degradative oxidation. Several authors stressed that this reaction depends mainly on storage conditions and the presence of other additives [12,17].

Table 1
Potassium sorbate (PS) concentration (theoretical and measured) in native corn starch films.

PS added to film formulation (g/100 mL filmogenic suspension)	Theoretical PS content in starch film (g/100 g starch film, dry basis)	Measured PS content in starch film (g/100 g starch film, dry basis)	
		pH non-adjusted	pH = 4.5
0.1	1.77	1.70 ± 0.06	1.76 ± 0.06
0.2	3.60	3.49 ± 0.06	3.59 ± 0.18
0.3	5.52	5.36 ± 0.09	5.39 ± 0.11
0.4	7.46	7.23 ± 0.07	7.34 ± 0.12
0.5	11.81	11.66 ± 0.05	11.70 ± 0.10

3.2. PS minimum inhibitory concentration

Fig. 2 shows the photographs of the cheese samples, placed on native corn starch films with glycerol (1.5% w/w) and PS at different concentrations (0.1–0.5% w/w) and stored at 20 ºC and 65% RH during 6 days.

PS minimum inhibitory concentration (MIC) was 0.3% w/w since cheese samples placed on active films containing this active agent concentration did not present visible fungi growth at the described storage conditions. Stanojevic and coworkers (2009), investigating antimicrobial effects of PS on selected food- spoiling bacteria and fungi, stressed that MIC varies depending on the kind of preservative and taxonomic characteristics of the microorganism species tested. They reported that MIC of PS resulted 10, 50 and 15 mg/mL against *Staphylococcus a*, *Candida albicans* and *Penicillium italicum*, respectively [18].

Despite a pH formulation effect would be expected on MIC values (since the PS non-dissociated form is the effective one to inhibit the microbial growth), differences between films without and with adjusted pH were not observed. Quintavalla and Vicini [19] stressed that the food pH influences the ionization (dissociation/association) of most active chemicals, and could change the antimicrobial activity of organic acids and their salts. In this particular case, the absence of the pH effect could be attributed to the proximity of the sorbic acid pKa (4.76) to the assayed dairy product pH (4.7–5.5), so the concentration of the non-dissociated sorbate form in the interface film-product would be similar in both cases.

3.3. Surface color

Fig. 3 shows the surface color parameters ΔE for active films containing the MIC (0.3%) of PS; in general, sorbate presence produced a significant ($p<0.05$) increase in ΔE. In the case of acetylated starch films, although the ΔE mean value of films with sorbate resulted higher than the corresponding to control one, this variation was not significant ($p>0.05$). The ΔE increase was attributed mainly to the increase of parameter b^*, indicating that active films resulted more yellow than the control samples due to sorbate oxidative browning [20]. However, despite this increase, film color development was relatively low and it is not expected to affect the product acceptability. Besides, the sorbate addition did not modify the luminosity of the starch films being the mean values 96.2, 94.3 and 95.2 for films based on native, acetylated and a mixture of both starches, respectively.

On the other hand, the suspension pH adjustment did not affect the surface color of the active films (Fig. 3). However, Famá and coworkers [20] informed that the yellow index of cassava starch films with 0.1–0.3% of PS decreased when the pH formulation diminished from 6.7 to 5.

3.4. PS diffusion

Fig. 4 shows the experimental results of PS diffusion from: (i) a solution and from (ii) a polymeric matrix based on native corn starch, both in contact with a semisolid medium (agar gel of $a_w \sim 0.8$). When active films were tested it was observed that 80% of the sorbate contained in the film matrix was released after 36 h, reaching 91% after 60 h. In the case of the sorbate diffusion from the solution after 36 h the 98.5% of the additive was released to the agar gel. Thus, the obtained results indicated that, as it was expected, starch films effectively retained for a longer period the active agent in the polymeric matrix.

In order to determine the PS diffusivity from the solution, experimental data of sorbate concentration in the different sections of the agar gel were determined (Fig. 5A). The additive diffused towards the agar gel; thus, in the case of the slice which was in contact with the sorbate solution (slice 1), PS concentration decreased 38% at the maximum assayed time. So, the antimicrobial action in the zone near to the product surface decreased rapidly. A similar tendency was described by

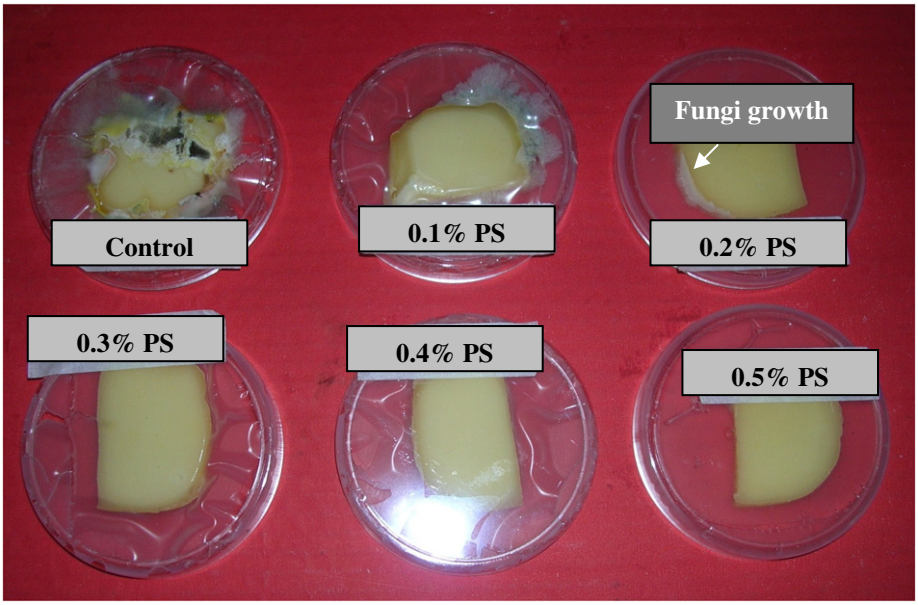


Fig. 2. Cheese samples stored at 20 °C and 65% RH placed onto native corn starch films with glycerol (1.5% w/w) and potassium sorbate (PS) at different concentrations (0.1–0.5% w/w).

several authors [3,21,22]. Thus, if this additive is applied by immersion or spray methods on the surface product it would be necessary to use a highly concentrated solution to ensure its antimicrobial activity during product storage. In the assays performed in this study, to reach a sorbate concentration of 1.86 mol/m^3 in the gel surface after 60 h, it was necessary to apply a solution whose concentration was 87.2 mol/m^3.

The usefulness of the mathematical model consisted in the possibility to predict the diffusion parameters and to provide information about the additive controlled release mechanisms.

The theoretical curves were fitted to the experimental PS concentrations to estimate diffusivity values. Calculations were performed to determine the best-fit diffusion coefficients by minimizing the sum of square of differences between experimental and calculated PS concentrations in the gel.

Fig. 5B shows the simulated concentrations curves obtained for the antimicrobial agent diffusion from the solution to the agar gel. This figure corresponds to an experiment where the sorbate initial concentration in the solution was 87.2 mol/m^3; considering a diffusion coefficient of PS in the liquid solution of 2×10^{-9} m^2/s, the sorbate diffusion coefficient in agar gel that minimized the differences between predicted and experimental PS concentration was 6×10^{-10} m^2/s. The asymptotic values predicted by the model resulted similar to those obtained experimentally (Fig. 5A).

Several authors had informed PS diffusivities in different food products [23,24]. For example, Ulloa and coworkers [24] reported sorbate diffusion coefficients between 3.9×10^{-9} and 8.3×10^{-10} m^2/s in mango slices from a sucrose syrup based solution.

Experimental data of PS concentration in the different agar gel sections when the additive diffused from the active starch films are shown in Fig. 6. Experimental profiles showed that after 60 h, sorbate concentration in the agar slice which was in contact with the film (slice 1) only decreased 9%, maintaining a constant value of 2.25 mol/m^3. These results demonstrated the active film efficiency to maintain their antimicrobial action on the food product surface, in comparison with the additive application by immersion or spray methods.

Most of the studies found in the literature describe and model the diffusive process of different additives from films to a liquid medium, [12,25,26]. However, these systems do not represent a real system in which the active film is in contact with a semisolid food product.

Besides, modeling the additive diffusion process from hydrophilic matrixes is of great interest for the pharmaceutical industry since

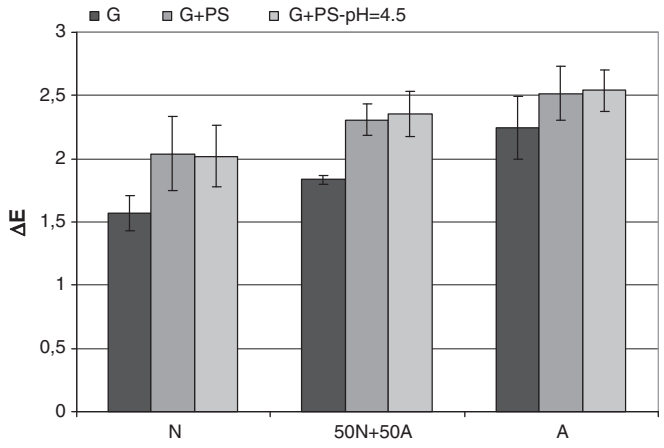


Fig. 3. Potassium sorbate addition effect on superficial color parameter ΔE of films based on: native corn starch (N), a mixture of native and acetylated corn starches in equal proportions (50 N + 50A) and acetylated one (A). All films were plasticized with 1.5% glycerol (G) and active films contained 0.3% potassium sorbate (PS).

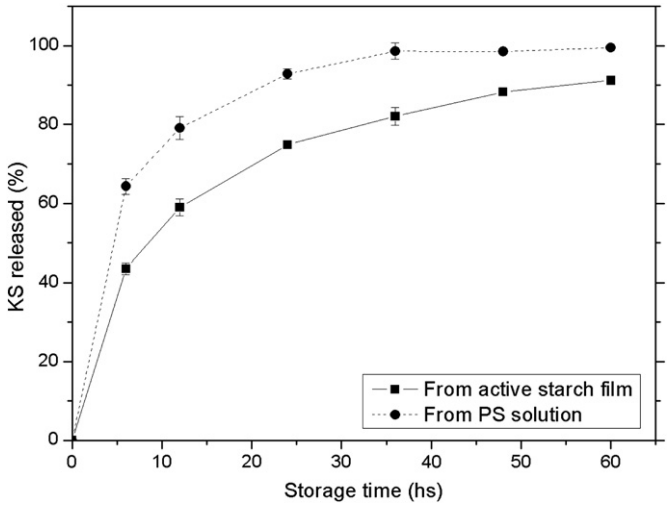
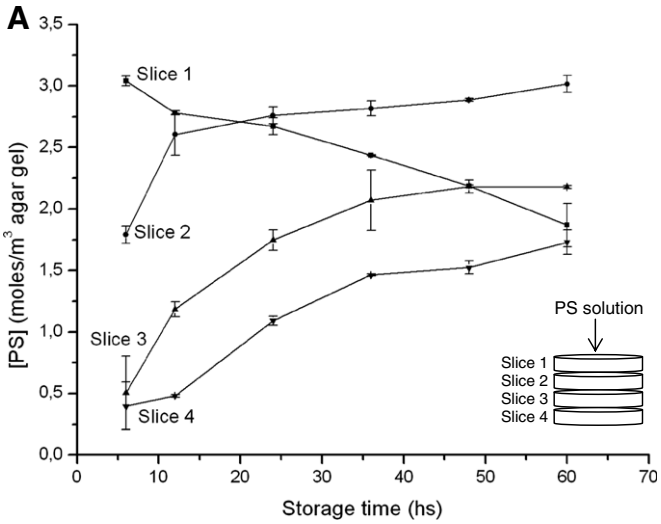
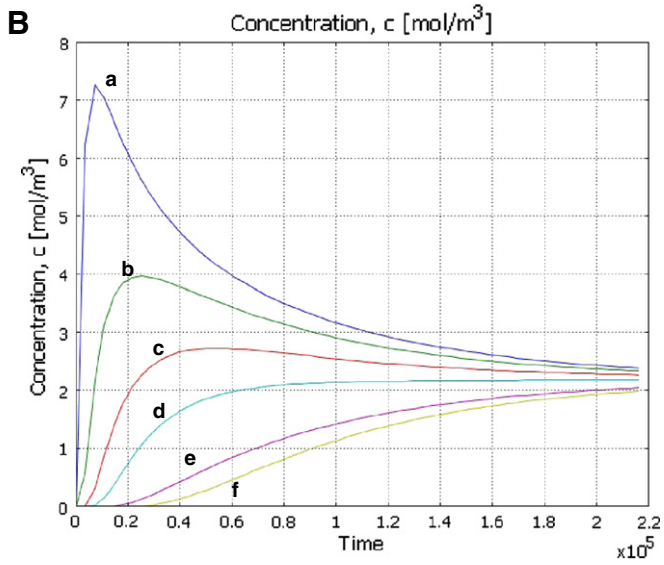


Fig. 4. Potassium sorbate (PS) release from an active native starch film and from a sorbate solution to a semisolid medium, as a time function.





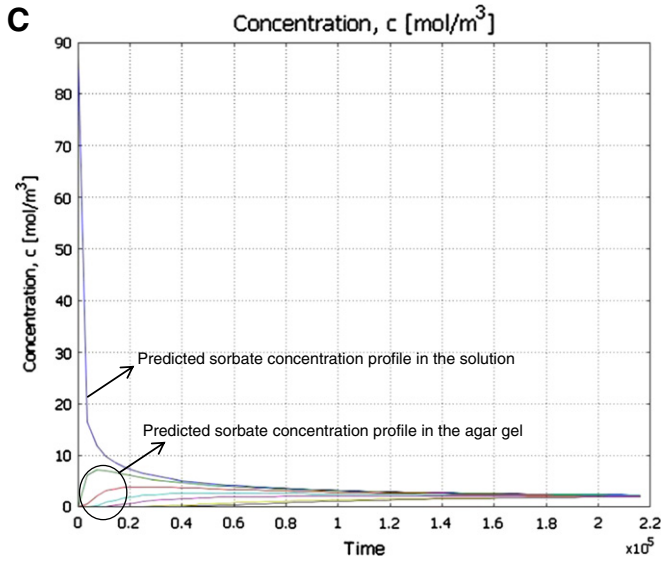


Fig. 5. Potassium sorbate diffusion from the solution. (A) Experimental sorbate concentration profiles in the agar gel. (B) Predicted sorbate concentration profiles in the agar gel: curves a and b correspond to slice 1, c and d to slice 2, e to slice 3 and f to slice 4 (time is expressed in seconds). (C) Predicted sorbate concentration in the solution and in the agar gel. Blue curve corresponds to solution sorbate concentration (to 0.0004 m from the interface) and the other curves correspond to the sorbate concentration at different gel positions (−0.0175 m; −0.015 m; −0.0125 m; −0.010 m; −0.005 m), time is expressed in seconds.

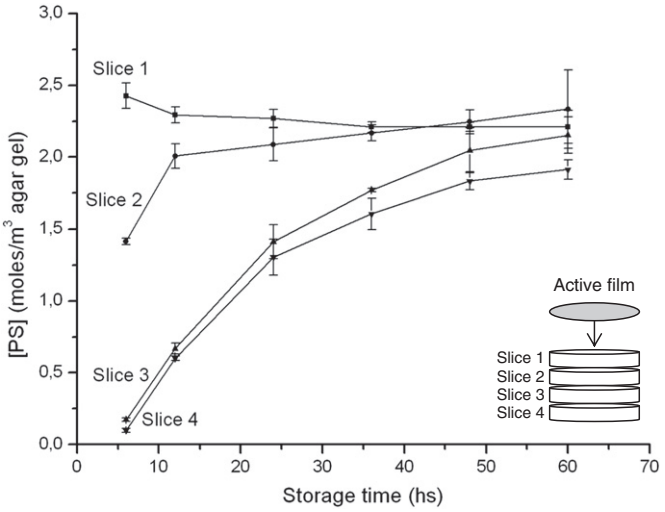


Fig. 6. Potassium sorbate diffusion from the active starch films: experimental sorbate concentration profiles in the agar gel.

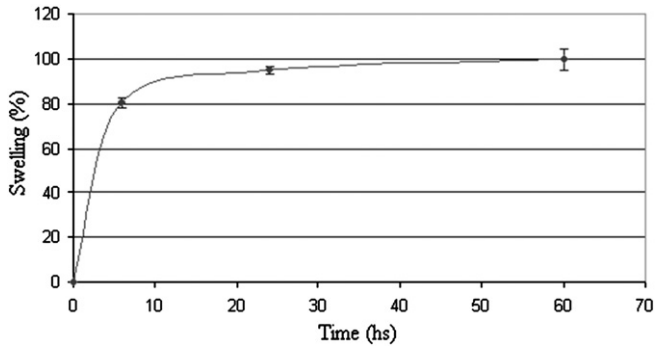


Fig. 7. Active starch films swelling in contact with the agar gel during the diffusive assay.

these materials are used for drugs controlled release [27,28]. In the present work, a theoretical model which considers the antimicrobial diffusion process from a film to a semisolid medium was developed. As it was described for PS diffusion from sorbate solution to agar gel, this model could be used to predict antimicrobial agent concentration profile inside the semisolid medium. For these simulations, the initial mean sorbate concentration in starch film should be 470 mol/m^3 and the PS diffusion coefficient in the agar gel 6×10^{-10} m^2/s (this value is the same obtained from the previous experiment where PS diffused from the solution).

The PS diffusivity in the starch films matrix that minimized the differences between predicted concentrations in each slice and the corresponding average experimental value was 0.3×10^{-13} m^2/s.

In the literature there is a wide variation among sorbate diffusion coefficients values reported for biodegradable films. Choi and coworkers [23] reported a value of 2.6×10^{-13} m^2/s for films of κ-carrageenan. Redl and coworkers [26] informed sorbate diffusion coefficients in gluten films and in bi-layer systems with beeswax of 7.6×10^{-12} and 2.7×10^{-16} m^2/s, respectively. In whey protein films the diffusion coefficients informed varied between 4.1 and 9.3×10^{-11} m^2/s, depending on the formulation [29].

Table 2
Sorbate potassium concentration in agar gel after being in contact 60 h with active corn starch based films.

Film composition	Potassium sorbates concentration in agar gel (mol/m^3)	
	pH non-adjusted	pH = 4.5
N-1.5G-0.3PS	8.61 ± 0.15	8.44 ± 0.57
50N + 50A-1.5G-0.3PS	8.57 ± 0.20	8.44 ± 0.01
A-1.5G-0.3PS	8.50 ± 0.70	8.32 ± 0.05

N: native corn starch; A: acetylated corn starch; 50N + 50A: mixture of native and acetylated corn starches in equal proportions; 1.5G: 1.5% w/w glycerol; 0.3PS: 0.3% w/w sorbate potassium.

Although the mathematical model adjusted satisfactorily, some differences between the experimental data and the predicted values were observed, since the proposed model consider constant the sorbate diffusion coefficient during the process. Fajardo and coworkers [16] stressed that Fick's law deviations were observed due to the hydrophilic matrixes swelling during the diffusion process. Several authors considered that this effect was particularly important in chitosan films which exhibit swelling degrees around 1015% when they were immersed in a liquid medium for short times [16,30,31].

In our experiments starch based films swelled during the diffusion assays (Fig. 7), indicating that the polymeric matrixes present variable

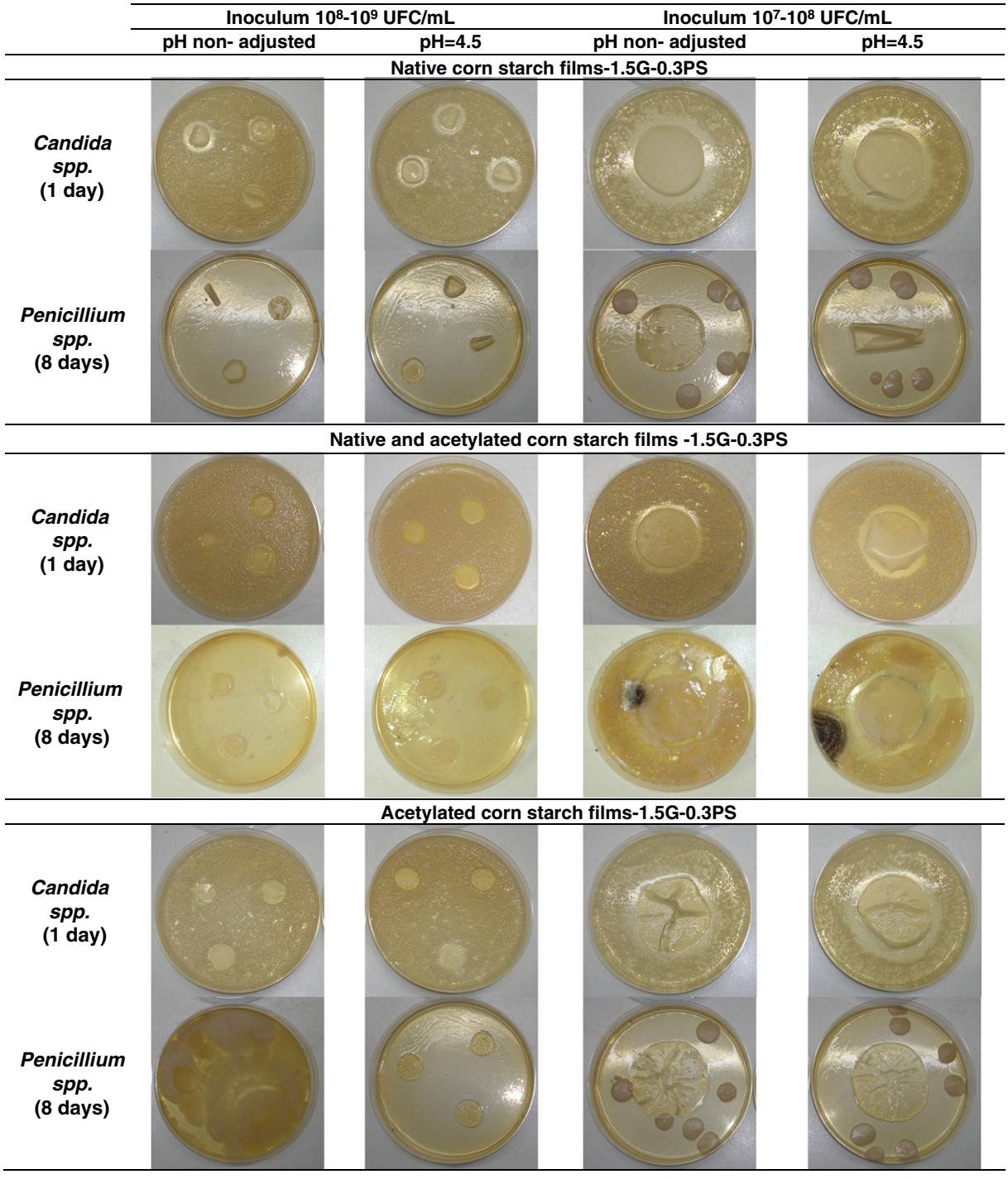


Fig. 8. Inhibition zones development of *Candida spp.* and *Penicillium spp.* in starch films with glycerol (1.5% w/w) and potassium sorbate (0.3% w/w PS).

characteristics. Buonacuore and coworkers [32] developed a complex model which describes the release kinetic of different antimicrobial agents (lysozyme, nisin and sodium benzoate) from a highly hydrophilic matrix to an aqueous medium. Thus, in future investigations it will be necessary to introduce in the theoretical model a variable sorbate diffusion coefficient in the starch film to consider the matrix swelling.

However it must be considered that the swelling of the biodegradable films in this kind of experiments when they are in contact with solid foods is lower than in the cases that the films are immersed in liquid media.

Finally, to evaluate the film formulation effect on the sorbate diffusion process, the additive concentration in the agar gel was measured after 60 h; the results are shown in Table 2. The starch type employed did not affect the sorbate concentration asymptotic values in the agar gel. Besides, non-significant differences ($p > 0.05$) were found between the results for the films without and with the adjusted pH. Similar results were found by Choi and coworkers [23] studying the sorbate diffusion from κ-carrageenan films to solutions with different pH. Besides, Quattara and coworkers [3] informed a similar trend for acetic and propionic acids diffusivities from chitosan films.

3.5. Active films antimicrobial capacity

Antimicrobial capacity of the active starch films was evaluated through the agar diffusion method. Fig. 8 corresponds to the photographs of the inhibition zones observed for *Candida spp.* and *Penicillium spp*. In general, the microbial growth inhibition and the effect of the formulation pH were better observed working with the most concentrated inoculums (10^7–10^8 CFU/mL).

The three active starch film formulations assayed inhibited *Candida spp.* growth and a positive effect of the pH adjustment to 4.5 was observed since the corresponding inhibition zones presented largest diameters (Fig. 8, Table 3).

As can be observed in Fig. 8, it was not possible to measure the inhibition zones for *Penicillium spp.*; therefore a visual inspection of the microbial growth and a qualitative description were done. For films based on native starch and the mixture of starches, when concentrated inocula were seeded, fungi development was not observed, regardless of the formulation pH (Fig. 8). In the case of acetylated films, although fungi grew, the pH adjustment effect was evident (Fig. 8). For diluted inocula fungi growth was observed regardless of films formulation; however, a positive effect of the formulation pH adjustment on the development of this microorganism was detected.

Sofos and Busta [33] have described extensively that potassium sorbate inhibits the development of a great number of bacteria (gram positive and negative, catalase positive and negative, aerobic and anaerobic, mesophilics and psycrotrophics). In the present work, the active film inhibitory action on *S. aureus* and *Salmonella spp* was evaluated. The selection of these microorganisms was based on their pathogenesis and their amenability on some diseases transmitted by foods. Both bacteria are facultative anaerobic, being *S. aureus* gram positive and *Salmonella spp.* gram negative.

Table 3
Inhibition zones measurements of *Candida spp.* in corn starch films with 1.5% w/w glycerol (1.5G) and 0.3% w/w potassium sorbate (0.3PS).

Film composition	Inhibition zone (mm)			
	Inoculum 10^8–10^9 UFC/mL		Inoculum 10^7–10^8 UFC/mL	
	pH non-adjusted	pH = 4.5	pH non- adjusted	pH = 4.5
N-1.5G-0.3PS	13.55 ± 0.12	15.71 ± 0.63	42.08 ± 1.14	43.53 ± 1.07
50N + 50A-1.5G-0.3PS	10	10	32.07 ± 1.11	36.22 ± 2.63
A-1.5G-0.3PS	10	10	36.38 ± 1.95	43.25 ± 0.36

N: native corn starch; A: acetylated corn starch; 50N + 50A: mixture in equal proportions of native and acetylated corn starches.

Native starch films with potassium sorbate inhibited *S. aureus* growth, being this effect 34% higher for films with pH = 4.5. A similar trend was observed for films based on the mixture of starches, increasing the inhibitory zone diameters 41% when the formulation pH was adjusted. Pranoto and coworkers [2] reported that chitosan films containing potassium sorbate also presented antimicrobial effectiveness against this microorganism, evaluated by the agar diffusion assay.

In the case of *Salmonella spp.* similar results were observed. The pH adjustment increased 22 and 50% the inhibitory zone diameter for starch films based on native and the mixture of starches, respectively. Active acetylated starch films were only able to inhibit the development of both bacteria in the contact zone between the film and the culture medium, regardless of the suspension pH. Active films based on the mixture of the starches presented the highest inhibitory capacity for the tested bacteria.

3.6. Antimicrobial capacity evaluation of active starch film on a dairy product

To analyze the effectiveness of the developed active films, shelf life of cheese packaged samples in contact with these films was evaluated through microbiological assays. Considering the antimicrobial capacity results, the active starch films based on the mixture of native and acetylated corn starches in equal proportions were selected for these assays.

Fig. 9 shows the microbial counts obtained for cheese samples deposited onto active films which were effective to inhibit molds and yeasts growth. Despite formulation pH adjustment led to the lowest count values, this decrease was not significant ($p > 0.05$).

Cheese samples shelf life stored under refrigeration conditions is mainly determined by microbial contamination. Fresh cheese is characterized to be a slightly fermented product with low acidity (pH ≈ 5), high water activity, low salt content (<3%) and with an electronegative redox potential (oxygen absence). These conditions led to the development of many microorganisms, specially molds and yeasts. In the present work shelf life of fresh cheese was defined as the time necessary to reach 10^6 CFU/g of sample. Psychrophilic microorganisms cause important economical losses as a result of lipolytic and proteolytic activities and may be present at a level of 10^6 CFU g^{-1} in milk and milk products [16,22]. When microbial counts exceed this limit, toxic substances may be produced in other products [34]. In the case of the control sample, the observed shelf life was 14 days while for cheese samples stored on active films it was extended to 17 days (21%), regardless of the formulation pH (Fig. 9). The low effect of decreasing the formulation pH to 4.5 could be attributed again to the similitude between the sorbic acid pKa with the product pH.

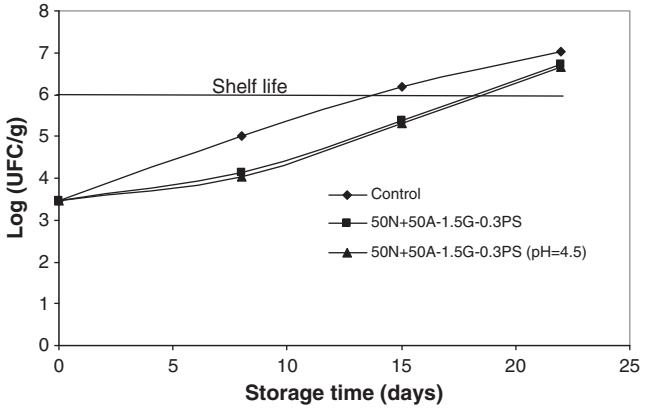


Fig. 9. Yeast and molds growth on cheese samples deposited onto corn starch films based on mixtures of equal proportions of native and acetylated corn starch (50 N + 50AS) plasticized with 1.5% glycerol (1.5 G) containing 0.3% potassium sorbate (0.3PS) with (pH = 4.5) and without pH adjustment. Samples were stored at 4 °C.

Moreover, Cerqueira and coworkers [35] stressed that galactomannan coatings were effective to prolong shelf life of Regional cheese. Likewise, several authors have used chitosan based films to extend the shelf life of different types of cheese under refrigeration or modified atmosphere storage [16,36,37]. On the other hand, other biodegradable materials can be used for this purpose, for example PLA was used to package Dambo cheese maintaining its quality attributes during 84 days [38].

4. Conclusions

Biodegradable films of native and acetylated corn starches, as well as a mixture of them in equal proportions containing potassium sorbate were developed and characterized. Formulation pH effect on films antimicrobial capacity was also evaluated. Sorbate content of active films decreased during storage due to its oxidative degradation. Active films resulted more yellowness and less transparent than films without potassium sorbate. Potassium sorbate minimum inhibitory concentration in starch films resulted 0.3% w/w, regardless of starch type and formulation pH. Sorbate inclusion in starch matrixes maintained a high antimicrobial concentration in product surface, where its action is required. Additive kinetic release was neither affected by starch type nor by formulation pH. Besides, experimental data of sorbate diffusion from active films and from solution to a semisolid medium were mathematically modeled. The corresponding sorbate diffusion coefficients in the different media were satisfactorily adjusted. This model assumes a constant initial mass of antimicrobial that diffuses along both media that are in contact; model solution allows to simulate non-stationary diffusion processes of different additives incorporated to polymeric matrixes, taking into account only preservative concentration, as well as, film and semisolid medium dimensions. Further researches should consider the swelling film effect on controlled release of active compounds.

Starch active films developed were able to inhibit *Candida spp.*, *Penicillium spp.*, *S. aureus* and *Salmonella spp.* growth. These microorganisms have a negative effect on food quality and are responsible of some foodborne diseases. The best performance was exhibited for films formulated with the mixture of both starches, regardless of the pH filmogenic suspension. These active films were effective to extend shelf life (21% from 14 to 17 days) of refrigerated cheese samples, regardless of formulation pH.

References

[1] H. Türe, M. Gällstedt, M. Hedenqvist, Food Res. Int. 45 (2012) 109–115.
[2] Y. Pranoto, S. Rakshi, V. Salokhe, LWT - Food Sci. Technol. 38 (2005) 859–865.
[3] B. Quattara, R. Simard, G. Piette, A. Begin, R. Holley, J. Food Sci. 65 (2000) 768–773.
[4] A. Cagri, Z. Ustunol, E. Ryser, J. Food Sci. 66 (2001) 865–870.
[5] Y. Li, B. Liu, Z. Zhao, F. Bai, Chin. J. Biotechnol. 22 (2006) 650–656.
[6] E. Chollet, I. Sebti, A. Martial-Gros, P. Degraeve, Food Control 19 (2008) 982–989.
[7] M. García, A. Pinotti, M. Martino, N. Zaritzky, in: M. Embuscado, K. Huber (Eds.), Edible Films and Coatings for Food Applications, Springer Science, India, 2009, pp. 169–210.
[8] O. López, M. García, N. Zaritzky, Carbohydr. Polym. 73 (2008) 573–581.
[9] O. López, N. Zaritzky, M. García, Stewart Postharvest Rev. 6 (2010) 1–8.
[10] O. López, C. Lecot, N. Zaritzky, M. García, J. Food Eng. 105 (2011) 254–263.
[11] E. Kristo, K. Koutsoumanis, C. Biliaderis, Food Hydrocolloids 22 (2008) 373–386.
[12] S. Flores, L. Famá, A. Rojas, S. Goyanes, L. Gerschenson, Food Res. Int. 40 (2007) 257–265.
[13] G. Mehyar, H. Al-Qadiri, H. Abu-Blan, B. Swanson, J. Food Sci. 76 (2011) 210–217.
[14] S. Sayanjali, B. Ghanbarzadeh, S. Ghiassifar, LWT - Food Sci. Technol. 44 (2011) 1133–1138.
[15] AOAC, in: Sorbic Acid in Wines Spectrophotometric Method, 1984, p. 229.
[16] P. Fajardo, J. Martins, C. Fuciños, L. Pastrana, J. Teixeira, A. Vicente, J. Food Eng. 101 (2010) 349–356.
[17] M. Gliemmo, C. Campos, L. Gerschenson, J. Food Sci. 69 (2004) 39–44.
[18] D. Stanojevic, L. Comic, O. Stefanovic, S. Solujic-Sukdolak, Bulg. J. Agric. Sci. 15 (2009) 307–311.
[19] S. Quintavalla, L. Vicini, Meat Sci. 62 (2002) 373–380.
[20] L. Famá, S. Flores, L. Gerschenson, S. Goyanes, Carbohydr. Polym. 66 (2006) 8–15.
[21] A. Reps, L. Drychowski, J. Tomasik, K. Winiewska, Pak. J. Nutr. 1 (2002) 243–247.
[22] I. Var, Z. Erginkaya, M. Güven, B. Kabak, Food Control 17 (2006) 132–136.
[23] J. Choi, W. Choi, D. Cha, M. Chinnan, H. Park, D. Lee, J. Park, Food Sci. Technol. 38 (2005) 417–423.
[24] J. Ulloa, G. Guatemala, E. Arriola, H. Escalona, L. Díaz, J. Food Eng. 91 (2009) 211–216.
[25] M. Ozdemir, J. Floros, J. Food Eng. 47 (2001) 149–155.
[26] A. Redl, N. Gontard, S. Guilbert, J. Food Sci. 61 (1996) 116–120.
[27] N. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Eur. J. Pharm. Biopharm. 50 (2000) 27–46.
[28] J. Siepmann, F. Siepmann, Int. J. Pharm. 364 (2008) 328–343.
[29] S. Hasan, S. Deniz, O. Murat, Dry. Technol. 24 (2006) 21–29.
[30] E. Azevedo, T. Saldanha, M. Navarro, A. Medeiros, M. Ginani, F. Raffin, J. Appl. Polym. Sci. 102 (2006) 3462–3470.
[31] D. Baskar, T. Sampath Kumar, Carbohydr. Polym. 78 (2009) 767–772.
[32] G. Buonocore, M. Del Nobile, A. Panizza, S. Bove, G. Battaglia, L. Nicolais, J. Food Sci. 68 (2003) 1365–1370.
[33] J. Sofos, F. Busta, in: A. Branen, P. Davidson (Eds.), Antimicrobials in Foods, Dekker, New York, 1983, pp. 141–176.
[34] L. Howard, T. Dewi, J. Food Sci. 60 (1995) 142–144.
[35] M. Cerqueira, M. Sousa-Gallagher, I. Macedo, R. Rodriguez-Aguilera, B. Souza, J. Teixeira, A. Vicente, J. Food Eng. 97 (2010) 87–94.
[36] M. Del Nobile, A. Conte, G. Buonocore, A. Incoronato, A. Massaro, O. Panza, J. Food Eng. 93 (2009) 1–6.
[37] D. Gammariello, S. Chillo, M. Mastromatteo, S. Di Giulio, M. Attanasio, M.A. Del Nobile, J. Dairy Sci. 91 (2008) 4155–4163.
[38] V. Holm, G. Mortensen, J. Risbo, Food Chem. 97 (2006) 401–410.