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Effect of potassium sorbate on antimicrobial and physical properties of starch–clay nanocomposite films

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ABSTRACT

Using fresh foods which undergo the least processing operations developed widely in recent years. Active packaging is a novel method for preserving these products. Active starch–clay nanocomposite films which contained potassium sorbate (PS) at a level of 0, 5, 7.5 and 10 g PS/100 g starch were produced and their physical, mechanical and antimicrobial properties were evaluated. In order to evaluate antimicrobial properties of films *Aspergillus niger* was used. The results showed that 5% of the PS did not produce antimicrobial property in the film, but by increasing the content of the additive in film formulation, antimicrobial effect increased. PS increased water permeability and elongation at break of the films, but decreased tensile strength. The rate of PS migration into the semi-solid medium in starch–nanocomposites was lower than starch films. This shows that nanocomposite films could retain their antimicrobial property for longer time.

1. Introduction

Active food packaging is an innovative solution to meet the continuous changes in current consumer demands and market trends. Active food packaging extends shelf life and improves the safety of the food by scavenging of oxygen, moisture or ethylene, and by promoting emission of ethanol, flavors, and antimicrobial agents (Appendini & Hotchkiss, 2002; Quintavalla & Vicini, 2002). Antimicrobial packaging is attracting increasing attention from the food and packaging industry, since the use of preservative packaging films offers several advantages compared with the direct addition of preservatives into food products. The incorporation of antimicrobial agents into polymeric films allows industry to combine the preservative functions of antimicrobials with the protective functions of the pre-existing packaging concepts (Persico et al., 2009).

Antimicrobial activity can be achieved by including pads containing volatile antimicrobial agents into packages, incorporating antimicrobial agents into polymers, coating antimicrobials onto polymer surfaces, immobilizing antimicrobials by chemical grafting, using polymers that are antimicrobial by themselves (Appendini & Hotchkiss, 2002).

Starches are polymers that naturally occur in a variety of botanical sources such as wheat, corn, potatoes and tapioca. It is a renewable resource widely available and can be obtained from different left overs of harvesting and raw material industrialization. They are useful for numerous applications in the food industry and their functional properties depend on the source but are also affected by other factors like chemical modifications, system composition, pH and ionic strength of the media (Fama, Rojas, Goyanes, & Gerschenson, 2005).

Sorbic and its potassium salt (sorbates) are considered GRAS additive and are active against yeast, molds, and many bacteria (Flores, Fama, Rojas, Goyanes, & Gerschenson, 2007; Flores, Haedo, Campos, & Gerschenson, 2007). These preservatives are unstable in aqueous solution and can suffer an oxidative degradation or can be metabolized by microorganisms under certain conditions of storage (Gerschenson & Campos, 1995; Sofos, 1989). The addition of sorbates to edible films has been proposed as a way of minimizing surface microbial contamination (Cagri, Ustunol, & Ryser, 2001; Chen, Yeh, & Chiang, 1996). To accomplish this objective, a certain concentration of the preservative must be present at the surface of the product. Reduction of the surface level due to diffuse into the food or due to degradation of the preservative must be taken into account when designing an antimicrobial film (Gliemmo, Campos, & Gerschenson, 2004).

Development of the polymer/clay nanocomposites is one of the latest revolutionary steps of the polymer technology. The nanocomposites obtained by the addition of the low percentage of clay to polymers exhibited an improvement in the properties such as

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barrier, thermal and oxidative when compared with traditional composites (Cyras, Manfredi, Ton-That, & Vazquez, 2008).

Potential effects of nanoclays in human health are not well-known. There has been no conclusive evidence for negative effects of nanoclays in human health. However, a few in vitro studies have been conducted to determine their potential toxicological effects. Maisanaba et al. (2013) investigated the cytotoxic effects of a non-modified clay (Cloisite_Na+) and an organoclay (Cloisite_30B) in the hepatic cell line HepG2. Their result showed that only Cloisite 30B has cytotoxicity.

The objective of this research was to study the mechanical, physical and antimicrobial properties of starch–clay nanocomposite films containing potassium sorbate, and to evaluate the release rate of potassium sorbate from starch starch–clay nanocomposite films into semi solid medium.

2. Materials and methods

2.1. Materials

Potato starch (12.2% moisture) was obtained from Alvand Company (Iran). The amylose and amylopectin contents are 18% and 82%, respectively. Na^+ montmorillonite (MMT) was acquired from Southern Clay Products (USA). Glycerol was purchased from Merck, Germany. Potassium Sorbate was supplied from sdfine- CHMLimi TED (India).

2.2. Preparation of films

On one hand, 4 g starch was added to 100 ml distilled water, which contained 0.3 g glycerol/g starch and gelatinized in 90 °C for 10 min. On the other hand, 0.2 g montmorillonite (0.05 g montmorillonite/g starch), was dispersed in 50 ml distilled water and was stirred under mechanical force in 1200 rpm for 30 min. The obtained mixture was sonicated for 30 min. Finally, the obtained mixture was spread onto Plexiglas plates (20 cm × 30 cm) and dried for 20 h at 50 °C. In order to produce antimicrobial films, 0.05, 0.075 and 0.1 g PS/g starch dissolved in 20 ml distilled water, which contained glycerol, then the rest of the steps was as like as above. For making a starch film without montmorillonite only the first solution was prepared and spread onto Plexiglas for drying. They were removed from casting plates and stored at 25 °C and 50% HR during a week.

2.3. Film thickness

Film thickness was measured with a handheld digital micrometer (Mitutoyo, Japan) having a sensitivity of 0.001 mm. Ten thickness measurements were taken on each testing sample in different points and the mean values were used in water vapor permeability and tensile properties calculations (Alboofetileh, Rezaei, Hosseini, & Abdollahi, 2013).

2.4. X-ray diffraction (XRD)

X-ray diffraction (XRD) studies of the samples were carried outusing aPW3050 Advance X-ray diffractometer (Philips, Netherlands) operating at CuK_{α} wavelength of 0.1539 nm. The samples were exposed to the X-ray beam with the X-ray generator running at 40 kV and 30 mA. Scattered radiation was detected at ambient temperature in the angular region (2θ) of 1–12° at a rate of 1°/min.

2.5. Water vapor permeability

Water vapor permeability (WVP) tests were conducted using ASTM method (E96, 2000), by using circular glass cups with internal diameter of 3 cm and depth of 3.5 cm. Each film sample was sealed on top of the cups containing 8 ml distilled water to provide RH = 100%. The cups were stored at 25 °C in a silica gel containing desiccator (RH = 0%). The RH inside the cup was always higher than outside, and water vapor transmission rate (WVTR) was determined from the weight loss of the cups. Changes in the weight of the cups were recorded to the nearest 0.0001 g and plotted as a function of time. WVTR (g/m^2 s) and WVP (g/m s Pa) were calculated using the following equations:

$$\mathrm{WVTR} = \frac{\Delta m}{\mathrm{A} \times \Delta t} \quad (1)$$

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times x}{p_1 - p_2} \quad (2)$$

where Δm: is the weight loss of the cup, A: exposed area (7.06×10^{-4} m^2), Δt: time of loss, x: the mean film thickness and p_1–p_2: is real vapor partial pressure difference (Pa) across the film.

2.6. Mechanical properties measurement

Mechanical tests including tensile strength (TS) and elongation percentage at break (E) were performed according to ASTM (D882-02, 2002) standard. The films were cut into 6 × 1 cm strips. Texture Analyzer (Zwick, model BZ2.5/TH1S, Germany) was used. Initial grip separation and crosshead speed were 40 mm and 50 mm/min, respectively. TS was calculated by dividing the maximum force by the initial area of the film and E% was calculated by dividing the extension at the moment of specimen rupture by the initial gauge length and multiplying by 100.

2.7. Surface color measurement

Color properties of films were measured using a color meter from Hunter Lab, USA. Measurements are expressed as L^* (lightness), a^* (red/green), and b^* (yellow/blue). The parameters were determined by placing film samples on a standard plate (L^* = 92.23, a^* = −1.29, and b^* = 1.19). Color difference (ΔE) was calculated with respect to standard plate parameters by using following Eq. (3) (Abdollahi, Alboofetileh, Rezaei, & Behrooz, 2013).

$$\Delta E = \sqrt{(\Delta l^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

2.8. Antimicrobial activity

2.8.1. Culture preparation

Aspergillus niger Persian Type Culture Collection (PTCC-5012), was obtained from the culture collection at the Iran Institute of Industrial and Scientific Research. The fungal cultures were cultivated on potato dextrose agar (PDA; Merck, Germany) slants for 10 days at 25 °C and the spores harvested with 10 ml of 1 ml/1000 ml tween 80 (Merck) solutions (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011). The spore suspension was adjusted with the same solution to give a final spore concentration of 10^5–10^6 spore/ml and was used the same day.

2.8.2. Antimicrobial test

The antimicrobial test was carried out according to the method developed by Li Shen, Min Wu, Chen, and Zhao (2010). The inhibitory zone test on semisolid medium was used for determination of the antimicrobial effects of films on *Aspergillus niger*. Films were cut into a disk (diameter = 20 mm) with a punch. During tests, 1 disk placed carefully into each petri dish containing semisolid medium (Potato Dextrose Agar), where 0.1 ml seeding culture had been spread. The petri dishes were then incubated at 25 °C for 72 h. The plates were examined to find the Inhibition zone of the film discs, and the diameter of the zone was measured with a sliding caliper in triplicate. The area of the whole zone was calculated then subtracted from the disk area and the difference in area was reported as the inhibition zone.

2.9. Potassium Sorbate determination

Official method AOAC (AOAC, 1984) for determination of sorbic acid with some modifications was employed. Samples 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. In order to draw calibration curve, potassium sorbate 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.

2.10. Potassium sorbate release in a semisolid medium

The method proposed by Flores and coworkers was employed. To study the diffusion of the potassium sorbate in a semisolid food model, film discs (1 cm diameter) were applied on the surface of 20 ml potato dextrose agar (Merck, Germany) contained in plates (diameter 9 cm) and with $a_w = 0.985$. Plates were incubated at 25 °C for 72 h.

At selected times (0, 4, 8, 16, 24, 32, 40 and 48 h), agar circles of 5 cm diameter were cut with the help of a cork borer and their potassium sorbate contents were determined. The area of agar cut included the agar area where the film discs have been deposited and also a safety zone evaluated in previous assays as exceeding the maximum area where sorbate diffusion occurred, under experimental conditions (Flores, Fama, et al., 2007; Flores, Haedo, et al., 2007).

2.11. Statistical analysis

The difference between factors and levels was evaluated by the analysis of variance (ANOVA). Duncan's multiple range tests were used to compare the means to identify which groups were significantly different from other groups ($p < 0.05$). All data are presented as mean ± SD.

3. Results and discussion

3.1. X-ray diffraction

In order to investigate the dispersion of the montmorillonite layers, X-ray diffraction analyses were performed on the composite. Fig. 1 shows the XRD patterns obtained for the starch, the MMT and the nanocomposite. Starch shows no peak in the study range while MMT exhibits a single 0 0 1 diffraction peak around 7.61°. In the composite film the 0 0 1 diffraction peak of the MMT (4.91°) shifted to lower angle. These results should indicate that either the glycerol or the polymer chains or both entered into the silicate layers forming intercalated starch/MMT nanocomposite, without reaching complete exfoliation. This could be due to the strong polar interactions between the hydroxyl groups present both in the polymer chain, in the glycerol and in the silicate layers (Cyras et al., 2008; Park et al., 2002).

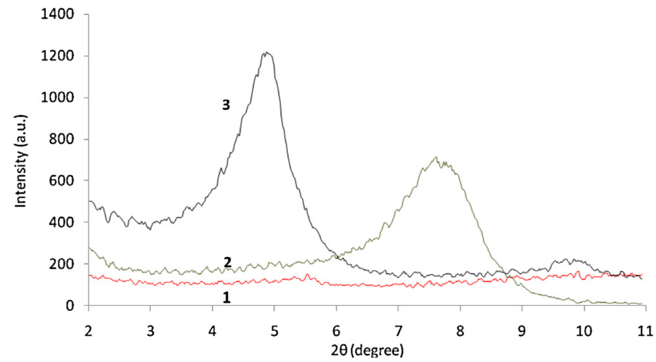

Fig. 1. XRD patterns of the starch (1), MMT (2) and starch–MMT nanocomposite (3).

3.2. Mechanical properties of films

Stress–strain test is one of the most popular tests for determination of the mechanical behavior of viscoelastic polymers. The effect of PS on these behaviors showed that, when the sorbate contents increase from 0 to 10%, tensile strength decreased. Fig. 2A. While elongation at break of the films increased significantly by increasing sorbate content (Fig. 1B). Films with sorbate showed lower tensile strength and more elongation at break compared with blank film.

Plasticizers changed mechanical properties of the films by decreasing intermolecular forces in starch films and laying among amylose chains. Sorbate acted as a plasticizer and decreased tensile strength and increased elongation at break. The reason is that, the

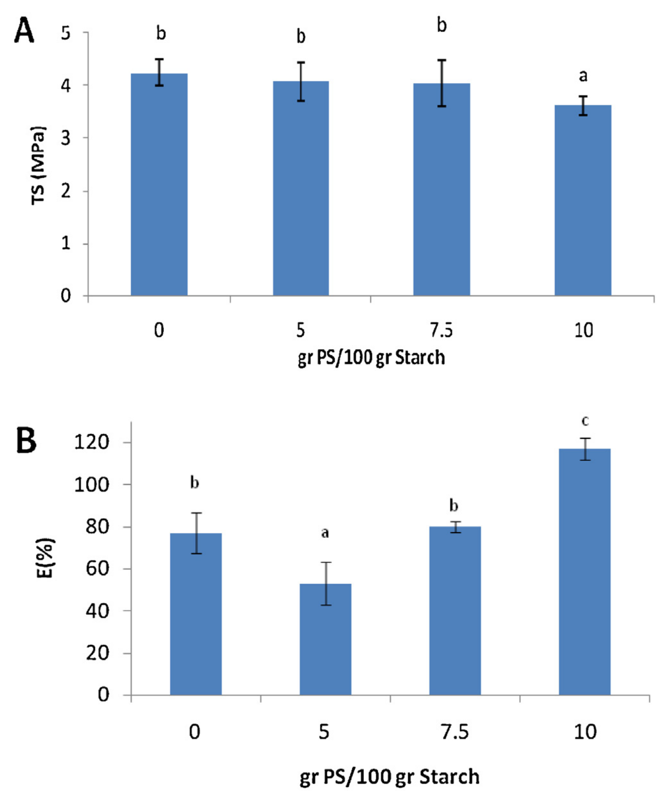

Fig. 2. Tensile strength (A) and elongation at break (B) of starch–clay nanocomposite films as a function of potassium sorbate (PS) content.

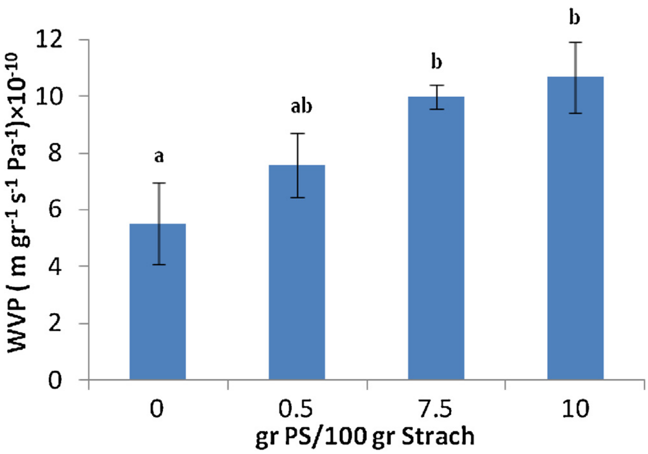

Fig. 3. Water vapour permeability (WVP) of starch–clay nanocomposite films containing potassium sorbate (PS).

linear sorbate chain could penetrate into the starch chains simply and in the result caused an increase in mobility of polysaccharide chains and in turn decreased tensile strength and increased elongation at break (Cagri et al., 2001).

3.3. WVP of the films

The results of WVP of the films showed in Fig. 3. Results showed that WVP increased by increasing sorbate content. Adding 5, 7.5 and 10% sorbate increased WVP of the films, 37, 81 and 94% respectively. This increment could be the result of increasing polar hydrophile groups in the films. Li Shen et al. By evaluating the effects of sorbate on the different properties of potato starch films showed that high sorbate content could increase WVP (Li Shen et al., 2010). According to the study of Mac Hugh et al., adding polar compounds may increase hydrophobicity and solubility of the films. Furthermore, such compounds could decrease density and loose and weak the structure which finally resulted in more mobility in amylose chains (Mc Hugh, Aujard, & Krochta, 1994).

3.4. Antimicrobial properties of films

Antimicrobial effect of films containing PS, on the base of the inhibition zone against experimental fungi is shown in Table 1. Antimicrobial materials penetrate agar from the films and show their effects. If zones do not emerge around the film discs, It shows no inhibition zone and then it is considered zero in measurement. In blank samples which did not have any antimicrobial agent, there was not any inhibition zone around the discs. It shows that starch–clay nanocomposites without any additive do not have antimicrobial effect. No antimicrobial effect of non modified montmorillonite has been shown in other researches. Rhim et al., evaluated the antimicrobial effects of chitosan films with natural MMT (Na^+ Cloisite) and modified MMT (Cloisite 30 B). According to the results of their research, Na^+ MMT did not show antimicrobial effect against bacteria, but modified MMT prohibits the growth of gram positive bacteria (Rhim, Hong, Park, & Ng, 2006). 5% of PS did not show antimicrobial effect, It means low content of sorbate could not be able to inhibit the growth of *A. niger*. This fungus resists against low concentration of sorbate. The reason for no antimicrobial effect of low concentration of sorbate could be related to the formation of hydrogen bonds between hydroxyl groups of starch and carboxyl groups of sorbate. The formation of these bonds limits the migration of sorbate from starch films (Li Shen et al., 2010). The least concentration of this material for inhibition of these fungi was 7.5%.The diameter of inhibition zone increased by increasing the concentration of sorbate, so the highest diameter of the zone was related to the film with 10% sorbate.

Table 1
Antimicrobial activity of starch–clay nanocomposites films incorporated with potassium sorbate against *Aspergillus niger*.

PS content (g/100 g starch)	Inhibitory zone (mm^2)
0	0.00 ± 0.00^a
5	0.00 ± 0.00^a
7.5	268.91 ± 42.35^b
10	532.08 ± 74.62^c

[a–c] Different lowercase letters in the same column under the same antimicrobial agent indicate significant differences ($p < 0.05$). Data show in mean ± standard deviation ($n = 3$).

3.5. Surface color

Table 2 shows the effect of different amounts of PS on color factors of nanocomposite films. The Addition of sorbate, decreased L^* in the films. So the transparency of the films decreased. In addition, ΔE increased significantly ($p < 0.05$) by adding sorbate. Increasing ΔE is basically related to b^* factor and its shows that yellowness active films are more the films without sorbate. The reason is related to the oxidation browning of sorbate in the films (Fama et al., 2005). Anyway increase of color was not so high that could decrease apparent acceptability of the films.

3.6. Potassium sorbate release

The amount of released sorbate from the film was determined by agar penetration method. As shown in Fig. 4, Nanocomposite films had higher ability for retaining PS in both 10 and 25 °C. In 25 °C, after 4 h, nanocomposite film, released only 40% of the initial PS, while this was more than 90% of starch film alone. Like starch films, nanocomposite films released sorbate at lower temperatures, more slower than high temperatures. Nanocomposite films could retain PS for longer time in 10 °C. These films released 80% of its initial sorbate after 48 h. The reason for low release rate in nanocomposite films is due to the nanoclay silicate layers and their agglomerates which could act as a spot or points for storing additives and a factor for retardation of their diffusion from film texture (Mascheroni, Guillard, Gostaldi, & Chalier, 2011). The migration rate of active compounds from packaging films has a strong relationship with storage temperature. The storage temperature could affect antimicrobial activity and the rate of the migration of them. The migration rate of antimicrobial substances increased with increasing temperature, while during storage at low temperatures, diffusion rate decreased. Migration kinetic of these substances should be in such a way that the concentration of antimicrobial additives be higher than the critical inhibitory concentration. Therefore temperature during storage, distribution, and handling could be effective in antimicrobial activity (Ojagh, Rezaei, Razavi, & Hosseini, 2010). The pH of the external media slightly influences the PS release rate, this effect might be related to the increase in the solubility of sorbate with pH (Flores, Fama, et al., 2007; Flores, Haedo, et al., 2007).

3.7. Stability of PS in films

Table 3 shows the potassium sorbate amounts of starch and its nanocomposite films with 10% PS after 60 days storage in petridishes at 50% RH and ambient temperature. There is a few differences between the initial amounts added and the measured PS content of films. Evaluation of the effect of storage time on PS content after 60 days, showed that in both kinds of films, PS was reduced. The amount of reduction in nanocomposites was lower than starch films. After 60 days storage, starch and its

Table 2
Surface color parameters of starch–clay nanocomposite films.

PS concentration (%W/W starch)	L^*	a^*	b^*	ΔE
0	89.40 ± 0.80^{b}	-1.29 ± 0.06^{c}	4.06 ± 0.15^{a}	4.80 ± 0.60^{a}
5	88.60 ± 0.12^{ab}	-1.77 ± 0.00^{b}	7.25 ± 0.01^{b}	7.70 ± 0.63^{b}
7.5	88.12 ± 0.03^{a}	-1.80 ± 0.03^{b}	7.67 ± 0.04^{b}	8.27 ± 0.21^{bc}
10	87.89 ± 0.22^{a}	-1.94 ± 0.03^{a}	8.76 ± 0.58^{c}	9.29 ± 0.61^{c}

[a–c] Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$). Potassium sorbate (PS) and color difference (ΔE).

Table 3
PS retention in films after 60 days storage.

Film type	PS added to film formulation (g/100 g starch)	Measured PS content in film (g/100 g starch)	PS content in film after 60 days (g/100 g starch)
Starch film	10	9.71 ± 0.32	7.53 ± 0.27
Starch–clay nanocomposite film	10	9.66 ± 0.24	8.76 ± 0.15

nanocomposite films loosed 22% and 9% of their initial sorbate amounts, respectively. The reason for the higher retention of sorbate in nanocomposite films is the inhibition of oxygen penetration which reduced. So sorbate in nanocomposite films exposed to lower amounts of oxygen and will retain more in film. Gutierrez and coworkers showed that the addition of MMT into carboxymethylcellulose films, decreased the permeability to water vapor, oxygen and carbon dioxide of them (Gutierrez, Echeverria, Ihl, Bifani, & Mauri, 2012). Other researchers reported the relation of storage conditions and other additives in formulation to the oxidation of sorbate (Flores, Fama, et al., 2007; Flores, Haedo, et al., 2007; Lopez, Giannuzzi, Zaritzky, & Garcia, 2013).

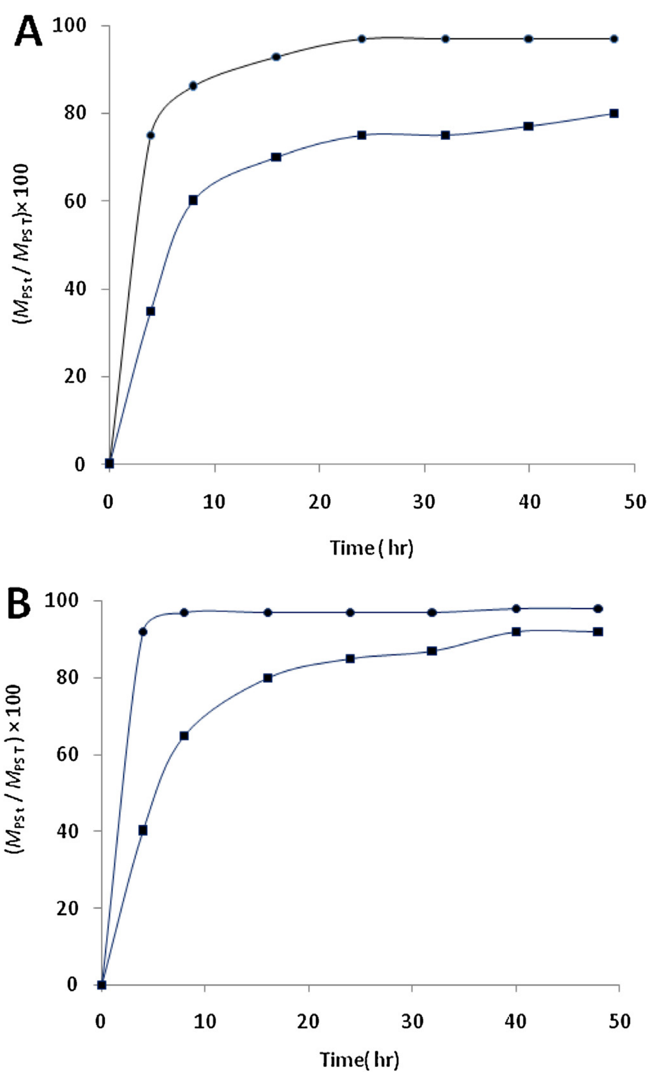

Fig. 4. Release of sorbates from starch films. *Panel A*: at 10 °C. (●) starch film; (■) nanocomposite film; *panel B*: at 25 °C. (●)starch film; (■) nanocomposite film. $M_{PS,t}$: amount of potassium sorbate released at time t; $M_{PS,T}$: amount of potassium sorbate contained in the film at time zero.

4. Conclusions

Adding sorbate to the starch–nanoclay films cause increase of WVP, elongation at break and decrease of tensile strength. The amounts of 7.5% and more could inhibit the growth of *A. niger* in vitro.

In both systems of antimicrobial packaging (film or coating), the concentration of antimicrobial agents in films and coatings are more than the amount that may add to foods but with the migration of these additives from surrounding film into the foods, the concentration of them will reduce in the packaging films. At the end, the film will be emptied from the antimicrobial agents.

Therefore the rate of migration should be controlled in order to inhibit the early evacuation of antimicrobial agents as a fast migration. The addition of silicate layers into the starch film, retard the release rate of antimicrobial agent from the film, and enables the film to retain its antimicrobial properties suitably. The presence of silicate layers could retard the oxidation of PS in the active films. Active starch–clay nanocomposite films could be applied successfully for preserving foods especially bakery products.

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