# *Effect of Natamycin on Physical Properties of Starch Edible Films and Their Effect on* Saccharomyces cerevisiae *Activity*

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**Food and Bioprocess Technology**
An International Journal

ISSN 1935-5130

Food Bioprocess Technol
DOI 10.1007/s11947-012-0960-0


Springer

Food Bioprocess Technol
DOI 10.1007/s11947-012-0960-0

ORIGINAL PAPER

# Effect of Natamycin on Physical Properties of Starch Edible Films and Their Effect on *Saccharomyces cerevisiae* Activity

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Received: 24 May 2012 / Accepted: 26 August 2012


**Abstract** The effectiveness of using a tapioca starch–glycerol matrix containing natamycin to control *Saccharomyces cerevisiae* activity in a model system was studied and the effect of the formulation on physico-chemical properties was also evaluated. The presence of natamycin tended to depress firmness at break and Young modulus and to increase strain at break. Colour was also affected by antimycotic presence. The importance of these changes will be determined by the characteristics of the product to which the antimicrobial film will be applied. The films developed were capable of acting as a hurdle against *S. cerevisiae* in food systems during storage: they acted as an effective reservoir of the antimycotic which was also available to prevent an external contamination. The films containing 1.85 mg natamycin/dm$^2$ of natamycin developed a fungistatic effect till 72 h of storage, while those with a 3.70-mg natamycin/dm$^2$ concentration developed a fungicidal action allowing the selection of the proper formulation according to the antimicrobial goal pursuit. As natamycin addition affects mechanical properties and colour of the films, it is advisable to use the lower natamycin concentration that allows the attainment of the goal pursued for film application.

**Keywords** Tapioca starch · Natamycin · Edible matrices · Physico-chemical properties · Antimicrobial properties

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## Introduction

Antimicrobial agents can be applied by dipping, spraying, or brushing to food surfaces for controlling microbial growth. However, these direct application techniques are laborious and have limited benefits (Ture et al. 2011), and the compound generally exhibits a rapid loss of activity due to a reduction of its active concentration resulting from interaction or reaction with food components. When the additive diffuses to the bulk of the food, a phenomenon of dilution also acts against its effectiveness. Incorporation of antimicrobials in foods by means of the use of edible films where they are entrapped collaborates to a decrease of their diffusion rate from the surface to the bulk of the product, thus assisting in the maintenance of high concentrations of the active ingredient where it is required (Kristo et al. 2008).

The use of natural antimicrobial compounds from a wide variety of natural sources is being explored as a means to improve the safety and stability of several foods while maintaining a natural, high quality and healthy product (Gould 1997). Natamycin, also known as pimaricin, is a polyene macrolide produced by *Streptomyces natalensis* and it is widely utilized in the food industry to prevent yeasts and moulds contamination of cheese and other non-sterile foods (El-Diasty et al. 2009; Gallo and Jagus 2007). It has been approved as a food additive in over 40 countries and has been considered as a GRAS (generally recognized as safe) product by the FDA (Koontz et al. 2003) and also designed as a natural preservative by the European Union (EEC No. 235). Natamycin acts by

Published online: 12 September 2012

binding the membrane sterols, primarily ergosterol, the principal sterol in fungal membrane, distorting the selectivity of the membrane permeability, and therefore, it is active against fungi but not against bacteria (te Welscher et al. 2010, 2008).

Several studies have reported that antimicrobial films based on different hydrocolloids and containing natamycin possess potential ability to inhibit the microorganisms on food products. Natamycin-impregnated cellulose-based films showed inhibitory effects against *Penicillium roquefortii* present on the surface of Gorgonzola cheese (de Oliveira et al. 2007). Its combination with nisin included into cellulose film formulations improved the shelf life of sliced mozzarella cheese (Dos Santos Pires et al. 2008). Chitosan coating containing natamycin decreased mould/yeast population on Saloio cheese after 27 days of storage (Fajardo et al. 2010). Also, Ture et al. (2011) showed that methylcellulose and wheat gluten films containing natamycin could have potential to be used in the prevention and control of toxigenic moulds on dairy products such as cheese samples, in combination with other preventive measures in a hurdle concept.

According to bibliography, the addition of antimicrobials like potassium sorbate or nisin to polysaccharide matrices increases the solubility, tensile strain and water vapour permeability of the films, stating the importance of evaluating the adequate concentration to be used for attaining the technological objective pursued without impairing to much other functionalities of the films as well as their integrity (Sanjurjo et al. 2006; Flores et al. 2007a).

To the best of our knowledge, scarce data exist pertaining to the activity of natamycin when incorporated in edible films, and essentially, no data at all encompassing incorporation in tapioca starch films.

Therefore, the purpose of this study was to evaluate the effectiveness of using a tapioca starch matrix containing natamycin to control *Saccharomyces cerevisiae* activity in a model system and also to analyse the effect of natamycin presence on the physico-chemical properties of the edible starch matrices studied.

## Materials and Methods

### Materials

Tapioca starch was provided by Industrias del Maíz S.A. (Argentina). Glycerol was provided by Mallickrodt (Argentina) and commercial natamycin (Delvocid® Salt) containing 50 % NaCl and 50 % natamycin was provided by DSM (The Netherlands) Argentina branch.

### Film Preparation

Mixtures of starch, glycerol and water (1.8:1:32.5, in weight) or of starch, glycerol, water and commercial natamycin were prepared. In this last case, 10 ml of water was replaced with a solution of natamycin of adequate concentration for obtaining a final concentration of 1.85 mgnatamycin/dm$^2$ (I), 3.70 mgnatamycin/dm$^2$ (II) or 9.25 mgnatamycin/dm$^2$ of film (III) in the system of pure natamycin. Starch gelatinization was performed at a constant rate of 1.5(±0.2)°C/min attaining a final temperature of 82 °C; for this purpose, a magnetic device was used and constant agitation was performed. Vacuum was applied to remove air from the gel. The slurry was dispensed in aliquots of 12 g in plates of 7 cm diameter. The drying of the films was performed at 37 °C during 48 h in a convection camera. Once constituted, films were peeled off from plates, and before evaluating film properties, samples were conditioned at 28 °C, over saturated solution of NaBr (water activity, $a_w \cong 0.575$) during 7 days.

### Physical Properties

#### *Sample Thickness*

Sample thickness was measured, using a digital micrometer (Mitutoyo, Japan), at three different positions in each specimen and to the nearest 0.01 mm; average and standard deviation (SD) are reported.

#### *Mechanical Properties*

For studying the tensile stress–strain behaviour, the strain rate was fixed in a value of 0.8 mm/s and the experiment was performed till rupture, recording the stress $\sigma$ ($F/A$, being F the force and A the area of the specimen), the strain $\varepsilon$ ($H/$long, being $H$ the deformation occurred and long the initial effective length of the sample) and the firmness ($F=\sigma/\varepsilon$) at break ($\sigma_b$, $\varepsilon_b$, $F_b$) from the tensile curves obtained. Young modulus was calculated as the slope of the linear region of the curves stress–strain. Samples used had a length of 60 mm and a width of 6 mm. The initial distance between grips was 20 mm. Assays were performed seven times for each sample and condition.

#### *Solubility in Water*

Solubility is defined (Gontard et al. 1992) as the percentage of film dry matter solubilized after 24 h of immersion in distilled water. The initial percentage of dry matter was determined by drying 2 cm diameter disks in a vacuum oven at 100 °C during 24 h. Additional disks were cut, weighed and immersed in 50 ml of distilled water, with periodic stirring, during 24 h at 25 °C. Not solubilized films were taken out and dried to determine the final weight of dry matter. Solubility is reported as the difference between initial and final dry matter with respect to initial dry matter.

Experiments were performed in two separate trials for each sample in duplicate.

*Colour Evaluation*

Film disks of appropriate diameter were rested on white background standard (Trezza and Krochta 2000). Measurements were performed in a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan) using an aperture of 1.5 cm in diameter. The exposed area was sufficiently great relative to the illuminated area to avoid any light trapping effect. The CieLab parameters: $L^*$, $a^*$ and $b^*$, and the yellow index (YI) were measured according to a standard test method (ASTM E1925 1995), in at least five positions randomly selected for each sample. Colour parameters range from $L=0$ (black) to $L=100$ (white), $-a$ (greenness) to $+a$ (redness) and $-b$ (blueness) to $+b$ (yellowness). Standard values considered were those of the white background. Calculations were made for D-65 illuminant and 2° observer.

The total colour difference ($\Delta E$; Hill et al. 1997) was evaluated for the samples treated with respect to their respective controls using the following equation:

$$\Delta E = \left[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right]^{1/2}, \; being:$$

$$\Delta L* = L* - L_0*$$

$$\Delta a* = a* - a_0*$$

$$\Delta b* = b* - b_0*$$

where $L_0^*$, $a_0^*$ and $b_0^*$ correspond to the values evaluated in the standard of comparison and $L^*$, $a^*$ and $b^*$ to values in the sample tested.

*Water Vapour Permeability*

Water vapour permeability (WVP) of films was determined gravimetrically at 25 °C using a modified ASTM E96-00 (2000) procedure.

The permeation cells (acrylic cups) had an internal diameter of 4.4 cm and an external diameter of 8.4 cm (exposed area=15.205 $cm^2$). They were 3.5 cm deep and contained $CaCl_2$ (0 % relative humidity (RH); 0 Pa water vapour partial pressure). Films were adjusted at the top of the cell with four screws located describing a cross, leaving a 7-mm air gap beneath the film. Seals of rubber and vacuum grease helped to assure a good seal.

The cup was placed in a temperature and RH controlled chamber (Ibertest, España) maintaining a temperature of 25 °C and a RH of 70 % (≅2,288 Pa water vapour partial pressure). After ≅16–20 h, a stationary water vapour transmission rate was attained, and from that moment on, changes in weight of the cell (to the nearest 0.1 mg) were recorded daily over a 24–48-h period. All tests were conducted, at least, in triplicate and WVP values were calculated using the WVP Correction Method described by Gennadios et al. (1994).

Microscopy Analysis

*Optical Microscopy (OM)*

The morphologies of starch films with and without natamycin were examined with an optical microscope (Olympus® BX43, Japan).

Tested samples were prepared by cutting cross sections of each type of film which were placed between slides and observed at different magnifications: ×40, ×100 and ×400. Images were taken with a digital camera (Q-Color 3C) and then analysed with an image analyser (Qcapture pro® 6.0, QImaging, Canada).

*Atomic Force Microscopy (AFM)*

The surface topography of the edible films C and III was studied with an atomic force microscope-AFM (NanoScope IIIa, Quadrex, Digital Instrument. Veeco, Melville, New York, USA) provided with a silicon cantilever of 0.06 N/m elastic constant operating at 300 kHz of resonance frequency. Films were scanned using the tapping mode and under nitrogen. Scan size was set to obtain 5.0×5.0 nm images. Two to three areas of each film were scanned using the tapping mode under nitrogen. 3D images were obtained with the software WSxM 4.0 Develop 11.3-Package (2007, Nanotec Electronica, Madrid, Spain) which was also used for image analysis (Horcas et al. 2007).

The quantitative parameter "roughness" was calculated with the data of the topographical micrographs by means of the previously mentioned software. The roughness average (Ra) is the mean of the difference, in absolute value, between the average height and the height of each single point of the sample. It shows how much rough the sample is. It is given by the following expression:

$$\mathrm{Ra} = \frac{\sum_{i=1}^{N} Z_i - \overline{Z}}{N} \; with \; \overline{Z} = \sum_{i=1}^{N} \frac{Z_i}{N} \quad (II.6)$$

being $Z_i$ the height value of each single point (nm), $Z$ the average height (nm) and $N$ the number of experimental points. It was also calculated the root mean square of the roughness,

Rq, which has the following expression (Ghanbarzadeha and Oromiehib 2008):

$$\mathrm{Rq} = \sqrt{\sum_{i=1}^{N} \frac{\left(Z_i - \overline{Z}\right)^2}{N}}$$

## Microbiological Assay

### *Strains and Growth Conditions*

*S. cerevisiae* (CBS 1171, strain collection SC) was grown in 150 ml Sabouraud broth at 28 °C in a continuously agitated temperature-controlled shaker (1.79×*g*) until early stationary phase was achieved.

### *Agar Diffusion Method*

The agar diffusion test was used to determine the antimicrobial effect of films on the test microorganisms. Briefly, 100 μl of inoculum containing $1\times10^6$CFU/ml of *S. cerevisiae*, prepared in the previous step, was spread on the surface of Petri dishes containing chloranphenicol glucose agar, (YGC, Biokar Diagnostics, France), with the pH adjusted to 5.2 (citric acid 50 % *w*/*w*).

Film disks (7 mm diameter) without natamycin, namely control (C), and films with different concentration of natamycin (I, II and III) were placed on plates previously inoculated. The plates were pre-incubated at 4 °C for 48 h and afterward incubated at 28 °C for 72 h (Hanušová et al. 2010). The inhibitory activity was quantified by measuring the total diameter (disk plus inhibition zone).

### *Barrier to Microbial Contamination*

The experimental design proposed by Sanjurjo et al. (2006) was adapted and applied with the object of testing the effectiveness of tapioca starch films containing natamycin against external yeast contamination. Petri dishes containing YGC agar with pH 5.2 were used to resemble a food product. Disks of 1.0 cm diameter were cut from films control, I, II and III and brought in contact with the surface of the agar. Immediately, 10 μl of a culture of *S. cerevisiae*, containing $1\times10^6$CFU/ml, was dispensed on the disks. Samples were incubated at 25 °C during 168 h and periodically sampled to test yeast viability.

The initial and surviving number of viable cells at different storage times was evaluated. Dilution drops (20 μl) were spotted in duplicated onto agar YGC and the number of CFU per milliliter was determined after incubation at 28 °C for 72 h. Enumeration of colonies was performed, and inhibition of microorganisms growth was expressed as log CFU per milliliter. Determinations were made in duplicate in two separate experimental runs.

### *Barrier to Microbial Contamination at Different Times*

Disks of 1.0 cm diameter were cut from films control, I, II and III, and brought in contact with the surface of the YGC agar. After different contact times (0, 6 or 10 days), 10 μl of a culture of *S. cerevisiae*, containing $1\times10^6$CFU/ml, was dispensed on the disks in order to resemble contamination during storage.

All the samples were incubated at 25 °C, and the inoculated disks were sampled at 0, 24, 48 and 72 h after inoculation to test yeast viability. Determinations were made in duplicate.

## Statistical Analysis of Data

Data were analysed through a two-way ANOVA with $\alpha$= 0.05 and Tukey was the post hoc test applied. Results are informed on the basis of their average and confidence interval for an $\alpha$=0.05. The results are reported based on their mean and standard deviation (Sokal and Rohlf 2000). The software GraphPad Prism®, version 5.01 (GraphPad Software, Inc., a privately held California corporation) was used for the treatment and analysis of data.

# Results and Discussion

## Physical Properties

### *Atomic Force Microscopy (AFM)*

Figure 1 show the AFM topographic images and the 3D topographic plots of films named control and III. Control film presented a more smooth and homogenous surface topography than film with natamycin which showed more peaks and depressions.

The AFM allowed obtaining the roughness parameters for the surfaces of films C and III. Both films, C and III, exhibited low values of roughness parameters: Ra= 14.00 nm±1.55 and 11.00 nm±2.07, respectively, and Rq=10.00 nm±1.02 and 9.00 nm±1.52, respectively, with non-significant differences ($p$>0.05) between them. Arzate-Vázquez et al. (2012) reported similar roughness parameters for a chitosan-based film, 8.60 and 11.26 nm for Ra and Rq, respectively.

### *Mechanical Properties*

Tapioca starch based edible films studied in the present work are retrograded starch systems that have been

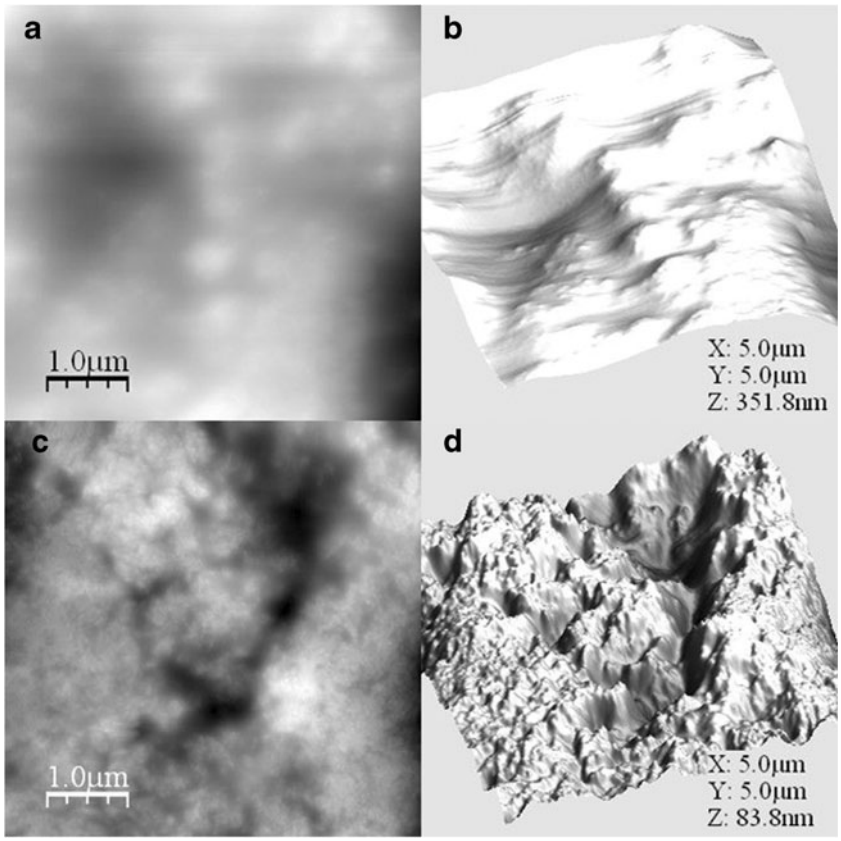


**Fig. 1** AFM images: **a** tapioca film C, *top view*; **b** tapioca film C, *3D view*, all scanned by a tapping mode; **c** tapioca film III, *top view*; **d** tapioca film III, *3D view*. (magnification—**a**, **c** 1 μm, **b**, **d** 5 μm)

plasticized with glycerol. Figure 2 shows the tensile stress ($\sigma$)–strain ($\varepsilon$) behaviour of the film III and control film, as an example. It can be observed that the deformation nature of the polymers at room temperature, under an applied load, was typical of ductile plastics in terms of the stress and strain. As generally occurs for those materials, the films exhibited two characteristic regions of deformation behaviour in their tensile stress–strain curves. At low strains (lower than 10 %), the stress increased rapidly with an increase in the strain and the initial slopes were steep in the elastic region, indicating the high elastic modulus of the materials. At higher strain (higher than 10 %), the films showed a slow increase in stress with strain. It is important to remark that the initial slope of the stress–strain curves, which is defined as the Young modulus depends on the temperature and the strain rate (Mano and Viana 2001).

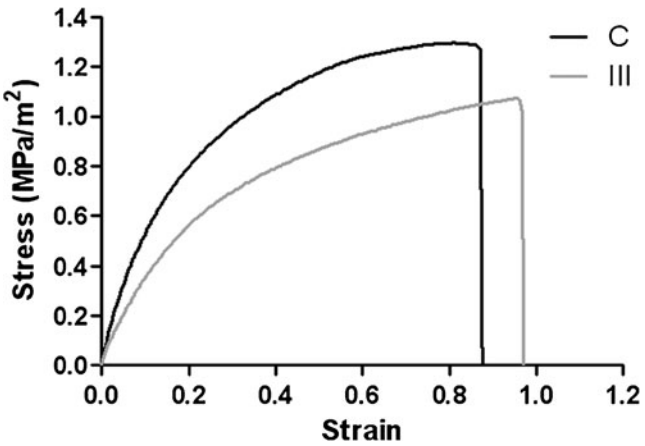


**Fig. 2** Stress–strain curves for systems (III) and without (C) natamycin

Table 1 clearly shows that the films without natamycin showed a higher firmness at break and Young modulus (this difference is only significant between control film and film II) and lower strain at break than the films with antimicrobial and that behaviour, in general, was independent on the natamycin content. With respect to stress at break although a trend to lower values was observed when the antimycotic was present, no significant differences were detected. Probably, antimicrobial presence produced less organized networks that showed higher deformation and lower firmness al break.

### *Solubility and Water Vapour Permeability (WVP)*

Film solubility and WVP did not show significant differences between formulations (Table 2).

The resistance of films to water, determined by the solubility in water test, is critical for the potential application of films. Sometimes, high water solubility is desired. This is the case when the film or coating will be consumed simultaneously with the food. However, in general, a high solubility might affect film integrity impairing its response as a

**Table 1** Textural parameters obtained from the tensile assay for films (I, II, III) and without (C) natamycin

| Condition | Stress at break (MPa/m$^2$) | Strain at break | Firmness at break (MPa/m$^2$) | Young modulus (MPa/m$^2$) |
|---|---|---|---|---|
| C | 1.349±0.1493$^a$ | 0.8375±0.08814$^a$ | 1.619±0.1808$^a$ | 6.192±0.9363$^a$ |
| I | 1.240±0.1687$^a$ | 1.018±0.3396$^{a,b}$ | 1.289±0.3494$^{a,b}$ | 5.318±0.6023$^a$ |
| II | 1.271±0.1636$^a$ | 1.147±0.1048$^b$ | 1.119±0.1987$^b$ | 4.936±1.022$^b$ |
| III | 1.202±0.02202$^a$ | 1.067±0.02658$^b$ | 1.127±0.03985$^b$ | 4.867±0.5405$^{a,b}$ |

Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p<0.05$)

barrier to contamination or its acting as reservoir of antimicrobial. Flores et al. (2007a) observed that the addition of potassium sorbate increased the solubility of tapioca starch edible films obtained by casting from ≈20 % to 30–35 %. They also informed (Flores et al. 2011) that the addition of nisin to tapioca starch edible films increased the solubility of film from ≈20 % to 32 %. On the contrary, data from the present research shows that the addition of natamycin did not increase the solubility of the films developed.

It has been reported a low oxygen transmission rate for polysaccharide based edible films and a high water vapour permeability. In general, incorporation of antimicrobials to edible films increases water vapour permeability, probably due to the loosening of the polymeric network. This trend was observed for the addition of potassium sorbate to chitosan/tapioca starch films (Vásconez et al. 2009) and of nisin to chitosan films (Pranoto et al. 2005). Concerning the values observed for water vapour permeability (WVP) of different edible films, Flores et al. (2007a, 2011) reported values of (0.6–2.0).$10^{-9}$g/s m Pa for different techniques of film production and in the presence or absence of potassium sorbate. In particular, the results obtained in this research are $4.10^{-9}$g/s m Pa which are higher than those previously mentioned and did not change with the addition of the antimicrobial natamycin.

### *Colour*

Table 3 shows colour parameters for studied films. It can be observed that, in general, films containing antimicrobial showed higher $L^*$, $b^*$ and YI and lower $a^*$ values. Additionally, this trend was enhanced in general, with natamycin concentration increase. It can be also observed that the parameter $a^*$ was negative for all films and $b^*$ showed positive values, showing a green-yellow colour for the films.

It can be observed on Table 3 that films containing the highest antimicrobial concentration showed the highest yellow index and the highest $\Delta E$.

**Table 2** Solubility and water vapour permeability (WPD) for films (I, II, III) and without (C) natamycin

| Condition | Solubility (%) | WVP (g/seg.m.Pa) |
|---|---|---|
| C | 21.81±7.42$^a$ | 3.92E-09±1.11E-11$^a$ |
| I | 17.98±6.20$^a$ | 3.71E-09±2.20E-10$^a$ |
| II | 24.40±4.24$^a$ | 3.91E-09±2.46E-10$^a$ |
| III | 18.72±6.65$^a$ | 3.68E-09±2.58E-11$^a$ |

Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p<0.05$)

## Antimicrobial Effectiveness of Films

### *Diffusion in Solid Medium*

Diffusion of natamycin occurred forms the edible matrices to the agar. Clear zones were observed around the samples of film, the fact that showed the inhibitory activity exerted by the antimicrobial and this activity was quantified by measuring the diameter of the clear zone.

Results obtained for *S. cerevisiae* showed that the tapioca starch-based films without antimicrobials could not inhibit the yeast growth at the film-medium interface. In the case of film I also a slight growth was observed below its surface. In contrast, the starch films II and III yielded a clear inhibition zone around the film disk, with a total diameter (disk plus inhibition zone) of 32 mm, showing antimicrobial diffusion to the solid medium. Pintado et al. (2010) studied the effect of nisin, natamycin and malic acid incorporated in whey protein films against *Yarrowia lipolytica* and *Penicillium* spp. strains. They observed that the film containing natamycin produced a zone of inhibition of 8.0 and 11.9 mm for these microorganisms.

It is important to state that in all cases the films increased in diameter due to swelling of the hydrophilic matrices, a trend that could influence the results observed (Flores et al. 2007b). Baumgartner et al. (2005) reported that when a hydrophilic matrix comes in contact with water, it swells due to hydration, forming a gel layer that acts as a diffusion barrier. Additionally, the thickness of the gel layer not only increases with the swelling time, but its polymer concentration profile is continuously changing, affecting the mesh size of the polymer network and thus the antimicrobial release.

**Table 3** Colour parameters for films (I, II, III) and without (C) natamycin

Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p<0.05$)

| Condition | $L^*$ | $a^*$ | $b^*$ | YI | $\Delta E$ |
|---|---|---|---|---|---|
| C | $88.32\pm0.45^{a}$ | $-1.20\pm0.03^{a}$ | $3.76\pm0.16^{a}$ | $6.77\pm0.31^{a}$ | $0^{a}$ |
| I | $88.29\pm0.35^{a}$ | $-1.29\pm0.02^{b}$ | $4.11\pm0.18^{a}$ | $7.41\pm0.35^{a}$ | $0.37\pm0.17^{b}$ |
| II | $88.62\pm0.40^{a,b}$ | $-1.41\pm0.03^{c}$ | $4.39\pm0.16^{b}$ | $7.84\pm0.31^{b}$ | $0.73\pm0.23^{c}$ |
| III | $88.70\pm0.53^{b}$ | $-1.58\pm0.03^{d}$ | $5.06\pm0.17^{c}$ | $9.02\pm0.36^{c}$ | $1.41\pm0.19^{d}$ |

These outcomes indicate the effectiveness of tapioca starch based films containing natamycin for acting as natamycin reservoir and for inhibiting the *S. cerevisiae* growth in a solid matrix, due to the liberation of the antimycotic from the film. Results are in agreement with those observed for other authors with different edible matrices. Franssen et al. (2004) showed that whey protein films have the ability to carry and release natamycin. Also Fajardo et al. (2010) demonstrated the efficacy of chitosan films to be a carrier for liberation of natamycin.

### *Film as Barrier to Microbial Contamination*

Several authors have assessed both the barrier properties and the diffusion of preservatives into different matrices (Flores et al. 2007b; Sebti et al. 2004). However, Cong et al. (2007) considered that the rationale for incorporating antimicrobials into food coatings is to prevent microbial growth on the surface where a large portion of spoilage and contamination occurs. One of the major potentials of this hurdle lies in the storage of semi-moist foods (Chen et al. 1996). According to this, it was tested the effectiveness of tapioca starch films containing natamycin against external yeast contamination. Results obtained during storage at 25 °C are shown in Fig. 3.

Tapioca starch films without antimicrobials (C) allowed yeast growth. Therefore, these films are not suitable to act as a barrier against an external contamination of *S. cerevisiae*. These results are similar to those of Basch et al. (2011) who studied the activity of tapioca starch films against *Zygosaccharomyces bailii*, observing that this film did not affect the growth of studied yeast.

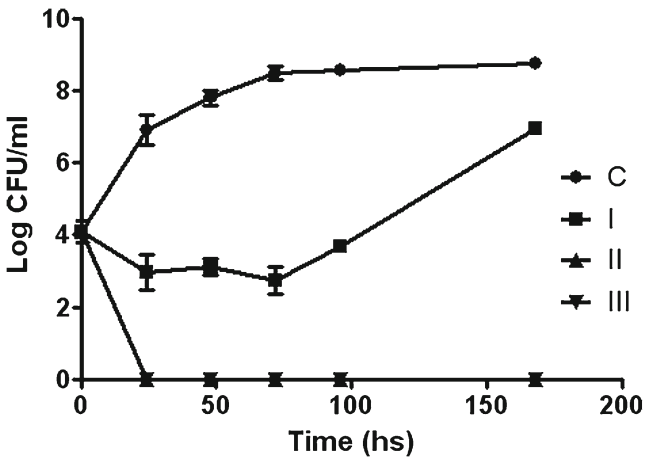


**Fig. 3** Tapioca starch films as barrier to external contamination by *Saccharomyces cerevisiae* during storage at 25 °C

For tapioca starch films containing natamycin, results showed that the preservative was available to prevent an external contamination of *S. cerevisiae*. The antimycotic effect exerted by the films increased with natamycin content of the films. Film I exerted a slight reduction in the first 75 h. Even though growth was thereafter restored, reaching at the end of the storage was a population of about two log cycles lower than the film C. Films II and III produced an important reduction and maintained a count <10 CFU/ml until the end of the evaluated period (>150 h).

Other authors studied the ability of films to act as barrier to external contamination with different matrices and antimicrobials. Ramos et al. (2012) studied the efficacy of edible films produced from whey protein isolate, glycerol and natamycin as antimicrobial agent. The authors showed through the viable cell counting assay that natamycin incorporated in the film led *Y. lipolytica* to depletion within 3 h of storage at 30 °C. Flores et al. (2007b) studied the performance of tapioca starch films containing potassium sorbate, an additive widely used as antimycotic agent in the food industry, observing that the preservative was available to act as a barrier for external yeast contamination, and exerted a bacteriostatic effect against *Z. bailii.*

### *Films as Barrier to Contamination at Different Times*

Food is normally susceptible to microbiological contamination through process, storage and distribution. The present assay was performed in order to resemble potential contamination during different stages of food processing.

The antimicrobial activity of films inoculated after different times of contact (a=0 day or b=6 days or c=10 days) of the films with the solid medium can be observed in Fig. 4. The activity of natamycin diminished when the contact period implemented previous to yeast inoculation was increased as can be noticed, clearly comparing the performance of the films II and III in Fig. 4a, b, c. It can be also remarked that films I for a 10-day contact time previous to

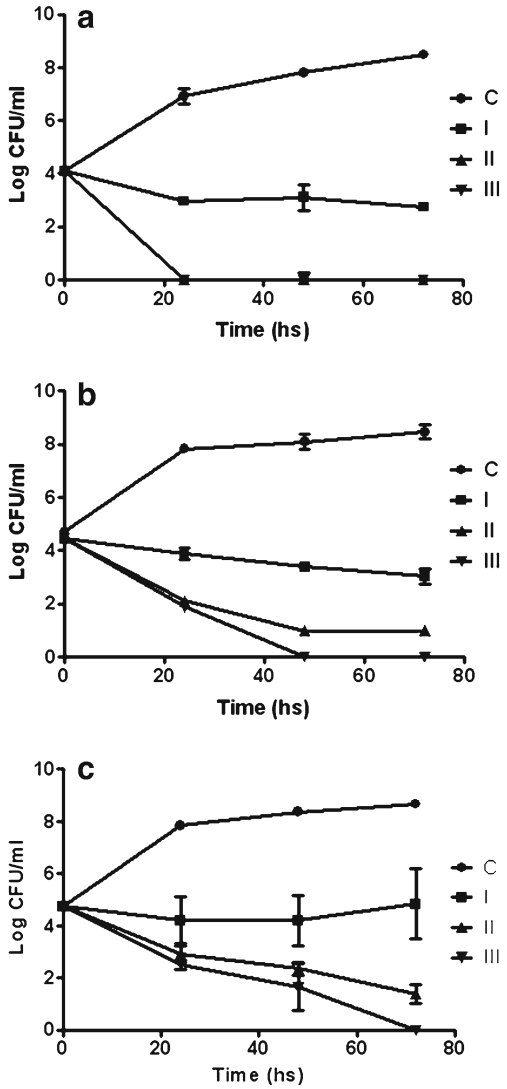


**Fig. 4** Tapioca starch films as barrier to external contamination by *Saccharomyces cerevisiae* at different times. **a** Inoculation after 0 days of contact. **b** Inoculation after 6 days of contact. **c** Inoculation after 10 days of contact

inoculation, also showed an increase at 72 h of yeast CFU per milliliter (Fig. 4c) trend that was not observed for smaller contact times previous to inoculation. Anyhow, it can be concluded that films with natamycin exerted a fungistatic (film I) or fungicidal (films II and III) action in this experiment while the control film could not inhibit yeast growth. In particular, films II and III maintained a good performance after 10 days of contact previous to inoculation.

Fajardo et al. (2010) studied the release of natamycin from chitosan-based edible films. They studied the diffusion process to different media: a solid food and liquid medium (PBS solution). In this last case, they obtained a higher diffusion coefficient than that obtained for the release to a solid medium. They attributed the increase of the diffusion coefficient to the swelling effect of the matrix in contact with a medium of high water activity. In the same way, in our experiments, the reduction of antimicrobial activity along the contact, with a medium of high water activity (Fig. 4), could be attributed to the swelling of the film matrix, which might allow a higher diffusion of natamycin producing a lower concentration of the preservative in the film determining a lower efficiency to act as a barrier to an external contamination.

Additionally, these results are in accordance with the optical microscopy observation along the contact period. The optical photomicrographs (Fig. 5) show the morphology of the matrix of control film and film III after different times of contact with a high $a_w$ (0.99) surface. The control films showed a homogeneous matrix (Fig. 5a) without changes throughout the entire test (not shown). However, film III presented irregularities in the matrix (Fig. 5b) showing inclusions of crystalline structures of natamycin, with straight and defined edges. During the contact with high $a_w$ surface, the structure of the natamycin crystals changed with contact period, losing its shape and decreasing in size (Fig. 5c). Probably, these crystals act as reservoirs of natamycin activity and the loose of their shape with time

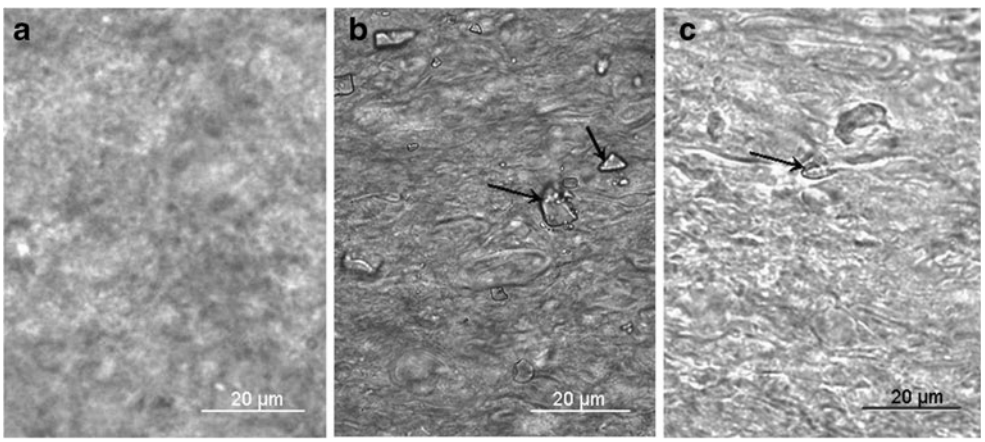


**Fig. 5** Optical photomicrography of superficial view of films C and III after different times of contact. **a** Film C after 0 days of contact. **b** Film III after 0 days of contact. **c** Film III after 10 days of contact (magnification ×400)

involves their incorporation in the film matrix helping to sustain natamycin antimicrobial activity along the assay compensating, at least partially, the loss of natamycin activity due to antimicrobial destruction through oxidative mechanisms (Thomas 1976).

## Conclusion

Tapioca starch-based films containing natamycin inhibited the *S. cerevisiae* growth in a solid matrix, showing to be an effective reservoir of the antimycotic. The preservative was also available to prevent an external contamination of *S. cerevisiae* and its effect increased with natamycin content; contaminations produced 10 days after film application on the food model could also be controlled showing sustainability of film protection.

The presence of natamycin tended to depress firmness at break and Young modulus and to increase strain at break. Colour was also affected by antimycotic presence.

The characteristics of the product to which the antimicrobial film will be applied and the final purpose of its use will balance these trends determining the proper concentration that is convenient to be used. But as films formulated are thought as conveyors of the antimicrobial and colour and mechanical properties being an important attribute in relation to consumer acceptance of food products, the benefits of using the smaller concentration of natamycin that is adequate for the goal pursuit are highlighted: film I can be applied for fungistatic effect during a period of 72 h, and for fungicidal effect for long storages, it is adequate to use film II.

**Acknowledgements** This study was financially supported by University of Buenos Aires (UBACyT 20020100100125), National Agency of Scientific and Technical Research (PICT 1172), and CONICET (PIP 531). The authors also wish to thank Industrias del Maíz S.A. (Argentina), Mallickrodt (Argentina) and DSM (Argentina).

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