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Natamycin and nisin supported on starch edible films for controlling mixed culture growth on model systems and Port Salut cheese

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ABSTRACT

Consumer demand for natural food additives has increased and, as a consequence, the use of natural antimicrobials like natamycin and nisin is being investigated. In the case of cheese, surface colonization by microorganisms constitutes a significant risk to consumer's health. In this study, the effectiveness of natamycin and nisin supported in tapioca starch films against *Saccharomyces cerevisiae* and *Listeria innocua* in a mixed culture present on the surface of a model system and of Port Salut cheese was evaluated. It was observed that the preservatives incorporated in starch films, controlled growth of both microorganisms present together on the surface of the cheese during storage. Additionally, the joint presence of nisin and natamycin was effective as a barrier against a mixed culture preventing an external contamination of cheese and of a model system, during storage. Hence, this film has great potential to be used as antimicrobial edible packaging.

1. Introduction

Cheese is a ready-to-eat food susceptible to physical, chemical and microbiological deterioration throughout storage and distribution (Cha & Chinnan, 2004). During processing, the pathogenic bacteria risk is diminished by the pasteurization of raw milk, the control of the length of maturation and of the storage temperature of cheese. This temperature together with some intrinsic properties such as pH, water activity and the presence of antimicrobial compounds produced by starter culture, constitute a 'hurdle' system. However, colonization of cheese surface by microorganisms constitutes a significant risk (Kousta, Mataragas, Skandamis, & Drosinos, 2010) due to its high water content and favorable pH for microbial growth (Conte, Angiolillo, Mastromatteo, & Del Nobile, 2013).

Several authors have reported that *Saccharomyces cerevisiae* is one of the spoilage microorganisms most frequently isolated from ripening cheeses which causes undesirable visual appearance and diminishes the shelf life of cheeses (Deak & Beuchat, 1996; Welthagen & Viljoen, 1998).

Listeria monocytogenes is a pathogenic microorganism, which has been associated with listeriosis produced by consumption of cheese (Mc Lauchlin, Mitchell, Smerdon, & Jewell, 2004). *Listeria innocua* has been used as a model microorganism for the pathogenic *L. monocytogenes*, because it is physiologically closely related (Soares Pinto et al., 2009). Also, both microorganisms can be isolated from cheeses.

Addition of antimicrobial agents reduces or prevents the growth of pathogenic and spoilage microorganisms (Franssen, Rumsey, & Krochta, 2004). In recent years, consumer demand for natural food ingredients has increased and, as a consequence, the use of natural antimicrobials from a wide variety of natural sources has begun to be explored (Gould, 1997; Tiwari et al., 2009).

Natamycin is currently used as an antimicrobial agent to prevent cheese surface spoilage. It is produced by *Streptomyces natalensis* and is commonly employed in dairy-based food products to prevent yeast and mould contamination (El-Diasty, El-Kaseh, & Salem, 2008). 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, Marcy, Barbeau, & Duncan, 2003) and also designed as a natural preservative by the European Union (EEC N° 235). It is known that it kills yeasts by specifically binding to

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ergosterol and without permeabilizing the plasma membrane. It inhibits vacuolar fusion through the specific interaction with ergosterol (Te Welscher et al., 2008, 2010). Therefore, it is active against fungi but not against bacteria.

Nisin is an antimicrobial peptide produced by strains of *Lactococcus lactis* subsp. *lactis*, recognized as safe for food applications by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives. Nisin exhibits antimicrobial activity towards a wide range of Gram positive bacteria, including *L. monocytogenes* (Martins, Cerqueira, Souza, Carmo Avides, & Vicente, 2010).

For controlling microbial growth, antimicrobials can be applied on food solid surfaces by different techniques: spraying, dipping or brushing. However, these direct application techniques have limited benefits (Ture, Eroglu, Ozen, & Soyer, 2011) and the antimicrobials generally exhibit a rapid loss of activity. Recently, the research community and the food industry showed an increasing interest in active edible films supporting antimicrobials for the purpose of enhancing food safety and extending food shelf life due to their potential to decrease antimicrobial 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. Also, edible films can be an alternative source for packaging materials development due to their biodegradability (Dos Santos Pires et al., 2008; Fajardo et al., 2010; Kristo et al., 2008; Ollé Resa, Gerschenson, & Jagus, 2013; Ture et al., 2011).

To the best of our knowledge there has been no research reported neither on the activity of nisin and natamycin incorporated together in tapioca starch films against a mixed culture nor on the behavior of this film in a real food system. Therefore, the aim of this study was to evaluate the effectiveness of natamycin and nisin supported in edible films against *S. cerevisiae* and *L. innocua* in a mixed culture present on the surface of a model system and of Port Salut cheese and as a barrier to an external contamination.

2. Materials and methods

2.1. Materials

Tapioca starch was provided by Industrias del Maíz S.A. (Argentina). Glycerol was provided by Mallinckrodt (Argentina). The antimicrobials, commercial natamycin (Delvocid® Salt) containing 50%*w*/*w* NaCl and 50%*w*/*w* natamycin, and commercial nisin (DelvoPlus®) containing 97.5%*w*/*w* NaCl and 2.5%*w*/*w* nisin, were provided by DSM (The Netherlands) Argentina branch. Port Salut cheese (La Serenisima®, Argentina) was purchased in a local supermarket.

2.1.1. Film preparation

Different mixtures of starch, glycerol, water and natural antimicrobial (each one alone or in combination) were prepared. For the sake of obtaining edible films with adequate mechanical properties, it was necessary to formulate the mixtures with different quantities of glycerol, the compound used as plasticizer. Previous trials suggested that the formulations described below were suitable.

2.1.1.1. Film with natamycin (NA). Starch, glycerol and water (1.8:1:32.5, in weight) were mixed to constitute the control system, named CNA. For preparing the film NA, 300 g of slurry were prepared, with the mixture previously stated, but 10 g of water was replaced by 10 g of a solution of natamycin of adequate concentration for obtaining a final concentration of 0.027 g natamycin/100 g slurry (or 9.25 mg natamycin/dm^2 of film).

2.1.1.2. Film with nisin (NI). Starch, glycerol and water (3:1:56, in weight) were mixed to constitute the control system, named CNI. For preparing the film NI, 300 g of slurry were prepared with the mixture previously stated, but 10 g of water was replaced by 10 g of a solution (pH2) of nisin of adequate concentration for obtaining a final concentration of 0.0068 g nisin/100 g slurry (or 2.31 mg nisin/dm^2 of film).

2.1.1.3. Film with nisin and natamycin (NANI). Starch, glycerol and water (2.5:1:46.5, in weight) were mixed to constitute the control, named CNANI. For preparing the film NANI, 300 g of slurry was prepared with the mixture previously stated, but 20 g of water was replaced by: i) 10 g of a solution of natamycin of adequate concentration for obtaining a final concentration of 0.027 g natamycin/100 g slurry (or 9.25 mg natamycin/dm^2 of film) and ii) 10 g of a solution (pH2) of nisin of adequate concentration for obtaining a final concentration of 0.0068 g nisin/100 g slurry (or 2.31 mg nisin/dm^2 of film).

In all cases, starch gelatinization was performed at a constant rate of ~1.5 °C/min attaining a final temperature of 82 °C. Vacuum was applied to remove air from the gel when necessary. 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 chamber. Once constituted, films were peeled off from plates and, before evaluating film properties, samples were conditioned at 28 °C, in a dessicator over saturated solution of NaBr (water activity, $a_w \cong 0.575$) for 7 d.

2.2. Microbiological assay

2.2.1. Strains and growth conditions

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

L. innocua (CIP 80.11) was grown in 150 ml tryptone soya broth with yeast extract (TSBYE, Biokar Diagnostics, France) at 28 °C in a continuously agitated temperature-controlled shaker overnight. Finally, 2 ml of inoculated broth was added onto fresh TSBYE and agitated 1 h till desired cell concentration.

For preparation of mixed culture, each of the microorganisms was grown in its corresponding broth to achieve desired density (specified for each assay). Thereafter, 30 ml aliquots of each culture were centrifuged at 10,000 rpm, each cell pellet was resuspended in 15 ml of TSBYE and both suspensions were mixed together.

2.2.2. 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 approximately 1×10^6 CFU/ml of *S. cerevisiae* or *L. innocua* or a mixed culture with a concentration of approximately 1×10^6 CFU/ml of each microorganism and prepared in the previous step, were spread on the surface of Petri dishes containing YGC agar (Biokar Diagnostics, France, agar selective for yeasts and moulds), TSYE agar (Biokar Diagnostics, France, unrestricted agar) and $TSYE_C$ agar (TSYE containing cycloheximide, Sigma–Aldrich, agar selective for bacteria), respectively.

Film discs (7 mm diameter) of all treatments (CNA, NA, CNI, NI, CNANI and NANI), 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 (disc plus inhibition zone). As in some cases, the halo observed was not completely transparent, a certain growth was suspected. In this case, it was performed an assay of growth in selective media (Oxford agar and

YGC agar) for checking the microorganism responsible for the lack of transparency in the agar.

2.2.3. Barrier to mixed microbial contamination at different times

Assays were performed using cheese samples and TSYE agar with pH 5.2 which resembled a food product, to test the efficacy of studied films as barriers to an external contamination of food surface with a mixed culture after food production. Additionally, with the objective to test the ability of the film to control food contamination along storage, an assay at 5 d of film-food contact was designed for the model system.

Disks of 1.0 cm diameter were cut from all films (CNA, NA, CNI, NI, CNANI and NANI), and brought in contact with the surface of the agar or the cheese. Then, 10 μl of mixed culture of *S. cerevisiae* and *L. innocua* containing 1×10^6 CFU/ml each, were dispensed on the disks. In the case of the 5 d film-food contact test, the procedure was performed after 5 d of contact. Samples were incubated at 25 °C during 168 h and periodically sampled, to test microorganism viability.

The initial and surviving number of viable cells at different storage times was evaluated in the films. Dilution drops (20 μl) were spotted in duplicate onto agar YGC and Oxford (Biokar Diagnostics, France, agar selective for *L. innocua*), and the number of CFU/ml was determined after incubation at 28 °C for 72 h and at 37 °C for 48 h, respectively. Enumeration of colonies was performed, and microorganism growth was expressed as logCFU/ml. In order to investigate if the film prevented the contamination of the agar or cheese during the assay, after removal of the film, each system was incubated at 28 °C for 24 h. Afterward, the appearance of colonies was checked. Determinations were made in duplicate in two separate experimental runs.

2.2.4. Cheese diffusion method for mixed cultures

The cheese diffusion test was used to determine the antimicrobial effect of films in a real food system (Port Salut cheese). Briefly, pieces of cheese (2.5 × 2.5 × 0.5 cm; 5 ± 0.3 g) were cut with a sterile knife and placed on sterile petri dishes. Afterward, 20 μl of the mixed culture of *S. cerevisiae* and *L. innocua* containing 1×10^6 CFU/ml each, were spread on the surface of cheese.

Film squares (5 cm side) of the different films (CNA, NA, CNI, NI, CNANI and NANI), were placed on cheese previously inoculated. Also a spray containing the same antimicrobial concentration than that containing in the films (SNA, SNI, SNANI) were tested.

The plates were incubated at 25 °C for 192 h. The initial and surviving number of viable cells in the cheese was evaluated at different times. For this, cheese samples were homogenized in peptone water (1:10) and dilution drops (20 μl) were spotted in duplicate onto agar YGC and Oxford, and the number of CFU/ml was determined after incubation at 28 °C for 72 h and 37 °C for 24 h, respectively. Enumeration of colonies was performed, and microorganism growth was expressed as logCFU/ml. Determinations were made in duplicate in two separate experimental runs.

2.3. Statistical analysis of data

Data were analyzed through two-way ANOVA with α: 0.05 and Tukey was the post-hoc test applied. Results are reported based on their mean and standard deviation (Sokal & Rohlf, 2000). The software GraphPad Prism®, version 5.01(Graph Pad Software, Inc., California) was used for the treatment and analysis of data.

3. Results and discussion

3.1. Agar diffusion method

Diffusion of natamycin and nisin occurred from the edible matrices to the agar as can be observed in Table 1. Clear zones were observed around the samples of film, 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 showed that the tapioca starch based films without antimicrobials (controls CNA, CNI, CNANI) could not inhibit the microorganism growth at the film–medium interface, indicating that neither starch nor glycerol exerted an antimicrobial effect. In contrast, the natamycin contained in the films and released into the agar (NA) was effective to inhibit the growth of *S. cerevisiae* in simple and mixed cultures. It is noteworthy that in the TSYE agar for the mixed culture, below the yeast's halo of inhibition, growth of bacteria was observed (shadiness), as these were not inhibited by natamycin released into the medium. These results are in agreement with those observed for other authors with different edible matrices. Fajardo et al. (2010) showed that chitosan films have the ability to support and release natamycin. Also Ramos et al. (2012) evaluated the incorporation of natamycin in films obtained from whey protein isolate. These authors observed through *in vitro* evaluation, that natamycin displayed a strong effect against yeast.

The nisin contained in the films and released into the agar (NI) was effective to inhibit the growth of *L. innocua*, regardless of the presence of *S. cerevisiae*, since the halo in the simple culture showed no significant differences with the mixed culture. It is remarkable that in the TSYE agar for the mixed culture, below the bacteria's halo of inhibition, growth of yeast was observed (shadiness), as these were not inhibited by nisin released into the medium. Basch, Jagus, and Flores (2013) performed an agar diffusion test using edible films containing nisin and also observed that they did not produce an inhibition zone against *Zygosaccharomyces bailii*.

Table 1
Diffusion assay. Diameter of inhibition zones (cm) in single and mixed cultures generated from films containing nisin (film NA), natamycin (film NA) or natamycin and nisin (film NANI).

		L. innocua	*S. cerevisiae*	Mixed culture (*S. cerevisiae* and *L. innocua*)
Film NA	YGC	W/G	$2.97 \pm 0.17^{a,A}$	$3.25 \pm 0.17^{a,A}$
	TSYE	W/H	$2.95 \pm 0.17^{a,A}$	$3.15 \pm 0.17^{a,A}$
	$TSYE_C$	W/H	W/G	W/H
Film NI	YGC	W/G	W/H	W/H
	TSYE	$1.84 \pm 0.07^{a,A}$	W/H	$1.95 \pm 0.07^{b,A}$
	$TSYE_C$	$1.84 \pm 0.07^{a,A}$	W/G	$2.04 \pm 0.40^{b,A}$
Film NANI	YGC	W/G	$2.85 \pm 0.07^{a,A}$	$3 \pm 0.07^{a,A}$
	TSYE	$1.85 \pm 0.07^{a,A}$	$2.95 \pm 0.07^{a,A}$	Double halo: $3.05 \pm 0.07^{a,A}$ and $1.85 \pm 0.07^{b,A}$
	$TSYE_C$	$1.85 \pm 0.07^{a,A}$	W/G	$2.25 \pm 0.40^{b,A}$

W/G: without growth; W/H: without halo. Different lowercase letters in the same column indicate significant differences ($p < 0.05$). Different capital letters in the same row indicate significant differences ($p < 0.05$).

The film containing nisin and natamycin was very effective in inhibiting the growth of *S. cerevisiae* in YGC and TSYE agar in simple culture, and the diameter of the halo is similar to that observed with the film NA. When the combined film was evaluated in the presence of a mixed culture, in YGC and TSYE agar, the halo for the yeast was similar to the one observed when *S. cerevisiae* was alone. The film NANI exhibited inhibition against *L. innocua*, showing an inhibition halo of 1.85 cm in the TSYE and $TSYE_C$ agar in simple or mixed cultures. When the film NANI faced a mixed culture in TSYE agar (unrestricted growth of both microorganisms), two halos were observed (Fig. 1). It was verified that the smallest corresponded to the inhibition of growth of *L. innocua* and the biggest to the one of *S. cerevisiae*. Both diameters were similar to those observed when the bacteria and the yeast were tested individually.

It can be concluded that the presence of nisin in the film did not affect the bioavailability of natamycin and vice versa.

Pintado, Ferreira, and Sousa (2010) studied the effect of whey protein films containing nisin and natamycin alone and in combination, against spoilage and pathogenic microorganism isolated from cheese surface. These authors observed the formation of halos with similar size to those obtained in the present study and reported no difference between the zones of inhibition obtained with nisin alone or in combination with natamycin against tested bacteria (*L. monocytogenes* and *Pseudomonas aeruginosa*), or between the zones of inhibition obtained with natamycin alone or in combination with nisin against tested yeasts (*Yarrowia lipolytica* and *Penicillium roquefortii*).

3.2. Barrier to mixed microbial contamination at different times

Several authors have evaluated the barrier properties of nisin and natamycin in different matrices (Cong, Zhang, & Dong, 2007; Fajardo et al., 2010; Ollé Resa et al., 2013; Pintado et al., 2010; Ture, Eroglu, Soyer, & Ozen, 2008).The present assay was adapted from the one designed by Sanjurjo, Flores, Gerschenson, and Jagus (2006) and applied with the object of testing the effectiveness of tapioca starch films containing natamycin and nisin against external microbiological contamination during different stages of food storage. Results obtained during storage at 25 °C are plotted in Fig. 2.

Antimicrobial activity was not observed in the control consisting of a tapioca starch film disks with different concentration of glycerol and without incorporation of antimicrobials (CNA, CNI and CNANI). Because of that only one control was presented in Fig. 2. Therefore, these films are not suitable to act as a barrier against an external contamination of *S. cerevisiae* and/or *L. innocua*.

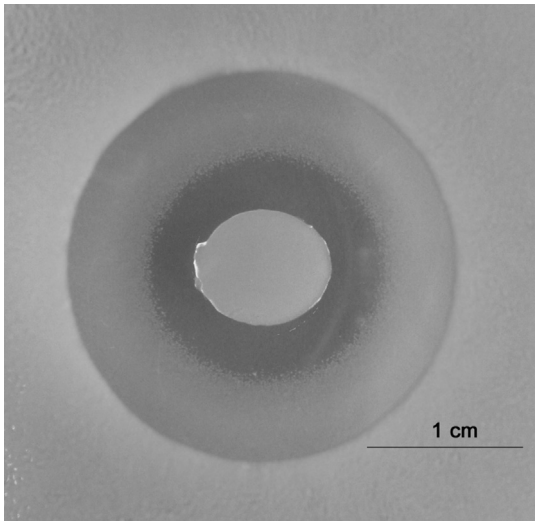

Fig. 1. Diffusion assay. Diameter of inhibition zone (cm) in mixed cultures generated from film containing natamycin and nisin (film NANI) in an unrestricted agar.

The natamycin present in films NA and NANI was effective to prevent an external contamination of *S. cerevisiae* even after 5 d of storage al 25 °C (Fig. 2a and b). Ollé Resa et al. (2013) also observed that tapioca starch film containing 9.25 mg of natamycin/dm^2 of film maintained a good performance after 10 d of contact previous to inoculation with *S. cerevisiae*.

Film NI had a similar behavior initially and at 5 d of storage, being only effective as a barrier against *L. innocua* during the first 48 h after inoculation. Afterward, the bacteria restored growth having at the end of the storage, a population similar to control film.

The activity of nisin present in film NANI changed with the contact period. It was extremely effective as a barrier to *L. innocua* (no growth until 196 h) when contamination was produced initially ($t = 0$; Fig. 2c). When contamination occurred after 5 d of contact it was observed an initial reduction of counts followed by a regrowth. This trend shows that film NANI presented a lower effectiveness than film NI (Fig. 2d). Probably, the changes occurred in film NANI during the 5 d of contact, affected nisin bioavailability in this film.

In this assay, additionally, all the films with and without antimicrobials prevented the contamination of the agar (data not shown). Furthermore, it is important to note that this barrier inhibits the growth of *S. cerevisiae* (NA and NANI) and controls the growth of *L. innocua* (NI and NANI) allowing the consumer to receive a safer product. It is important to mention that a similar barrier assay was performed in cheese with films with and without antimicrobials (data not shown) observing similar results to the ones previously reported for the food model.

3.3. Cheese diffusion method for mixed cultures

This assay was performed only with a mixed culture because this is a more realistic situation faced to during cheese preservation and storage.

Again, antimicrobial activity was not observed in the control consisting of a tapioca starch film disks with different concentration of glycerol and without incorporation of antimicrobials (CNA, CNI and CNANI), showing a similar behavior. Therefore, only the results of one control were presented in Fig. 3. Therefore, these films are not suitable to act as reservoir of antimicrobials and for inhibiting *S. cerevisiae* or *L. innocua* or its combination.

The use of film NI exerted no effect on yeast growth (Fig. 3a). It can be observed at 24 h that film NANI and spray NANI (SNANI) decreased the initial yeast counts in 2 log cycles, reassuming afterward the growth. At the end of the storage (196 h) the presence of natamycin (spray NA, spray NANI, film NA and film NANI) presented *S. cerevisiae* counts of ~1.5 cycles lower than the control and film NI, being film NANI the most effective one. Ollé Resa, Jagus, and Gerschenson (2014) studied the effectiveness of natamycin supported in tapioca starch films against different yeasts present in cheese as simple culture. These authors observed that a film containing 9.25 mg of natamycin/dm^2 of film, exerted an initial fungicidal effect against *S. cerevisiae*. However, growth was thereafter restored, reaching at the end of the storage a population of about 5 log cycles lower than that presented in cheese without antimicrobial. Probably, the reason of the different behavior observed of the initial response of *S. cerevisiae* could be attributed to the different initial cell concentration in the two studies. Also, in the present assay, the yeast was in a mixed culture instead of a single one.

When *L. innocua* growth was evaluated, it could be observed that the spray NI and spray NANI produced an instantaneous

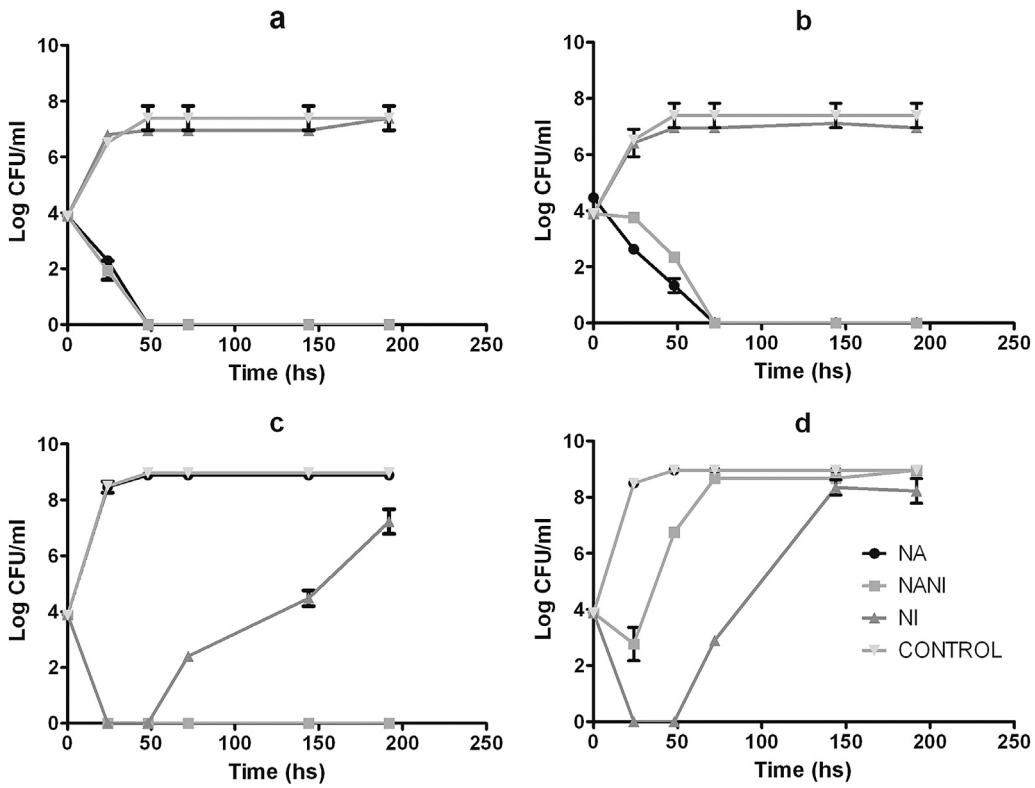

Fig. 2. Tapioca starch films as barrier to external contamination by mixed cultures at different times at 25 °C. a,c: Inoculation after 0 days of contact, b,d: Inoculation after 5 days of contact, a,b: *Saccharomyces cerevisiae*, c,d: *Listeria innocua*.

decrease of its counts and at 24 h of storage, spray and films NI and NANI determined a count lower than 10 CFU/ml (Fig. 3b). These trends show that initially, the presence of natamycin did not affect nisin availability. For longer times, the bacteria in the presence of spray NI and film NI reassumed growth. At the end of the storage (196 h) film NI is more effective for the bacteria showing 3.29 log counts lower than the spray NI and 7.66 log counts lower than the control cheese. The cheese treated with spray NANI and film NANI showed counts lower than 10 CFU/ml till end of storage resulting in an excellent hurdle for *L. innocua* control. It must be remarked that film NA and spray NA showed an unexpected bacteriostatic effect on *L. innocua* in this mixed culture.

The yeast *S. cerevisiae* responds to increased external osmolarity by enhanced production and intracellular accumulation of glycerol to counterbalance the osmotic pressure (Blomberg & Adler, 1992; Mager & Varela, 1993). Ethanol is produced as a result to ensure reoxidation of the NADH formed during the synthesis of glycerol, in the frame of a redox-equilibrated process (Michnick, Roustan, Remize, Barre, & Dequin, 1997). As the commercial natamycin has a support containing 50% of NaCl and the assay was performed in cheese presence which behaved as a nutrient medium, it can be postulated that the production of ethanol by the yeast could reduce the growth of *L. innocua*, giving origin to the trends observed concerning bacteriostatic effect of NA. Oh and Marshall (1993) observed that ethanol at concentrations up to 1.25% did not inhibit growth of *L. monocytogenes*, but growth was strongly inhibited in the presence of 5% ethanol.

Albertin et al. (2011) found that nutrient presence in growth medium is one of the main factors that control ethanol biosynthesis. Probably, the absence of nutrients in the barrier assay (no contact with a rich nutrient source as cheese) precluded ethanol formation showing a different trend concerning bacterial growth.

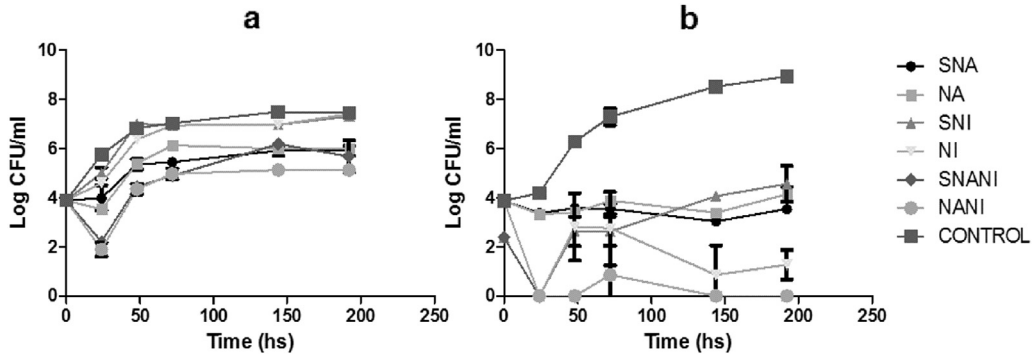

Fig. 3. Cheese diffusion method. Growth of mixed cultures during storage at 25 °C, a: *Saccharomyces cerevisiae*, b: *Listeria innocua*.

4. Conclusion

The results obtained in the agar diffusion test showed the positive effect of incorporation in the film of two antimicrobials, since the presence of nisin did not affect the bioavailability of natamycin and vice versa.

All the films tested as barrier prevented the contamination of the agar and cheese. Additionally, the films containing natamycin (NA and NANI) inhibited the growth of *S. cerevisiae* and NI and NANI controlled the growth of *L. innocua*, allowing the consumer to receive a safer product.

Moreover, the film containing both antimicrobials (NANI) controlled *S. cerevisiae* and *L. innocua* growth, present together on the surface of Port Salut cheese during storage.

The overall results indicate that the incorporation of nisin and natamycin together in tapioca starch matrices can be used to control contamination by a mixed culture in a food model system and in Port Salut cheese. Hence, this film has great potential to be used in antimicrobial packaging.

However, future studies of the effectiveness of these films need to be performed at lower temperatures. On the other hand, physico-chemical and mechanical evaluation of the films are planned to attain their complete characterization.

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