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Effect of natamycin, nisin and glycerol on the physicochemical properties, roughness and hydrophobicity of tapioca starch edible films

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ABSTRACT

In this paper, films based on tapioca starch and containing nisin, natamycin and glycerol were characterized in relation to their physicochemical properties, roughness and hydrophobicity. The content of glycerol affected the mechanical properties of the films studied and the roughness and it was observed an increase in WVP with the increase in glycerol content. The addition of antimicrobials affected the mechanical properties, being nisin the one that produced the greater decrease in the Young modulus. The color was highly affected by the joint presence of natamycin and nisin, which increased the yellow index. The contact angle increased with antimicrobial addition indicating a decrease in hydrophilicity. Nisin also affected the roughness of the films. Water vapor permeability was slightly reduced by the presence of natamycin. It was observed that water vapor permeability and contact angle were correlated with the roughness of the films.

1. Introduction

An increased interest in environmental protection has motorized in recent years, the development of renewable, biodegradable and compostable packaging. The biodegradability is an important attribute of the materials for their contribution to environmental protection. The compostability attribute allowing disposal of the packages in the soil is very important for biopolymeric materials because recycling is energy expensive.

According to the European Bioplastics association, bioplastics made with renewable resources can be biodegradable and compostable, so they can act as fertilizers and soil conditioners [1].

Most recently, the food industry showed an increasing interest in antimicrobial edible films to enhance food safety and to extend food shelf life due to their contribution to the decrease of 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 [2]. Nowadays, polysaccharide, protein and lipid biopolymers are being intensively studied with the purpose of developing edible films that can potentially serve as coating materials for environmentally friendly packaging [3]. Films with antimicrobial activity based on controlled preservative release provide a promising form of active packaging systems applicable in food processing [4–7]. The addition of antimicrobial agents can reduce or even prevent the growth of pathogenic and spoilage microorganisms [8]. Bacteriocins and fungicide substances incorporated in edible films have been tested for their capability to control spoilage and pathogenic microorganisms in food systems. Nisin is a bacteriocin produced by *Lactococcus lactis* that shows a broad spectrum of activity against Gram-positive bacteria. It has been widely used as a safe and natural preservative in the food industry [9,10]. Natamycin is a fungicide agent classified as a macrolide polyene, produced during fermentation by the bacterium *Streptomyces natalensis* and is commonly employed in dairy-based food products to prevent yeast and mould contamination [11,12].

Tapioca is a significant crop in South America, and it is an economical source of starch [13]. Tapioca starch-based edible films exhibit appropriate physical characteristics because they are odorless, tasteless, colorless and impermeable to oxygen. Glycerol is one of the most popular plasticizers used in edible film-making techniques due to stability and compatibility with hydrophilic biopolymer chains and its addition is necessary to improve the film flexibility [14,15].

Although extensive information on the antimicrobial properties of natamycin and nisin is available in the literature, scarce data exist in relation to the activity of natamycin and nisin incorporated together in tapioca starch films against a pool of different microbial species, and no data at all are available in relation to the physicochemical properties of edible films containing both antimicrobials. Therefore, the objective of this study was to evaluate the effect of nisin, natamycin and glycerol on physicochemical properties, roughness and hydrophobicity

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of tapioca starch films for the sake of contributing to their characterization and safe use.

2. Materials and methods

2.1. Materials

Tapioca starch was provided by Industrias del Maíz S.A. (Argentina). Glycerol was provided by Mallickrodt (Argentina). The antimicrobials, commercial natamycin (Delvocid® Salt) containing 50% w/w NaCl and 50% w/w natamycin and commercial nisin (Nisin®) containing 97.5% w/w NaCl and 2.5% w/w nisin, were provided by DSM (The Netherlands) Argentina branch.

2.1.1. Film preparation

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

For films 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 was prepared, with the mixture previously stated, but 10 g of water was replaced by 10 g of a solution of natamycin of adequate concentration (0,815 g/100 g) for obtaining a final concentration of 0.027 g natamycin/100 g slurry (or 9.25 mg natamycin/dm^2 of film).

For films 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 was prepared with the mixture previously stated, but 10 g of water was replaced by 10 g of a solution (pH 2) of nisin of adequate concentration (0,204 g/100 g) for obtaining a final concentration of 0.0068 g nisin/100 g slurry (or 2.31 mg nisin/dm^2 of film).

For films 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 (0,815 g/100 g) 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 (pH 2) of nisin of adequate concentration (0,204 g/100 g) 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 camera. Once constituted, films were peeled off from plates, and before evaluating film properties, samples were conditioned at 28 °C, over a saturated solution of NaBr (water activity, aW ≅0.575) during 7d.

2.2. Films properties

2.2.1. 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. These values were used for water vapor permeability and stress calculation.

2.2.2. Mechanical properties

For studying the tensile stress–strain behavior, the strain rate was fixed in a value of 0.8 mm/s, and the experiment was performed till rupture, recording the stress σ (F/A, being F the force and A the area of the specimen), the strain ε (H/long, being H the deformation occurred and long the initial effective length of the sample) and the firmness at break ($\boldsymbol{F}_b = \sigma_b/\varepsilon_b$), which is calculated on the basis of the stress (σ_b) and the strain at break (ε_b) evaluated from the tensile curves obtained. The Young modulus was calculated as the slope of the initial linear region of the curves stress vs 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 ten times for each sample and condition.

2.2.3. Color evaluation

Film disks of appropriate diameter were rested on white background standard [16]. Measurements were performed in a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan) using an aperture of 1.5-cm 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 D1925, 1995), in at least five positions randomly selected for each sample and for D-65 illuminant and 2° observer. Color parameters range from $L^* = 0$ (black) to $L^* = 100$ (white), $-a^*$ (greenness) to $+a^*$ (redness) and $-b^*$ (blueness) to $+b^*$ (yellowness).

The total color difference (ΔE) was evaluated for the films containing antimicrobials with respect to their respective controls using the following equation [17]:

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

$$\Delta L^* = L^*\text{-}L_0{}^*$$

$$\Delta a^* = a^*\text{-}a_0{}^*$$

$$\Delta b^* = b^*\text{-}b_0{}^*$$

where $L_0{}^*$, $a_0{}^*$ and $b_0{}^*$ correspond to the values for the control and L^*, a^* and b^* to the values for the sample containing antimicrobials.

2.2.4. Contact angle and wettability

The contact angle of the films was estimated by the sessile drop method, based on the optical contact angle method, following the Choi and Han [18] procedure. A droplet of distilled water (3 μl) was deposited on the film surface with an automatic piston syringe. Contact angle measurements were carried out using a tensiometer (Sinterface PAT-1, Germany) equipped with a CCD camera. The digital camera was placed horizontally in order to capture the drop image. An image analyzer software (Profile Analysis Tensiometer PAT-1, Germany) was used to measure the angle formed between the surface of the film in contact with the drop and the tangent to the drop of liquid at the point of contact with the film surface. Both film surfaces were tested, and twenty measurements were performed on each side of the film.

According to Zisman [19], in systems having a surface tension lower than 100 mN/m (low-energy surfaces), the contact angle formed by a drop of liquid on the solid surface will be a linear function of the surface tension of the liquid, γ_{LV} (where phase V is air saturated with the vapor of liquid L). If θ is the contact angle between the liquid, for which the γ_{LV} is known, and some solid, the interaction can be described in terms of the reversible work of adhesion, W_a, as

$$W_a = \gamma_{LV}(1 + \cos\theta)$$

It is also possible to define the cohesion coefficient (Wc), or work of cohesion, by

$$W_c = 2\gamma_{LV}$$

which is related to the reversible work required to separate a liquid into two parts. While the adhesion forces cause the liquid to spread on the surface, the cohesion forces cause the liquid to contract. The balance between W_c and W_a gives the spreading coefficient, Ws, according to

$$W_s = W_a – W_c$$

The spreading coefficient or wettability, represents the ability of a given liquid to spread on a solid surface. The maximum value that can be obtained for this coefficient is zero [20].

2.2.5. Water vapor permeability

Water vapor permeability (WVP) of films was determined gravimetrically at 25 ºC 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 vapor 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 cups were placed in a temperature and RH-controlled chamber (Ibertest, Spain) maintaining a temperature of 25 ºC and an RH of 70% (≅2288 Pa water vapor partial pressure). After ≅16–20 h, a stationary water vapor transmission rate was attained, and from that moment and on, changes in weight of the cell (to the nearest 0.1 mg) were recorded daily over a 24- to 48-h period. All tests were conducted, at least, in triplicate, and WVP values were calculated using the WVP Correction Method described by Gennadios [21].

2.2.6. Roughness (Rq)

The surface topography of the edible films with and without antimicrobials were 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. Scan size was set to obtain 5.0 μm × 5.0 μm images. Two to three areas of each film were scanned using the tapping mode under nitrogen. The 3D images were obtained with the software WSxM 4.0 Develop 11.3-Package (2007, Nanotec Electronica S.L., Madrid, Spain), which was also used for image analysis [22].

The quantitative parameter roughness (Rq) was calculated with the data of the topographical micrographs by means of the previously mentioned software and using the following expression [22,23]:

$$Rq = \sqrt{\frac{\sum_{ij}\left(Z_{ij}-\overline{Z}\right)^2}{N}}$$

with:

$$\overline{Z} = \frac{\sum_{ij} Z_{ij}}{N}$$

being Z_{ij} the height value of each single point (nm), $\overline{Z}$ the average height (nm) and N the number of experimental points.

2.3. Statistical analysis of data

Data were analyzed for differences through two-way ANOVA with $\alpha = 0.05$, and Tukey was the post hoc test applied. Results are reported based on their mean and standard deviation [24].

The correlation between contact angle, Rq and WVP was analyzed through the Pearson product moment coefficient, widely used as a measure of the degree of linear dependence between two variables [25].

The software GraphPad Prism®, version 5.01 (Graphpad Software, Inc., a privately held California corporation) and the software Statgraphics Centurion XV (V. 15.2.06, 2007) were used for the treatment and analysis of data.

3. Results and discussion

The thickness of the films CNA and NA showed values of (0.284 ± 0.078) mm and (0.270 ± 0.030) mm, respectively. The thickness values of CNANI, NANI, CNI and NI were significantly lower, showing values of 0.198 ± 0.029 mm, 0.192 ± 0.013 mm, 0.204 ± 0.026 mm and 0.179 ± 0.010 mm, respectively.

3.1. Mechanical properties

Edible films should possess adequate mechanical strength and extensibility, so as to maintain integrity and withstand the external stresses that prevail throughout food processing, handling and storage, and they must also possess durability, when used to separate layers of homogeneous food [26]. The stress at break is a measure of the strength of the film, while the strain at break is a measure of the stretchability of the films before breaking. Both properties are important features to look for in a packaging material [27].

As can be seen in Fig. 1, CNA was the control film with less stress at break, probably because of its higher content of glycerol that affected the polymer network. Comparing the three control films, it could be concluded that the increase in plasticizer concentration in the film-forming solution produced less stiff and rigid, and hence more extensible films. In general, the addition of antimicrobials lowered the stress at break and increased the strain at break, which can be ascribed to the plasticizing action of the antimicrobial studied [28].

Films NANI and NI had similar values of stress and strain at break showing lower stress and greater deformation at break than NA, probably due to the strong plasticizing character of nisin.

As can be observed in Table 1, the Young modulus of CNA was not significantly modified by the presence of natamycin (NA). Ollé Resa et al. [5] reported that the presence of natamycin into a tapioca starch edible film did not significantly change the Young modulus, strain at break and stress at break. On the contrary, the addition of nisin to CNI significantly modified the modulus giving origin to the edible film NI with lower values for this parameter. Probably, it is the nisin presence the reason for the lower moduli of NANI in comparison to CNANI. Basch et al. [29] observed in edible films constituted by a blend of tapioca starch and hydroxypropyl methylcellulose (HPMC), that nisin addition caused a decrease of the stress at break and of the Young modulus. Sanjurjo et al. [30] also observed a similar behavior in the study of edible films based on tapioca starch and containing nisin. It can be concluded that nisin acted as a plasticizer affecting the formation of the starch network and, consequently, the mechanical properties of the films.

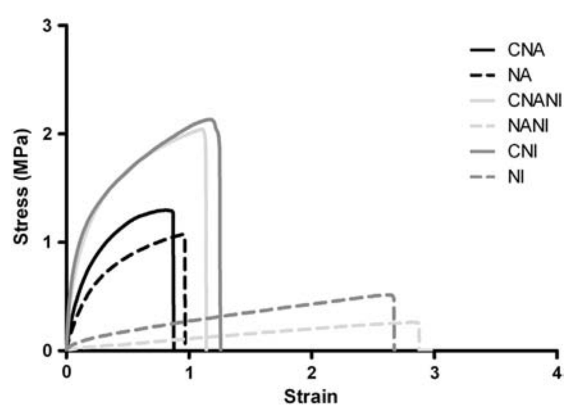

Fig. 1. Stress–strain curves for films with (NA, NANI, NI) and without (CNA, CNANI, CNA) antimicrobials.

Table 1
Textural parameters obtained from tensile assays for films with (NA, NANI and NI) and without (CNA, CNANI and CNA) antimicrobials.

Condition	Stress at break (MPa)	Strain at break	Firmness at break (MPa)	Young modulus (MPa)
CNA	1.263 ± 0.117[a]	0.833 ± 0.111[a,c]	1.527 ± 0.146[a]	6.638 ± 0.757[a]
NA	1.187 ± 0.012[a,b]	1.060 ± 0.029[a,b]	1.120 ± 0.009[b]	5.328 ± 0.609[a]
CNANI	1.637 ± 0.308[b,c]	1.480 ± 0.301[b]	1.106 ± 0.043[b]	11.250 ± 1.963[b]
NANI	0.273 ± 0.021[d]	2.823 ± 0.307[d]	0.097 ± 0.009[c]	0.315 ± 0.024[c]
CNI	1.935 ± 0.294[c]	1.180 ± 0.315[b,c]	1.647 ± 0.248[a]	12.320 ± 2.607[b]
NI	0.432 ± 0.097[d]	2.752 ± 0.387[d]	0.153 ± 0.039[c]	0.842 ± 0.439[c]

NA, film with natamycin; NI, film with nisin; NANI, film with natamycin and nisin; CNA, control for film NA; CNI, control for film NI; CNANI, control for film NANI.
Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p < 0.05$).

3.2. Color evaluation

Color of edible films is an important feature as consumers are attracted by the external appearance of the food matrix, both at the time of purchase and at the time of ingestion [31].

Table 2 shows the color parameters obtained. It can be seen that with the addition of nisin to the films (NI vs. CNI), parameters b^* and YI increased and L^* decreased. This phenomenon occurred to a greater extent by the joint addition of natamycin and nisin (NANI vs. CNANI). Basch et al. [29] observed similar effects in relation to nisin addition to edible films based on tapioca starch and HPMC. The addition of natamycin (NA) produced a smaller change in these parameters when comparing to the film without antimicrobial (CNA) as observed in Table 2. The same trend was previously reported by Ollé Resa et al. [5].

The parameter a^* tended to remain unchanged with the variation of glycerol as can be concluded by comparison of controls (CNA, CNI and CNANI) or with the addition of nisin (NI vs CNI) or natamycin plus nisin (NANI va CNANI). However, the addition of natamycin (NA vs CNA) increased a^*. The value of ΔE for systems NA, NI and NANI evaluated with respect to their controls, showed that the greater color change was observed when the antimicrobials were jointly added (NANI) probably due to the occurrence of reactions between both compounds or due to physical effects that can be attributed to their joint presence.

3.3. Water vapor permeability (WVP)

The water vapor permeability is the most extensively studied property of edible films mainly because of the importance of the water in food deteriorative reactions [32]. Table 3 shows the water vapor permeability (WVP) values for the different studied films.

It can be observed that WVP increased significantly with glycerol concentration (CNI < CNANI < CNA). Ghanbarzadeh et al. [33] and Ghasemlou et al. [34] observed that the WVP of zein films and kefiran films increased with glycerol concentration. Glycerol is a relatively small hydrophilic molecule, which can be inserted between adjacent polymeric chains, decreasing intermolecular forces and increasing molecular mobility in the film matrix. The increased mobility results in greater free volume and segmental motions, which facilitates the migration of water vapor molecules through the film [35]. Additionally, at a high concentration, glycerol can cluster with itself increasing the inter-chain spacing due to the inclusion of glycerol molecules between the polymer chains effect that may promote water vapor diffusivity through the film and hence accelerate the water vapor transmission [36].

There were no significant differences between WVP values of films with and without NI or NI plus NA (CNANI vs NANI and CNI vs NI). Grower et al. [37] observed the same trend for methylcellulose films with different nisin concentrations. Anyhow, it was observed a significant difference for films with and without natamycin (CNA vs. NA) in which the addition of the antimicrobial reduced the WVP value. Ollé Resa et al. [5] reported similar values of WVP for films with the same composition to CNA and NA but no significant differences were observed for both films.

3.4. Contact angle and wettability

The contact angle is the most common measure of wettability or surface hydrophobicity [38]. High contact angles (θ > 70°) indicate a hydrophobic surface and low contact angles (θ < 20°) are characteristic of hydrophilic surfaces [39]. Results reported in Table 3 showed that increasing glycerol concentration significantly diminished initial contact angle from ~30° for film CNI (1.7% glycerol) to ~7° for film CNA (2.8% glycerol). These results showed that the surface hydrophobicity of tapioca starch edible films decreased with the increase of glycerol concentration, which can be ascribed to the hydrophilic character of glycerol as informed by Carneiro-da-Cunha et al. [40]. A similar result was observed by Ahmadi et al. [41], who stated that increasing the glycerol in *psyllium* edible films produced a reduction of the contact angle of the deposited water droplet.

On the other hand, it can be seen in Table 3 that the addition of antimicrobials increased the contact angle (NA vs CNA, NANI vs CNANI and NI vs CNI), indicating that the hydrophobicity of the films increased. Bierhalz et al. [42] analyzed the contact angle formed over an alginate film containing natamycin and informed that the presence of the antimicrobial in the films led to higher contact angles, highlighting that the tendency to water sorption decreased and that the surfaces became more hydrophobic. However, to the best of our knowledge, no research has been previously conducted in relation to the evaluation of contact angle/hydrophobicity for edible films containing nisin or both antimicrobials together.

Table 2
Color parameters for films with (NA, NANI and NI) and without (CNA, CNANI and CNI) antimicrobials.

Condition	L^*	a^*	b^*	YI	ΔE
CNA	88.35 ± 0.45[a,c]	−1.19 ± 0.032[a]	3.86 ± 0.23[a,b]	6.98 ± 0.47[a,b]	0[a]
NA	88.92 ± 0.62[a,c]	−1.54 ± 0.051[b]	4.76 ± 0.36[a]	8.45 ± 0.71[a]	1.29 ± 0.32[b]
CNANI	89.07 ± 1.07[a]	−1.25 ± 0.059[a]	2.99 ± 0.25[b]	5.17 ± 0.54[b]	0[a]
NANI	85.41 ± 1.61[b]	−1.18 ± 0.55[a]	12.30 ± 2.12[c]	23.60 ± 4.20[c]	10.00 ± 2.36[c]
CNI	89.20 ± 1.05[a]	−1.27 ± 0.049[a]	2.94 ± 0.19[b]	5.04 ± 0.42[b]	0[a]
NI	87.99 ± 1.13[c]	−1.21 ± 0.18[a]	7.36 ± 1.15[d]	13.85 ± 2.45[d]	4.67 ± 1.35[d]

NA, film with natamycin; NI, film with nisin; NANI, film with natamycin and nisin; CNA, control for film NA; CNI, control for film NI; CNANI, control for film NANI.
Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p < 0.05$).

Table 3
Water vapor permeability (WVP), roughness (Rq) and contact angle for films with (NA, NANI and NI) and without (CNA, CNANI and CNA) antimicrobials.

Condition	WVP (g/seg mPa)	Rq (nm)	Contact angle (°)
CNA	3.920e−09 ± 1.414e−11[a]	10.86 ± 1.97[a]	7.80 ± 3.33[a]
NA	3.685e−09 ± 2.121e−11[b]	9.93 ± 1.55[a]	29.00 ± 7.06[b]
CNANI	1.885e−09 ± 6.718e−11[c]	17.79 ± 2.35[b]	16.89 ± 9.24[c]
NANI	1.034e−09 ± 6.576e−11[c]	32.85 ± 1.55[c]	55.60 ± 7.33[d]
CNI	7.650e−10 ± 8.485e−12[d]	26.78 ± 2.15[d]	31.71 ± 3.01[b]
NI	7.980e−10 ± 4.243e−11[d]	39.78 ± 1.98[e]	60.85 ± 2.87[d]

NA, film with natamycin; NI, film with nisin; NANI, film with natamycin and nisin; CNA, control for film NA; CNI, control for film NI; CNANI, control for film NANI.
Mean and standard deviation are reported. Different letters in the same column indicate significant differences ($p < 0.05$).

Wettability is an important property of edible films because it allows to understand the interfacial film–water interaction and is related to water resistance [43]. In Fig. 2, it can be observed that by increasing glycerol concentration (Panel a), films with better wettability values (Fig. 2b) were obtained and that the addition of antimicrobials reduced the wettability values (Fig. 2b). It can also be observed the images of water drops formed on each film studied.

According to results obtained, it can be concluded that films NANI and NI, which showed a higher contact angle and lower wettability indicating a higher hydrophobicity, will be more adequate than film NA for the covering of foods with high lipid content, as cheeses.

3.5. Roughness (Rq)

The surface morphology of the starch matrices was dependent on the glycerol concentration as can be observed in the AFM images presented in Fig. 3. The roughness values are related to the surface irregularities and are listed in Table 3. An increase in glycerol concentration produced a reduction in the roughness of studied films (roughness $_{CNA}$ < roughness $_{CNANI}$ < roughness $_{CNI}$). Glycerol acting as a plasticizer influenced starch network formation increasing smoothness of the matrices obtained.

There were not significant differences in roughness values between films with and without natamycin (CNA vs. NA). Ollé Resa et al. [5] and Arzate-Vázquez et al. [44] reported similar roughness values for tapioca starch based films and chitosan-based films with or without natamycin. The increase of roughness observed for NANI and NI films in comparison to CNANI and CNI can be attributed to the presence of nisin and it can be hypothesized that the presence of this antimicrobial, while affecting the starch network, increased surface irregularities. La Storia et al. [45] observed for polyethylene films a considerable increase of the surface roughness with the presence of nisin.

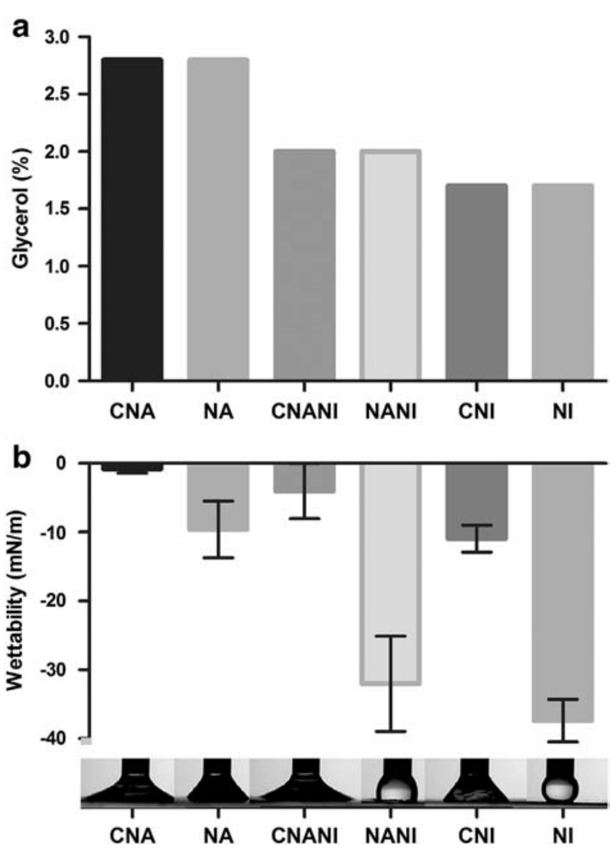

Fig. 2. Glycerol content (panel a) and wettability (panel b) for films with (NA, NANI and NI) and without (CNA, CNANI and CNA) antimicrobials. Images of the water droplets deposited on the films are also shown.

3.6. Correlation between studied parameters

In order to evaluate the relation between contact angle, Rq and WVP, a correlation analysis was performed. The resultant Pearson product moment correlation coefficients can be observed in Table 4. It is also shown, in bold, the statistical significance (significance level: 0.05) of the estimated correlations expressed though the p value.

The interfacial properties between a liquid and a polymer component are characterized by the surface energies of each phase and the contact angle between them [46]. According to Bico et al. [47] and Good et al. [48], for hydrophilic surfaces, the roughness improves the wetting of the surfaces determining a decrease of contact angle. Anyhow, in the present research, it could be observed that the Rq and the contact angle showed a significant and positive correlation coefficient (Table 4). Muscat et al. [38] studied the film forming behavior and hydrophobicity of high amylose (HA) starch in the presence of three different natural waxes (beeswax, candelilla wax and carnauba wax) in the presence and absence of Tween-80, and also observed that the higher contact angle values were obtained when carnauba wax and Tween-80 were present and that this trend was related to the higher surface roughness of these films.

Table 4 shows a significant and negative correlation coefficient between Rq and WVP. In a material without defects like pinholes or cracks, the primary mechanism for gas and water vapor flow through a film or coating is an activated diffusion. This means that the permeate dissolves in the film matrix at the higher concentration side, diffuses through the film, driven by a concentration gradient, and evaporates from the other surface [49]. Escamilla-García et al., [50] characterized chitosan-zein edible films and observed that the largest roughness produced the smallest permeability. Bosquez-Molina et al. [51] studied the surface morphology and the water vapor permeability (WVP) of coatings casted from candelilla wax/mineral oil-in-mesquite gum emulsions. They observed that the $CaCl_2$ addition determined an increase of the surface roughness, and that this roughness contributed to a better "coupling" of the structural material around the lipophilic dispersed phase resulting in more compact films with better properties against water vapor diffusion and lower WVP. Consequently, the negative correlation coefficient observed for Rq and WVP in the present research can be attributed to the impairment of the interfacial relation between water and the film surface with the increase in its roughness. This impairment might be responsible for the decrease in the water solubility and/or diffusion in the film, decreasing the WVP.

4. Conclusions

The increase in plasticizer concentration produced less stiff and rigid films and, in general, an increase in water vapor permeability.

The surface roughness of the films decreased with the addition of glycerol and increased with the presence of nisin.

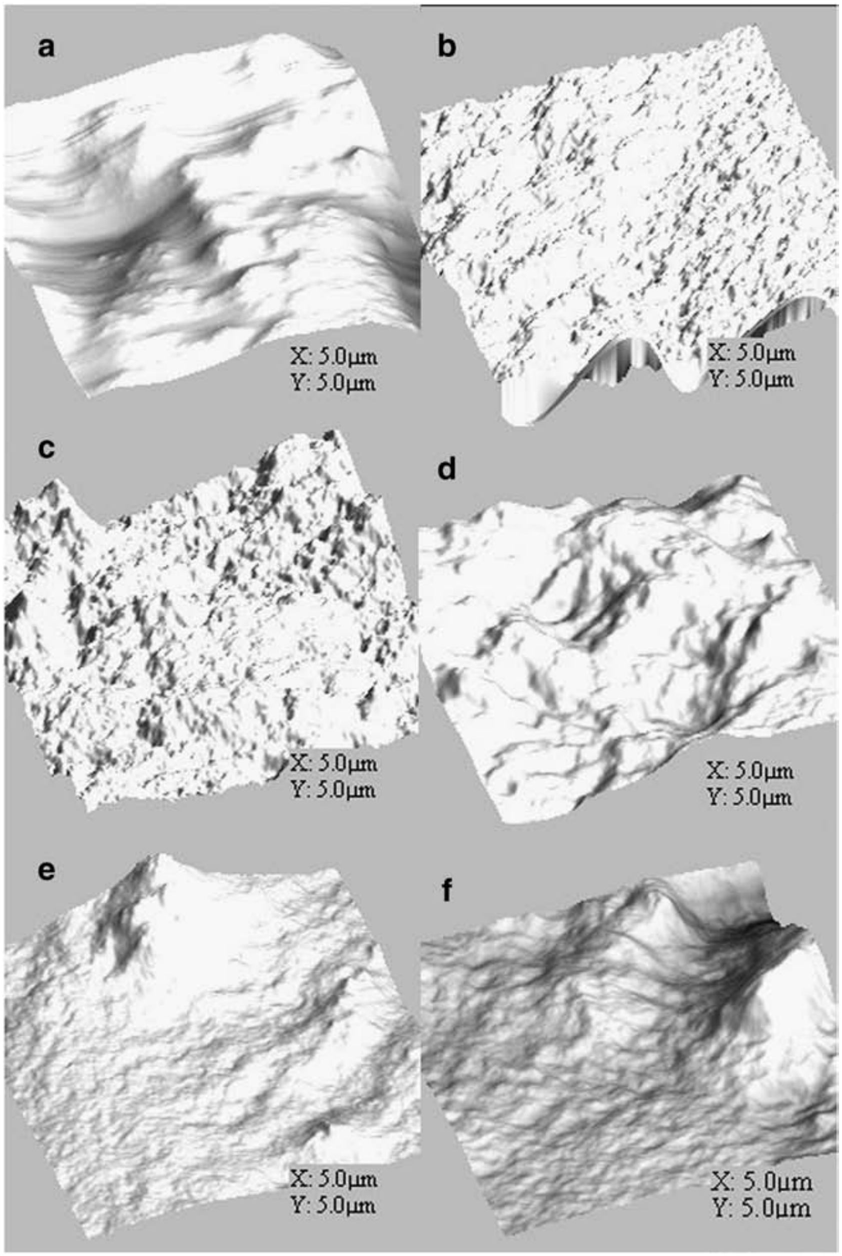

Fig. 3. AFM images (three-dimensional view) for different films. Panel a: CNA; panel b: NA; panel c: CNANI; panel d: NANI; panel e: CNI; panel f: NI.

The films containing nisin showed the lowest wettability but this antimicrobial adversely altered the mechanical parameters and increased the yellow index of the films especially when natamycin was also present.

Table 4
Pearson product moment correlation between contact angle, roughness (Rq) and water vapor permeability (WVP) for films with (NA, NANI and NI) and without (CNA, CNANI and CNA) antimicrobials.

	Contact angle	Rq	WVP
Contact angle		0.8853 **0.0190***	−0.7136 **0.1113**
Rq	0.8853 **0.0190***		−0,9005 **0.0144***
WVP	−0.7136 **0.1113**	−0.9005 **0.0144***	

The statistical significance is presented in bold.
* Significant coefficients ($p < 0.05$), $n = 6$.

The different quantities of glycerol used for obtaining adequate handling properties for the formulations proposed in this research, as well as the presence of different antimicrobials for controlling yeast and bacteria population, gave origin to films with different morphologies that affected the water vapor permeability and the wettability. Also, different textural properties and color were observed. The film safe use as antimicrobial support might be conditioned by the adequacy of these parameters for each specific application. Moreover, additional research must be done with films containing different concentrations of natamycin and/or nisin to evaluate if the present conclusions can be generalized to other formulations.

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