Food Packaging and Shelf Life 10 (2016) 54–59

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: http://www.elsevier.com/locate/fpsl

Biodegradable films obtained from triticale (*x Triticosecale Wittmack*) flour activated with natamycin for cheese packaging

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ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 22 April 2016
Accepted 1 September 2016
Available online xxx

Keywords:
Triticale flour film
Food active packaging
Antimicrobial activity
Natamycin
Barrier and mechanical properties
Cheese packaging

ABSTRACT

The objective of this study was to investigate the properties of films made from triticale flour activated with natamycin for cheese packaging. Films control and with different concentration of natamycin were prepared. Moisture content, water solubility, color, puncture test, water vapor permeability and microstructure of films and diffusion coefficients of natamycin were determined. The addition of natamycin reduced solubility values and water permeability, increases L*, b* and ΔE, whereas it did not significantly affect the moisture content of the films or puncture force. The analysis of the microstructure indicated that natamycin was incorporated homogenously onto the films. The diffusional exponents were between 0.5 and 1 (non-fickian diffusion). The antifungal activity of the active triticale flour films against *Candida albicans* and *Aspergillus niger* were demonstrated by agar diffusion test. In the case of *C. albicans* the zone of inhibition was greater at higher concentrations, while against *A. niger* there were no significant differences. In addition, we studied the performance of active films through testing soft cheese, covered partly with control and activated films and stored 14 days at 4 °C and at room temperature. Visual inspection showed growth of mold on cheese surfaces that were not covered and on the coated with film control, while those covered with activated films did not show mold growth. In conclusion, the addition of natamycin to triticale flour films allows obtaining a material suitable for use in food packaging as active container of cheese to inhibit deterioration.

1. Introduction

Biodegradable films and coatings can act as food packaging or barrier to external factors and improve the shelf life and quality of food products. Numerous bio-based materials have been developed in order to reduce the dependency of industry and society on limited fossil resources and replace them with (annual) re-growth of raw materials (Scarlat, Dallemand, Monforti-Ferrario, & Nita, 2015). Bio-based packaging polymer films are prepared mainly from polysaccharides, proteins and/or lipids (Gutierrez, Morales, Pérez, Tapia, & Famá, 2015; González & Alvarez Igarzábal, 2013). They are generally biodegradable, non-toxic and edible and in certain circumstances, they can replace synthetic polymers. In addition, they can serve as carriers of antimicrobial and antioxidant agents (Balaguer et al., 2014). Due to foods are often susceptible to microbiological deterioration during storage and distribution, a suitable selection of packaging materials can prevent loss of food quality. Nowadays the food industry show a growing interest in active films to improve food safety and shelf life. The concept of food active packaging have been innovated by the development of biodegradable or edible films with the incorporation of antimicrobial additives (Falguera, Quintero, Jimenez, Muñoz, & Ibarz, 2011). Therefore, biodegradable films can act as barrier to external factors and improve the shelf life and quality of food products. The properties of a film are important for its packaging efficiency. The addition of an antimicrobial agent may cause changes in its polymeric structure, affecting the mechanical and barrier properties (Bierhalz, Altenhofen da Silva, & Guenter Kieckbusch, 2012; Ramos et al., 2012).

Natamycin is a natural polyene produced during fermentation by the bacterium *Streptomyces natalensis* with fungicide activity, which is employed in food industry in order to prevent mold and yeast contamination (Ollé Resa, Jagus, & Gerschenson, 2014). Natamycin has been approved as a food additive in over 40 countries and it has been considered as a natural preservative by

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http://dx.doi.org/10.1016/j.fpsl.2016.09.003
2214-2894/

the European Union (EEC N° 235) and as GRAS (generally recognized as safe) product by the FDA (Koontz, Marcy, Barbeau, & Duncan, 2003). Natamycin can be applied on food surfaces using techniques like spraying, dipping, brushing or by the use of films activated where the natamycin is entrapped. This kind of films constitute a promising form of antimicrobial protection in food preservation (Ollé Resa et al., 2014) but the commercial available products are in general based on polyvinyl alcohol or polyvinyl acetate which safety has been discussed (Pintado, Ferreira, & Sousa, 2010).

Cheese is a ready-to-eat food characterized by different composition. The shelf life of cheeses is limited due to fungal and bacterial development on its surface during handling and storage, which may reduce its quality.

Triticale (x *Triticosecale* Wittmack) is the hybrid between wheat (*Triticum* ssp.) and rye (*Secale* ssp.). Triticale has a combined advantage of high wheat yield potential and disease and environmental tolerance of rye. It is a cereal adapted to less favorable soil conditions and it is suitable for low input agriculture because of lower demand in the application of pesticides. Although there is an insufficient demand for its use as foodstuffs, this cereal may be suitable for forage, energy, bioethanol production (Jørgensen, Deleuran, & Wollenweber, 2007; Marković et al., 2014; Pejin et al., 2009) and its proteins for film formation (Aguirre, Borneo, & León, 2013a; Aguirre, Borneo, & León, 2013b). Nowadays, triticale is grown in more than 30 countries worldwide on about 3.7 million hectares in total, producing over 12 million tons/year (Pejin et al., 2015).

The objective of this work was to prepared triticale flour films containing natamycin as active agent, investigate its effect on the properties of activated films and evaluate their antimicrobial effectiveness.

2. Materials and methods

2.1. Materials

Triticale (variety Buck TK 205) flour (moisture content, 13.25 ± 0.02 g $100\,g^{-1}$, protein content, 8.88 ± 0.02 g $100\,g^{-1}$, ash content, 0.61 ± 0.02 g $100\,g^{-1}$, particle size: pass through a US Standard Sieve No. 100) was donated by Campeloni Semillas S.A. (Córdoba, Argentina). Commercial Natamycin 100% (Proquiga, S.A) was used. Chemical reagents were purchased from *Sigma-Aldrich Chemie Gmbh (Munich, Germany)* and were of analytical grade. Cheese purchased in a local supermarket was used for the evaluation of the activity of the films in a real food.

2.2. Films formation

Triticale flour was used as film-forming component. Therefore, the cereal grain's total composition (excluding the pericarp) is used to obtain the film (Valderrama Solano & Rojas de Gante, 2014). Film-forming solutions for control films were obtained by dispersion of triticale flour (4.0 g/100 mL) in water for 15 min (pH 10.7). Glycerol (30 g/100 g flour), used as plasticizer, was added and the resulting dispersion was then magnetically stirred for 15 at 75 °C. In the case of activated triticale flour films, natamycin was added to the film forming solution (FS) for obtaining different final concentration of 0.02 g natamycin/100 mL FS (5 mg natamycin/dm^2 film), 0.04 g natamycin/100 mL FS (10 mg natamycin/dm^2 film) and 0.08 g natamycin/100 mL FS (20 mg natamycin/dm^2 film). Films were prepared by using the casting technique. Measured volumes of the film-forming solution were poured onto a horizontal flat silicon tray (12 cm diameter). Films were dried at 40 °C in an oven with air circulation. Dry films were peeled off the casting surface and preconditioned prior to the characterization in an environmental chamber at 25 °C and 52% relative humidity (RH). RHs were obtained using saturated salt solutions of $Mg(NO_3)_2$ for at least 72 h prior to testing.

2.3. Film thickness

The thickness of the films was determined with a micrometer Schwyz SC1. The average value of six thickness measurements at different locations on each film sample was used in all calculations.

2.4. Moisture content and film solubility

Moisture content (MC) and solubility in water (S) of control and activated triticale flour films were determined according to Aguirre, Borneo, and León (2011). Triplicate measurements of MC and S were conducted for each type of film and an average was taken as the result.

2.5. Mechanical properties

The force at the breaking point of the triticale films was determined by the puncture test using a texture analyzer Instron Texturometer. The films were fixed on a still flat surface with a 10 mm diameter hole and perforated with a P/2N probe (needle probe), moving at 1 mm/s until the film broke. All determinations were made five times. Mean and standard deviations were calculated.

2.6. Color evaluation

Measurements of color were performed on white background with a Minolta colorimeter (Minolta CM-508d, Tokyo, Japan). The CieLab parameters (L^*, a^* and b^*) were measured in at least five positions randomly selected for each sample. 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 natamycin with respect to the control using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

For every film incorporated with different amount of natamycin, four samples were taken and on each film sample, four readings were made on each side.

2.7. Water vapor permeability (WVP)

WVP was measured gravimetrically according to the method reported by Aguirre et al. (2011). Each film sample was sealed over a circular permeation cup containing silica gel (desiccant at relative humidity (RH) of 0%). The permeability cups were 3.5 cm in diameter. The air gaps between the silica surface and the films were less than 4 mm. The cups were kept in hermetically pre-equilibrated and close chambers containing a saturated solution of $Mg(NO_3)_2$ at 25 °C in order to maintain a RH difference of 52%. The RH inside the cell was always lower than the outside, and water vapor transport was determined from the weight gain of the permeation cell. Cups were periodically weighted and water vapor transfer rates (WVTR, $g\,m^{-2}\,s^{-1}$) of films were determined from the slope of weight gain versus time plots using: $WVTR = (\Delta m\,A^{-1}\,\Delta t^{-1})$, where Δ m is weight gain of permeation cell (g), A is the exposed area and Δt is time. Water vapor permeability (WVP, $g\,m^{-1}\,s^{-1}\,Pa^{-1}$) were calculated using the following equation:

$$WVP = (WVTR \,.\, X)/\Delta P \quad (2)$$

where X is film thickness and ΔP is vapor partial pressure difference (Pa) across the film.

2.8. Surface morphology

The surface morphology of the triticale flour films was observed by scanning electron microscopy (SEM) using a FE-SEM Σigma scanning electron microscope (Facultad de Matemáticas, Astronomía y Física, Universidad Nacional de Córdoba, Córdoba, Argentina). The samples were mounted on the stub and then were prepared accordingly with a fine gold layer before obtaining the micrographs.

2.9. Diffusion coefficient determination

Triticale flour films were cut into 3 × 3 cm square pieces and immersed in 150 mL of water with stirring at 25 °C. Samples of the solution were taken out at different time intervals to determine the amount of natamycin released from the films. Natamycin concentration in the solution surrounding the film was determined using UV spectroscopy at 317 nm. Approximately 80 samples taken at 5-min intervals were taken. Standard curves were used to determine concentrations. The mass of natamycin released at time t, Mt, was calculated and plotted as $Mt/M\infty$ as a function of time, where $M\infty$ is the maximum amount released by the triticale film so that $Mt/M\infty$ is the fractional natamycin release. Diffusion coefficients were determined using the Fick's Law involved in the diffusion process for a planar system by fitting the early portion of the release curve ($Mt/M\infty < 0.60$) to the Power Law Model (Eq. (3))

$$Mt/M\infty = k\ t^n \quad (3)$$

Where k is a kinetic constant that characterizes the macromolecular arrangement of the polymer matrix, t is the elapsed time and n is the diffusional exponent characteristic of the natamycin release mechanism. A value of n = 0.5 confirms that Fickian diffusion is observed and the release rate is linear to t0.5. A value of n = 1 indicates that transport and the release rate is directly proportional to time and values of $0.5 < n < 1.0$ indicates that anomalous (non -Fickian) transport is the predominating mechanism (Bierhalz et al., 2012).

2.10. Antimicrobial activity. Agar diffusion test

The agar diffusion test was used to determine the antimicrobial activity of the films containing natamycin against *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). The triticale flour films were cut into a disc (diameter 6 mm) with a punch. Film discs without natamycin (control, C) and with different concentration of natamycin were placed on the Petri plates containing agar YGC (BK 007 HA, Biokar Diagnostic, France) which had previously been seeded with 100 μL of inoculum containing 1.5×10^8 CFU/mL of each target microorganism. The plates were incubated at 25 °C for 5 days. There were 2 replicates for each microorganism. Afterwards, the antimicrobial activity of each film was evaluated by observing the formation of zones of inhibition. The width of the inhibition zone around each film disc was measured with Image J (http://rsbweb.nih.gov/ij/) (Abramoff, Magalhaes, & Ram, 2004).

2.11. Activity evaluation of triticale flour films in contact with cheese samples

Evaluation or triticale flour films antimicrobial activity was carried on commercial soft cheese. Pieces of soft cheese were partially covered with triticale flour films and stored up to 14 days at room temperature and in a refrigerator at 4 °C to reproduce the typical shelf life storage conditions of cheese. Each sample was observed and photographed at different times to evaluated the performance of activate films on the basis of molds growth (Fajardo et al., 2010).

2.12. Statistical analyses

Moisture content, water solubility and water permeability were determined in triplicate while mechanical properties were performed five times. Analysis of variance (ANOVA) was carried out to test mean differences and Tukey's multiple comparison test was used to find out which means were statistically and significantly different ($p < 0.05$). Statistical data and data fitting (modelling) were performed using the InfoStat statistical software version 2014 (Di Rienzo et al., 2014).

3. Results

3.1. Film appearance

All triticale flour films formulated were homogeneous, flexible and transparent and they could be easily be separated from the casting plates. The thickness of the films was 130 ± 16 μm and no statistical differences were observed with respect to control films without natamycin. There was no visible pores or cracks on the surface of films. Appearance of the film side facing the casting plate was bright, whereas the other side was dull. Similar results were reported previously (Ramos et al., 2012) in whey protein isolate films.

3.2. Moisture content, film solubility and water vapor permeability

Incorporation of natamycin into triticale flour films did not significantly affect the moisture content (MC) values relative to control films (Table 1). This was also observed in wheat gluten and methyl cellulose films (Türe, Eroğlu, Özen, & Soyer, 2009) chitosan films (Fajardo et al., 2010), alginate films (Bierhalz et al., 2012) and whey protein isolate films (Ramos et al., 2012). However, when

Table 1
Moisture content, solubility, water vapor permeability (WVP) and puncture force of triticale flour films without natamycin (control) and containing natamycin.

Natamycin concentration (g/100 mL FS*)	Moisture content (%)	Solubility (%)	WVP × 10^{11} ($g\,m^{-1}\,s^{-1}\,Pa^{-1}$)	Puncture force (N)
0	16.7 ± 2.09^a	22.2 ± 0.70^a	8.21 ± 0.98^a	1.77 ± 0.24^a
0.02	18.7 ± 1.15^a	19.8 ± 0.59^b	2.06 ± 0.28^b	1.87 ± 0.4^a
0.04	16.5 ± 0.98^a	20.7 ± 0.62^b	1.12 ± 0.15^c	1.87 ± 0.42^a
0.08	16.9 ± 2.49^a	18.6 ± 0.50^c	1.02 ± 0.12^c	2.00 ± 0.30^a

Reported values are means (n = 3) ± standard deviation. Different letters as superscripts in a column indicate significant differences ($p < 0.05$). FS: film forming solution.

natamycin (a molecule with a low hydrophilic nature) was incorporated in triticale flour films, a statistically significant decrease of solubility in water was observed with regard to the control films (Table 1). Water insolubility is usually required for commercial films (Galus & Kadzinska, 2016). The decrease observed in water solubility is an indicator of stable network in triticale flour films with natamycin. Activated triticale films (with natamycin) had more stability in water showing lower water solubility that control films, but all the films lost part of their integrity after immersion (24 h) in water.

Regarding water vapor permeability, it was observed (Table 1) a significant difference for triticale films with and without natamycin. The incorporation of natamycin reduced the WVP value of triticale flour films. This result may be related to the low water solubility of the molecule of natamycin. Ollé Resa et al. (2014) observed the same trend in tapioca starch edible films.

3.3. Mechanical properties

Results of the puncture tests of triticale flour films, control and containing natamycin, are shown in Table 1. Compared with other biodegradable films, triticale flour films exhibit good mechanical properties, with puncture force (PF) values from 1.77 to 2 N. These data agreed with other authors that observed values of PF of 1 and 2.34 N in amaranth flour films (Tapia-Blácido, Sobral, & Menegalli, 2005; Villaman Dieguez, Pellisari, Sobral, & Menegalli, 2015).

The incorporation of natamycin did not produced statistically significant differences in the values of puncture force of active films relative to the triticale control films. These results show that natamycin, when incorporated into triticale flour film do not destabilize the stable structure of the film network and do not increase the free volume and mobility of the polymeric chains. Ollé Resa et al. (2014) also reported that the incorporation of natamycin in tapioca starch films did not significantly change their mechanical properties.

3.4. Color evaluation

Color of films is an important feature because the consumers are attracted by the external appearance of products. The color parameters obtained in triticale flour films are shown in Table 2. All the films shown high lightness (L* parameter) values, positive values of parameter b* and negative values of parameter a*. The incorporation of natamycin increased all parameters when comparing with control film. Values of total color difference (ΔE) increased significantly for triticale flour films incorporated with natamycin. The addition of natamycin also increased the a* values of tapioca starch edible films (Ollé Resa et al., 2014) and L* values of whey protein isolate films (Ramos et al., 2012).

3.5. Surface morphology

Micrographs of the surface of triticale films observed by scanning electron microscopy (SEM) are shown in Fig. 1. The incorporation of natamycin in triticale flour films did not causes dramatic changes on the films surface. This uniform distribution observed corroborate the trends found for mechanical properties of active triticale films compared to the control films.

3.6. Diffusion coefficient determination

The comparison among films indicate that the release of natamycin from 0.08% natamycin films was slower than the other films. The natamycin in 0.02 and 0.04% natamycin films was depleted in approximately 5 h, while this period increased to 6.6 h for 0.08% natamycin films. The more rapid release from 0.02 and 0.04% natamycin films also explains the higher water solubility values observed in Table 1.

Diffusional coefficients of natamycin from triticale flour films for the Power Law Model (Eq. (3)) were obtaining by plotting fractional natamycin release M_t/M_∞ vs t and are shown in Table 3. All the films presented a diffusional exponent *n* between 0.5 and 1. These values indicated a mechanism of anomalous diffusion, in which the deviation from Fickian behavior indicates that the

Table 2
Color parameters for triticale flour films without natamycin (control) and containing natamycin.

Natamycin concentration (g/100 mL FS*)	L*	a*	b*	ΔE
0	86,36 ± 0,56[a]	−0,06 ± 0,01 [a]	0,80 ± 0,08 [a]	
0.02	87,37 ± 0,41 [b]	−0,21 ± 0,10 [b]	1.08 ± 0,28 [a]	1,06 [a]
0.04	87,55 ± 0,66 [b]	−0,49 ± 0,16 [c]	1,99 ± 0,39 [b]	1,73 [b]
0.08	87,89 ± 0,25 [b]	−0,80 ± 0,09 [d]	3,52 ± 0,74 [c]	2,92 [c]

Reported values are means ± standard deviation. Different letters as superscripts in a column indicate significant differences ($p < 0.05$). FS: film forming solution.

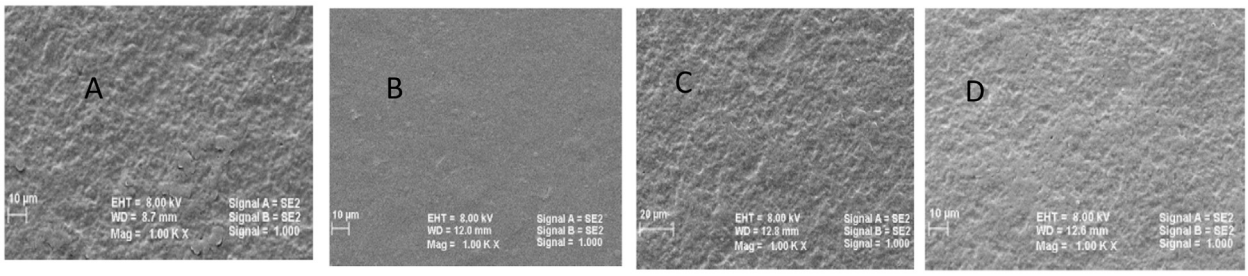

Fig. 1. Scanning electron micrograph (SEM) of surface of triticale flour films. A: Control film. B: film containing 0.02% natamycin. C: film containing 0.04% natamycin. D: film containing 0.08% natamycin.

Table 3
Diffusion exponent (n), diffusional constant (k) and determination coefficient (R^2) of the natamycin release from triticale flour films without natamycin (control) and containing natamycin, modelated by Eq. (3).

Natamycin concentration (g/100 mL FS*)	n	k (h^{-n})	R^2
0.02	0.6047	0.3761	0.9702
0.04	0.7555	0.2849	0.9988
0.08	0.7475	0.2500	0.9907

*FS: film forming solution.

Table 4
Antimicrobial activity of triticale flour films without natamycin (control) and containing natamycin, expressed as inhibition zone (cm).

Natamycin concentration (g/100 mL FS*)	Inhibition zone (cm)	
	Candida albicans	*Aspergillus niger*
0	0	0
0.02	2.02 ± 0.03 [a]	2.68 ± 0.31 [a]
0.04	2.22 ± 0.01 [b]	2.71 ± 0.11 [a]
0.08	2.30 ± 0.05 [c]	2.99 ± 0.09 [a]

Reported values are means ($n = 3$) ± standard deviation. Different letters as superscripts in a column indicate significant differences ($p < 0.05$). FS: film forming solution.

polymer relaxation phenomenon is prominent and may affect the release of natamycin from the film during the first moments of the process. An anomalous diffusion mechanism in alginate/pectin films was also observed by Bierhalz et al. (2012).

3.7. Agar diffusion test

Natamycin blocks fungal growth by binding to ergosterol present in the fungi plasma membranes almost exclusively. Triticale flour films with natamycin as active agent were effective to control the growth of *C. albicans* and *A. niger* when tested in a disc-diffusion assay (Table 4). Only activated films inhibited the growth of microorganisms: antimicrobial activity was not observed in the control film (without natamycin). The results shown a region of inhibition larger than that covered by activated films due to the diffusion of natamycin through the triticale films. The statistical comparison between the diameters obtained with different concentrations of natamycin in films showed that in the case of *C. albicans* the zone of inhibition was greater at higher concentrations, while against *A. niger* there were no significant differences (Table 4). The diameter of the inhibition disc is the result of the balance between the rate of antimicrobial diffusion to the agar and the rate of microorganism growth. Considering that the diffusion of natamycin is the same for *C. albicans* and *A. niger* because was used the same media for their growth, the different diameters observed were associated with different growth rate and sensitivity to natamycin for the different fungi evaluated.

3.8. Activity evaluation of triticale flour films in contact with cheese samples

We studied the efficiency and applicability of active triticale flour films through testing soft cheese covered partly with control and natamycin activated films stored 14 days at room temperature and at 4 °C. Related to cheese samples stored at room temperature, Fig. 2 shows growth of mold on the part of cheese samples that were not covered and on the cheese surfaces coated with film control, while those covered with activated films did not show mold growth. Regarding to samples stored 14 days at 4 °C, triticale flour films activated with natamycin were also able to inhibit the mold growth on the surface of soft cheese at that temperature. Fig. 3 shows that the inhibitory effect was observed for all the activated triticale films, but not for control film. The picture obtained on days 7 and 14 showed that deteriorations signs and the appearance of mold were observed on the surface of cheese without coating and coated with control film. However, the surface covered with activated films did not show mold growth (Fig. 3).

4. Conclusions

The presented study proved that antimicrobial agent natamycin can be incorporated in triticale flour films. Incorporation of natamycin into triticale flour films did not significantly affect the moisture content and water vapor permeability values but it decreased the solubility in water and water vapor permeability and increased color parameters relative to control films.

Triticale flour films have the ability to carry and release natamycin (with a non -Fickian mechanism), and that the films of triticale activated with natamycin have great potential as active container of cheese to inhibit deterioration. Therefore, the active triticale flour film developed could provide functional properties in packaging design and be an efficient antimicrobial food packaging.

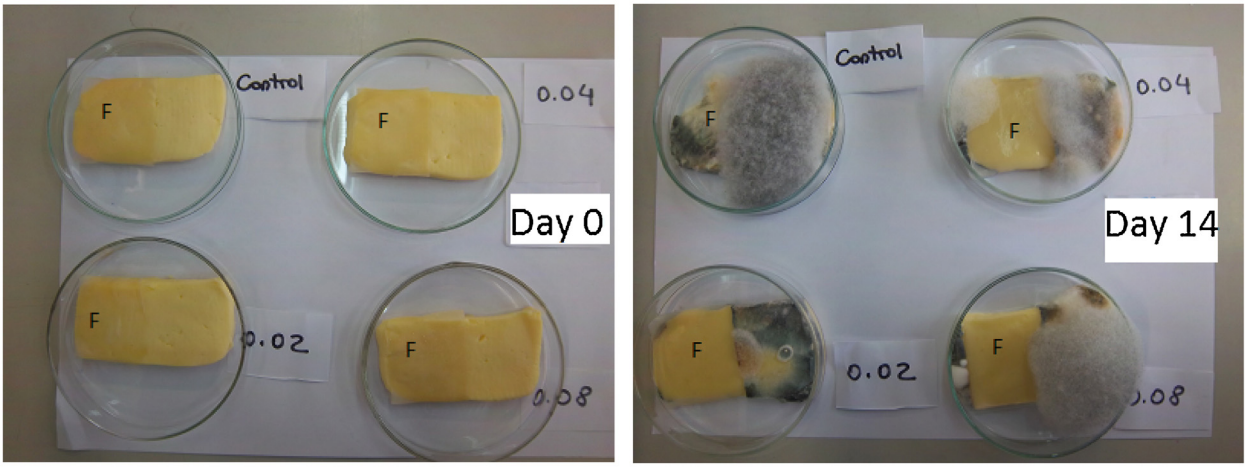

Fig. 2. Samples of soft cheese partially coated with triticale flour films (control and with different concentration of natamycin) at 0 and 14 days of storage at room temperature. F: film; 0.02: film containing 0.02% natamycin; 0.04: film containing 0.04% natamycin; 0.08: film containing 0.08% natamycin.

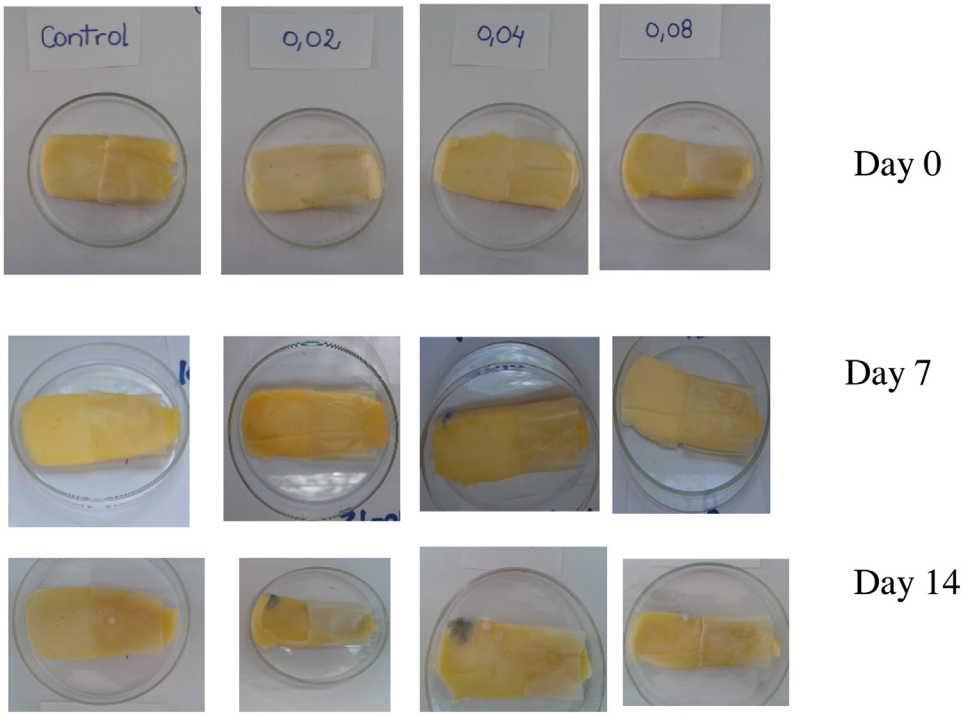

Fig. 3. Samples of soft cheese partially coated with triticale flour films (control and with different concentration of natamycin), at 0 and 14 days of storage at 4 °C. 0.02: film containing 0.02% natamycin; 0.04: film containing 0.04% natamycin; 0.08: film containing 0.08% natamycin.

Acknowledgement

Authors thank to Universidad Nacional de Córdoba, Argentina, for the financial support.

References

Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*, 36–42.

Aguirre, A., Borneo, R., & León, A. (2011). Properties of triticale flour protein based films. *LWT – Food Science and Technology*, *44*, 1853–1858.

Aguirre, A., Borneo, R., & León, A. (2013a). Properties of triticale protein films and their relation to plasticizing–antiplasticizing effects of glycerol and sorbitol. *Industrial Crops and Products*, *50*, 297–303.

Aguirre, A., Borneo, R., & León, A. (2013b). Antimicrobial: Mechanical and barrier properties of triticale protein films incorporated with oregano essential oil. *Food Bioscience*, *1*, 2–9.

Balaguer, M. P., Fajardo, P., Gartner, H., Gomez-Estaca, J., Gavara, R., Almenar, E., et al. (2014). Functional properties and antifungal activity of films based on gliadins containing cinnamaldehyde and natamycin. *International Journal of Food Microbiology*, *173*, 62–71.

Bierhalz, A. C. K., Altenhofen da Silva, M., & Guenter Kieckbusch, T. (2012). Natamycin release from alginate/pectin films for food packaging applications. *Journal of Food Engineering*, *110*, 18–25.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M., & Robledo, C. W. (2014). *InfoStat version 2014*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. http://www.infostat.com.ar.

Fajardo, P., Martins, J. T., Fuciños, C., Pastrana, L., Teixeira, J. A., & Vicente, A. A. (2010). Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *Journal of Food Engineering*, *101*, 349–356.

Falguera, V., Quintero, J. P., Jimenez, A., Muñoz, J. A., & Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology*, *22*, 292–303.

Galus, S., & Kadzinska, J. (2016). Whey protein edible films modified with almond and walnut oils. *Food Hydrocolloids*, *52*, 78–86.

González, A., & Alvarez Igarzábal, C. I. (2013). Soy protein- Poly (lactic acid) bilayer films as biodegradable material for active food packaging. *Food Hydrocolloids*, *33*, 289–296.

Gutierrez, T. J., Morales, N. J., Pérez, E., Tapia, M. S., & Famá, L. (2015). Physico-chemical properties of edible films derived from native and phosphated cush-cush yam and cassava starches. *Food Packaging and Shelf Life*, *3*, 1–8.

Jørgensen, J. R., Deleuran, L. C., & Wollenweber, B. (2007). Prospects of whole grain crops of wheat: Rye and triticale under different fertilizer regimes for energy production. *Biomass and Bioenergy*, *31*, 308–317.

Koontz, J., Marcy, J., Barbeau, W., & Duncan, S. (2003). Stability of natamycin and its cyclodextrin inclusion complexes in aqueous solution. *Journal of Agricultural and Food Chemistry*, *51*, 7111–7114.

Marković, M., Markov, S., Grujić, O., Mojović, L., Kocić-Tanackov, S., Vukašinović, M., et al. (2014). Microwave as a pre-treatment of triticale for bioethanol fermentation and utilization of the stillage for lactic acid fermentation. *Biochemical Engineering Journal*, *85*, 132–138.

Ollé Resa, C. P., Jagus, R. J., & Gerschenson, L. N. (2014). Effect of natamycin, nisin and glycerol on the physicochemical properties, roughness and hydrophobicity of tapioca starch edible films. *Materials Science and Engineering C*, *40*, 281–287.

Pejin, D., Mojovic, L. J., Vucurovic, V., Pejin, J., Dencic, S., & Rakin, M. (2009). Fermentation of wheat and triticale hydrolysates: A comparative study. *Fuel*, *88*, 1625–1628.

Pejin, J. D., Mojovic, L. V., Pejin, D. J., Kocic'-Tanackov, S. D., Savic, D. S., Nikolic, S. B., et al. (2015). Bioethanol production from triticale by simultaneous saccharification and fermentation with magnesium or calcium ions addition. *Fuel*, *142*, 58–64.

Pintado, C. M. B. S., Ferreira, M. A. S. S., & Sousa, I. (2010). Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. *Food Control*, *21*, 240–246.

Ramos, O. L., Silva, S. I., Soares, J. C., Fernandes, J. C., Poças, M. F., Pintado, M. E., et al. (2012). Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. *Food Research International*, *45*, 351–361.

Scarlat, N., Dallemand, J.-F., Monforti-Ferrario, F., & Nita, V. (2015). The role of biomass and bioenergy in a future bioeconomy: Policies and facts. *Environmental Development*, *15*, 3–34. http://dx.doi.org/10.1016/j.envdev.2015.03.006.

Türe, H., Eroğlu, E., Özen, B., & Soyer, F. (2009). Physical properties of biopolymers containing natamycin and rosemary extract. *International Journal of Food Science and Technology*, *44*, 402–408.

Tapia-Blácido, D., Sobral, P. J. A., & Menegalli, F. C. (2005). Development and characterization of edible films based on amaranth flour (Amaranthus caudatus). *Journal of Food Engineering*, *67*, 215–223.

Valderrama Solano, A. C., & Rojas de Gante, C. (2014). Development of biodegradable films based on blue corn flour with potential applications in food packaging. Effects of plasticizers on mechanical, thermal, and microstructural properties of flour films. *Journal of Cereal Science*, *60*, 60–66.

Villaman Dieguez, M. C., Pellisari, F. M., Sobral, P., & Menegalli, F. C. (2015). Effect of process conditions on the production of nanocomposite films based on amaranth flour and montmorillonite. *LWT – Food Science and Technology*, *61*, 70–79.