Assessment of functional properties and antimicrobial efficiency of polymer films with lacquer layer containing natamycin in cheese packaging

KRISTÝNA HANUŠOVÁ – JAROSLAV DOBIÁŠ – MICHAL VOLDŘICH

Summary

Polymer films coated with commercially available lacquers containing natamycin were studied to evaluate natamycin migration, mechanical and barrier properties and antimicrobial efficiency in cheese packaging. The amount of released natamycin was at maximal level from 0.60 mg·dm^{-2} ± 0.11 mg·dm^{-2} to 1.21 mg·dm^{-2} ± 0.15 mg·dm^{-2}, depending on the tested films. Mechanical properties of the films with lacquer coatings did not significantly change whereas oxygen and carbon dioxide permeability was decreased in these films. Natamycin released from the polyvinylchloride (PVC) film provided inhibitory effect against microorganisms isolated from the surface of cheese Blaťácké zlato on agar plates. The PVC film was insufficient for the packaging of cheese Blaťácké zlato during ripening. Conversely, the PVC and polyamide/polyethylene (PA/PE) films were able to inhibit the fungal growth on the surface of wrapped cheeses Blaťácké zlato, Eidamská cihla and Gaston Oregano during storage.

Keywords

active packaging; antimicrobial films; natamycin; migration; cheese

Antimicrobial packaging based on preservative release represents a promising form of active packaging systems applicable in food processing. Different types of such innovative concepts and their potential application have recently been described, for example by Coma [1], Kerry et al. [2] and Mastromatteo et al. [3].

Natamycin (earlier named pimaricin) is a natural antifungal agent classified as a polyene macrolide antibiotic. It is produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. It is used as a food additive (E235) for the surface treatment of hard, semi-hard and semi-soft cheeses and dry cured sausages because of its activity against yeasts and moulds [4, 5].

Only dissolved natamycin has antifungal activity. However, the amphoteric character of natamycin causes its low solubility in most solvents and water, which is, on the contrary, advantageous for the surface treatment of food due to its low migration into the foodstuffs [4]. The solubility is increased at high or low pH values [6]. Nevertheless, natamycin is highly susceptible to inactivation under these conditions, in particular at pH values lower than 3 and higher than 9 [4, 7]. In an acid medium, natamycin is decomposed to mycosamine and at least three inactive compounds (amphoteric aponatamycin, acidic di-natamycinolidediol, non-ionic di-decarboxy-anhydronatamycinolidediol), all of which have intact lactone rings [8]. At high pH values, the lactone is rapidly saponified, forming the biologically inactive natamycoic acid [6]. The stability of natamycin is also affected by light, oxidants, chlorine and heavy metals [4]. Data concerning the influence of different pH on natamycin migrating from the antimicrobial packages have not been published yet.

The surface of cheeses is a good substrate for the growth of moulds capable of forming mycoto-

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xins, which may be a major factor limiting the shelf-life. However, there is a limited number of mould species that are capable of causing spoilage of cheese [9, 10]. *Penicillium* is a genus most frequently isolated from naturally contaminated cheese rind. These microorganisms include psychrotrophic and mycotoxigenic strains that could proliferate during cold storage [11]. Therefore prevention of mould growth, in particular during ripening and storage, is necessary in the production of cheese and thus preservatives such as natamycin or sorbate are applied [10].

Nevertheless, resistance of moulds and yeasts to common preservatives, e.g. to frequently used sorbate, is a serious problem in the food industry [12]. Some species of *Penicillium* isolated from cheese, for example, are capable of growing in the presence of potassium sorbate at concentrations as high as 7.1 $g \cdot l^{-1}$; ca. 5.0 $g \cdot l^{-1}$ of sorbic acid [13] and 12.0 $g \cdot l^{-1}$ [14]. Moreover, some mould species from genera *Penicillium, Aspergillus, Fusarium, Mucor, Geotrichum* and *Trichoderma* can degrade sorbic acid to volatile 1,3-pentadiene, which is responsible for off-flavours in food products such as cheese [13–18]. On the other hand, natural resistance to polyenes such as natamycin does not occur among fungi, because of the mode of action of these chemical agents based on irreversible binding to ergosterol in fungal cell membrane [19–22].

In the future, economic losses due to spoilage and health risks associated with the growth of pathogenic or mycotoxin-producing moulds will remain important issues. The increasing incidence of sorbate-resistant and heat-resistant moulds and trend towards minimally processed food will demand enhaced preservation systems. The use of packaging films that contain antimicrobial agents could provide advantages over their direct addition into foods. Incorporation of antimicrobial agents into films or coverage of films with coatings containing preservatives adds new functionality to packaging. To assess the ability of a polymer film to act as an antimicrobial carrier and effective hurdle against microorganisms, diffusion coefficients for the selected antimicrobials and mechanical and barrier properties of films should be determined [23, 24].

The aim of this study was to (i) evaluate the migration of natamycin under various conditions (pH, temperature) from films prepared on printing lines in two Czech companies, (ii) assess mechanical and barier properties of these films and (iii) test their antimicrobial efficiency for cheese packaging under pilot-plant conditions.

MATERIALS AND METHODS

Packaging materials

1. Coextruded polyamide/polyethylene film 1 (PA/LDPE, thickness 70 μm; INVOS, Svárov, Czech Republic) was coated with the polyvinyldichloride (PVdC) lacquer Kombilack L-1917 (Rotoflex, Grenchen, Switzerland) on the polyethylene side of the film on flexography printing line in INVOS company. Commercial preparation Delvocid (100 g; DSM Food Specialities, Delft, The Netherlands) containing 50% w/w of pure natamycin and 50% w/w of lactose was dispersed thoroughly in 500 ml of butan-2-one and mixed with 1 kg of lacquer. The thickness of the final coating layer was 5.0 μm ± 0.9 μm. Natamycin content in the dry coating of the final film was 16.7% w/w, i.e. 9.3 $mg \cdot dm^{-2}$. In the following text this film is termed PA/PE-1 film.
2. Laminated polyamide/polyethylene film 2 (PA/LDPE, thickness 70 μm; Martin Peroutka, polygrafická výroba, Buštěhrad, Czech Republic) was coated with the polyvinylchloride lacquer Rotoflex L-1414A (Rotoflex) on the polyethylene side of the film on flexography printing line in Martin Peroutka, polygrafická výroba company. The lacquer for coating was prepared in the same way as mentioned above for the PA/PE-1 film. The thickness of the final coating layer was 4.1 μm ± 1.0 μm. Natamycin content in the dry coating of final film was 19.3% w/w, i.e. 7.3 $mg \cdot dm^{-2}$. In the following text this film is termed PA/PE-2 film.
3. Heat-shrinkable polyvinylchloride film (PVC, thickness 25 μm; Martin Peroutka, polygrafická výroba) was coated with the polyvinylchloride lacquer Rotoflex L-1414A in the same way as PA/PE-2 film. The thickness of the final coating was 4.0 μm ± 1.1 μm. Natamycin content in the dry coating of the final film was 19.3% w/w, i.e. 7.1 $mg \cdot dm^{-2}$. In the following text this film is termed PVC film.

Cheese

The following cheeses were used in the study:

1. Blaťácké zlato (Madeta, Řípec, Czech Republic) – a soft cheese (the content of solids and fat 51% w/w and 24.5% w/w, respectively) produced portioned (120 g) and packaged in modified atmosphere or wrapped in markets.
2. Eidamská cihla (Plastcom Mlékárna Příšovice, Příšovice, Czech Republic) – a semi-hard cheese of Edam type (the content of solids and fat 56% w/w and 25.2% w/w, respectively) produced portioned (200 g) and vacuum-packaged.

3. Gaston Oregano (Plastcom Mlékárna Příšovice) – a semi-hard cheese of Edam type with oregano (the content of solids and fat 56% w/w and 25.2% w/w, respectively) produced portioned (200 g) and vacuum-packaged.

Determination of film and lacquer layer thickness

Thickness of films and lacquer layers was measured with a micrometer (SE 051, type D2M; Lorentzen and Wettre, Kista, Sweden) around the testing area (1 dm^2) at ten random points and averaged.

Determination of water vapour, oxygen and carbon dioxide permeability

Water vapour permeability of films was measured in accordance with the ASTM E96/E96M-10 standard test methods for water vapor transmission of materials [25], known as the "cup method". The samples of films with and without lacquer layer were tested. The samples were conditioned at 85% relative humidity and temperature 23 °C in the climatic cabinet (Eratis ICH 200, E.R.A.T.I.S., Bouloc, France) during the test period of 10 days. The weight changes of samples were measured and plotted against time.

Oxygen and carbon dioxide permeability of films was measured in accordance with ASTM D1434 – 82(2009)e1 standard test method for determining gas permeability characteristics of plastic film and sheeting [26] using manometric gas permeation tester (Lyssy L100-5000, Systech Illinois, Johnsburg, Illinois, USA). The samples of films with and without lacquer layer were tested at temperature 23 °C. The area of tested film was 50 cm^2.

Measurement of mechanical properties

Tensile strength and strength at break of films were measured in accordance with ASTM D882-10 standard test method for tensile properties of thin plastic sheeting [27] using materials testing instrument (Instron Model 5544 Load Frame, Instron, Norwood, Massachusetts, USA) with pneumatic side action grips. Films were cut to 150 mm × 15 mm strips and tested in the machine and cross direction. Initial grip distance was 100 mm and crosshead speed was set to 250 $mm \cdot min^{-1}$. The test was finished after rupture of the film or at achieving the extension of 500 mm.

Peel strength was measured in accordance with 180 degree peel test (suggested by Instron company) using the instrument mentioned above. The sheets of tested films were sealed using impulse foot sealing machine (type K600; Singar Sealer, Taipei, Taiwan). Films were cut to 100 mm × 15 mm strips with seal welds in the middle of the sample. Initial grip distance was 50 mm and crosshead speed was set to 250 $mm \cdot min^{-1}$. The test was finished after peeling off the seal weld of the film or at achieving the extension of 500 mm.

Natamycin migration test

The migration of natamycin from PA/PE-1, PA/PE-2 and PVC films into distilled water was studied under the following conditions: 72 cm^2 of the film was fixed in a glass cell (Helendahl cuvette) and poured over with 50 ml of distilled water, so that both sides of the tested film were in contact with simulants. Testing cells were wrapped by aluminium foil to prevent light access and shaken (1 shake per second = 1 Hz) in a water bath at 23 °C for 168 h. Samples (0.5 ml) were withdrawn with a pipette in pre-determined times, maximally twelve times from one cuvette.

The influence of pH and temperature on natamycin migration was also observed in the case of PA/PE-1 film. The migration tests were performed into 3% acetic acid (pH = 2.7) and 1 $mmol \cdot l^{-1}$ sodium hydroxide (pH = 10.5) at 23 °C or into distilled water at 4 °C and 30 °C. The residual migration of natamycin was also tested during storage of packaged cheeses. The samples of films in tight contact with packaged cheeses were taken and the amount of released natamycin was determined by migration test into distilled water at 23 °C after 48 h according to the procedure mentioned above.

Amount of released natamycin was determined by a HPLC isocratic method (modular chromatograph Gynkotek, Germering, Germany, including high pressure pump P580, autosampler GINA 50 and UV detector UVD 170 S, column Waters Nova-Pack C18, 3.9 mm × 300 mm (Waters, Milford, Massachusetts, USA), software Chromeleon 6.3 Build 576 (Dionex, Sunnyvale, California, USA)), using acetonitrile : acetate buffer 35:65 (v/v, pH 4.3) as a mobile phase, wavelength λ=304 nm, flow rate 0.4 $ml \cdot min^{-1}$, temperature 23 °C.

Inhibitory efficiency of the films against microorganisms

The antimicrobial efficacy of the PVC film was tested against moulds and yeasts isolated from the tested cheese during storage tests by agar diffusion method. Three round samples of the tested film (4.5 cm in diameter) were placed with the active surface up on potato dextrose agar (PDA) agar (Oxoid, Basingstoke, United Kingdom) in Petri dishes. "Soft PDA agar" (i.e. PDA agar with PDA broth in the ratio of 1:1 (w/w)) was inoculated with the tested microorganisms (10^5 CFU per 1 ml of test agar). The tested films on the standard

PDA agar were covered with inoculated "soft PDA agar" forming 3 mm layer. After solidifying, the agar plate was placed in a thermostat at 25 °C for 5 days. Then, the inhibitory zones formed round the film samples were evaluated.

Cheese packaging and storage tests under the pilot-plant conditions

The cake of cheese Blaťácké zlato (1.5 kg) was packaged in PVC film before ripening in one of the Czech cheese-producing companies. Sachets from PVC film with cheese were heat-shrinked and sealed in the wrapping machine. The packaged cheese was stored in the ripening room at a temperature of 6–8 °C. The fungal growth on the surface of cheese packaged in the films was observed during ripening. The moulds as well as yeasts were isolated from the ripening cheese and identified according to PITT and HOCKING [28] and SAMSON and FRISVAD [29].

Portioned cheese Blaťácké zlato (100 g) obtained from a Czech supermarket was vacuum-packaged using vacuum packaging machine (Tecnovac S100DGT; Tecnovac, Grassobbio, Italy) in the PVC film in the laboratory. The level of vacuum was set to 99%. The samples were stored in the refrigerator at the temperature 4 °C. The mould growth on the surface of the cheese was observed and compared with the cheese commercially packaged in the modified atmosphere or wrapped in a stretching film in the supermarket.

Sliced cheeses Eidamská cihla and Gaston Oregano (100 g) were packaged in vacuum (90%) and modified atmosphere (30% CO_2, 70% N_2) in PA/PE-2 film in a Czech dairy. The tested cheeses were stored in a refrigerator at a temperature of 4 °C and the mould growth on the surface of cheeses was observed for 6 months. The cheeses packaged in a commercial PA/PE film without lacquer coating were used as a control.

Statistical analysis

Five replicate samples were analysed for the determination of natamycin migration. Correction for simulant volume change during sampling was included in calculation of the final level of natamycin migration. Five replicate samples of each film were tested for determination of water vapour permeability, and three replicate samples were tested for oxygen and carbon dioxide permeability. At least ten replicate samples were measured for assessment of mechanical properties of films. Three replicate tests were carried out for the evaluation of antimicrobial activity of the films against microorganisms. Two series of experiments (ten replicate samples) were peformed for storage tests of packaged cheese under the pilot-plant conditions.

The results are given as mean value (x) and standard deviation (SD) in the following text and standard deviations are expressed by error bars in the figures. The difference between results of measurement of mechanical properties of films with and without lacquer coating was evaluated statistically using Student's t-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Natamycin migration

The ability of natamycin to release from lacquer coatings, as well as mechanical and barrier properties of films were evaluated in the first part of this study. The maximum amount of natamycin was released into distilled water after 24 h at a temperature of 23 °C from all films and the levels of released natamycin were stable during the migration time (Fig. 1). The maximum natamycin release was determined to be 0.79 $mg \cdot dm^{-2} \pm 0.08$ $mg \cdot dm^{-2}$ for PA/PE-1 film, 0.60 $mg \cdot dm^{-2} \pm 0.11$ $mg \cdot dm^{-2}$ for PA/PE-2 and 1.21 $mg \cdot dm^{-2} \pm 0.15$ $mg \cdot dm^{-2}$ for PVC film. The results indicate that 8.2–17.0% of the amount of the preservative added in the lacquer were released into distilled water. This could be caused by the low solubility of the preservative in water, which is aproximately 30–50 $mg \cdot l^{-1}$ [10, 30]. However, the amount of released natamycin was only 0.008–0.09 $mg \cdot dm^{-2}$ after re-extration of the extracted films into distilled water. This means, that low solubility of natamycin in distilled water had no effect on the amount of released natamycin in this case, because the solution was not completely saturated during migration. Therefore the low levels of migration

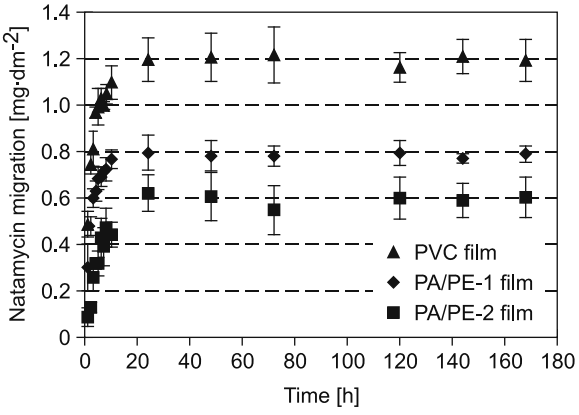

Fig. 1. The course of natamycin migration from the PA/PE-1, PA/PE-2, PVC films into distilled water at 23 °C during 168 h.

were most likely caused by strong fixation of natamycin in the lacquer, thereby subsequent release could be blocked. The diffusion modelling and calculation of diffusion coefficients were already described by HANUŠOVÁ et al. [31, 32].

The stability of natamycin releasing from PA/PE-1 film into solutions with various pH was tested during migration at 23 °C (Fig. 2). The maximum amount of released natamycin was only at the level of 0.091 mg·dm^{-2} ± 0.005 mg·dm^{-2} into 3% w/v acetic acid (pH = 2.7) after 9 h. The amount decreased to 0.027 mg·dm^{-2} ± 0.007 mg·dm^{-2} during 168 h. Concerning the release into 1 mmol·l^{-1} sodium hydroxide (pH = 10.5), the course of migration was similar that in case of 3% w/v acetic acid. The maximuml amount of released natamycin was at a level of 0.76 mg·dm^{-2} ± 0.04 mg·dm^{-2} after 9 h, but the released amount decreased to 0.46 mg·dm^{-2} ± 0.03 mg·dm^{-2} during 168 h. The amount of released natamycin after 168 h was only 3.4% at lower pH and 58.2% at higher pH in comparison with values obtained during migration to distilled water (pH = 7). We assume, that release and decomposition could occur simultaneously at pH values lower than 3 and higher than 10 [4–6]. With regard to pH of common foodstuffs, the antimicrobial activity of films with natamycin should be in particular considered at packaging the foods with higher pH, in which several common preservatives are nearly ineffective.

The influence of various temperatures (4 °C, 23 °C, 30 °C) on the preservative migration from PA/PE-1 film was assessed (Fig. 3). The release of natamycin was slower in the first 72 h at 4 °C than at 23 °C. Nevertheless, the levels of migration were equal after 72 h and the maximum amount of released natamycin was 0.79 mg·dm^{-2} ± 0.08 mg·dm^{-2}. At a temperature of 30 °C, the course of migration was similar to migration at 23 °C, but the amount of released natamycin was 0.62 mg·dm^{-2} ± 0.02 mg·dm^{-2}, i.e. lower by about 21.5%. This could be caused by simultaneous migration and decomposition of dissolved natamycin at higher temperatures. However, most foodstuffs are stored at the lower temperatures that did not influence natamycin decomposition. A question arises whether the slower release of the agent at lower temperatures would be sufficient to inhibit the growth of psychrophilic microorganisms at the beginning of storage.

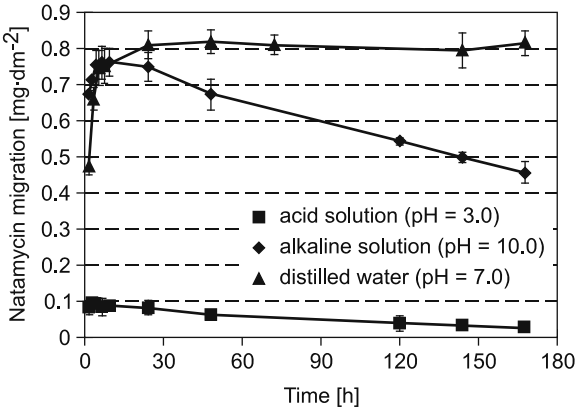

Fig. 2. The course of natamycin migration from the PA/PE-1 film into distilled water, acid and alkaline solution at 23 °C during 168 h.

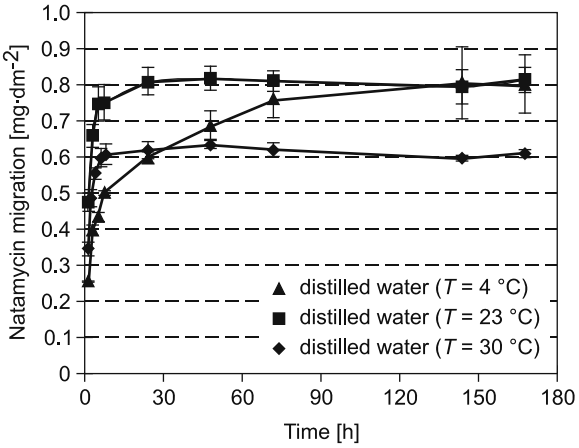

Fig. 3. The course of natamycin migration from the PA/PE-1 film into distilled water at temperatures of 4 °C, 23 °C and 30 °C during 168 h.

Mechanical and barrier properties

Mechanical and barrier properties of films with and without lacquer containing natamycin are presented in Tab. 1. Because the original film without the lacquer used for preparation of PA/PE-1 film was not available from the producer, only the results obtained for PVC and PA/PE-2 films with and without lacquer were statistically compared. Lacquer coating on the PVC and PA/PE-2 films did not cause major changes in their mechanical properties, i.e. tensile strength and strength at break. There were also no statistically significant differences between the water vapour permeability of the PVC film coated with lacquer and films without coating. However, the decrease in permeability was statistically significant in case of PA/PE-2 film. Oxygen and carbon dioxide permeability were decreased by the application of lacquer coating on the surface of PVC and PA/PE-2 films. This could be attributed to barrier effect of the additional lacquer layer. The decrease in permeabil-

Tab. 1. Mechanical and barrier properties of films coated with and without lacquer containing natamycin.

Film	Thickness [μm]	Mechanical properties				Peel strength [N]	Barrier properties		
		Tensile strength [MPa]	Strength at break [N]	Tensile strength [MPa]	Strength at break [N]		Oxygen permeability [ml·m^{-2}·d^{-1}·MPa^{-1}]	Carbon dioxide permeability [ml·m^{-2}·d^{-1}·MPa^{-1}]	Water vapour permeability [g·m^{-2}·d^{-1}]
		Machine direction		Cross direction					
PVC with lacquer	29.0 ± 1.1	50.4 ± 5.2^{a}	22.7 ± 2.3	51.3 ± 4.3	24.6 ± 2.1	12.5 ± 0.8	8630 ± 30^{a}	33400 ± 1800^{a}	53.7 ± 7.6
PVC without lacquer	25.0 ± 1.0	63.4 ± 4.4	24.7 ± 1.7	58.4 ± 7.0	23.6 ± 3.3	11.8 ± 1.6	9820 ± 490	39200 ± 800	52.5 ± 5.0
PA/PE-2 with lacquer	74.1 ± 1.0	36.6 ± 4.0	39.5 ± 4.3^{b}	28.7 ± 4.0	31.0 ± 4.4	24.7 ± 0.6	353 ± 1^{b}	887 ± 16^{b}	6.7 ± 0.2^{b}
PA/PE-2 without lacquer	70.0 ± 0.7	33.1 ± 5.2	22.5 ± 3.8	31.6 ± 2.7	33.4 ± 2.6	23.2 ± 1.3	610 ± 15	1195 ± 43	7.6 ± 0.5

Results are given in the form of mean value ± standard deviation.
a – results significantly different compared to values determined for PVC film without lacquer (α = 0.05), b – results significantly different compared to values determined for PA/PE film without lacquer (α = 0.05).

ity of PVC film was much smaller than in case of PA/PE-2 film.

Efficiency of antimicrobial film in packaging of cheese during ripening

During cheese ripening, the atmospheric conditions in the package are characterized by increasing carbon dioxide levels and decreasing oxygen levels. Films suitable for ripening should therefore avoid oxygen access to prevent mould growth on the cheese surface and should allow carbon dioxide and other gases to permeate through the film to avoid bag swelling and food acidification. The application of the PVC film was tested for packaging of Czech traditional soft cheese Blaťácké zlato considering that this cheese has already been investigated [31].

The PVC film was ineffective in preventing mould growth during ripening of Blaťácké zlato in a Czech cheese-producing company. Despite continual releasing of natamycin (Fig. 4) and despite the high carbon dioxide permeability of the film, a mould spoilage developed after 4 weeks of storage (Fig. 5A), whereas this cheese ripens commonly for 4–6 weeks without evidence of spoilage. The obtained results were similar to those of YILDIRIM et al. [33]. They described that casein coatings containing natamycin could suppress fungal growth on the surface of Kashar cheese approximately for 1 month during ripening. Different results were published by VAR et al. [34] who described that combined application of natamycin and PVC packaging material was able to

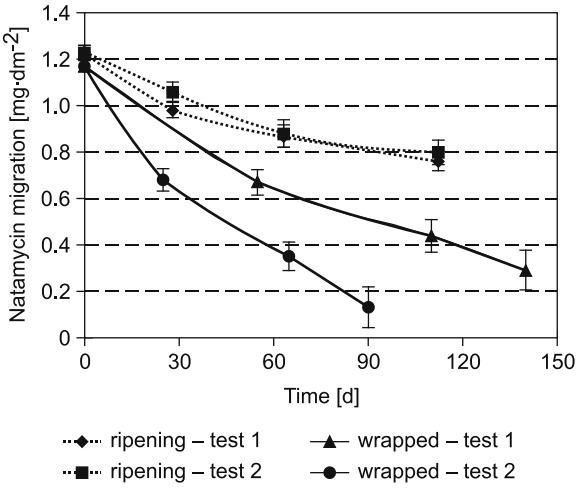

Fig. 4. The levels of natamycin released from the PVC film taken from the ripening and wrapped cheese Blaťácké zlato during storage time.

Migration test was performed into distilled water at 23 °C for 48 h.

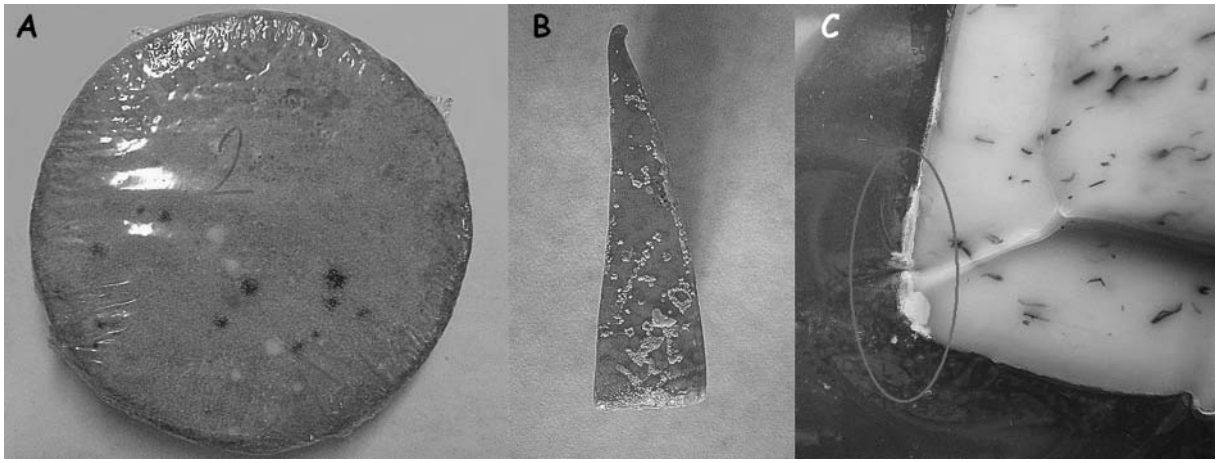

Fig. 5. Efficiency of antimicrobial film in packaging of cheese.

A – Cake of cheese Blaťácké zlato packaged in an antimicrobial PVC film with visible mould growth after 4 weeks of ripening, B – control sample of portioned cheese Blaťácké zlato wrapped in a commercial foil with extensive mould growth after 3 weeks, C – the fungal growth on the surface of vacuum-packaged control samples of cheese Gaston Oregano developed during storage for 3 months.

prevent mould growth on Kashar cheese ripened for 5 months. Nevertheless, the efficiency of antimicrobial films with natamycin ultimately depend on the amount of released natamycin, quality of contact with a cheese surface, barrier properties of films and environmental conditions in ripening room [35, 36].

The moulds as well as yeast isolated from the surface of cheese Blaťácké zlato during ripening were identified (Tab. 2). Fungal isolates found on the cheese belonged to genera *Penicillium* and *Cladosporium*, whereas *Penicillium* species were predominant. These species have already been reported to occur on refrigerated cheeses and in the environment of warehouses and cheese factories [37–43]. Salt-tolerant *Debaryomyces hansenii*, which commonly occurrs in brine and during ripening of cheeses [10, 40], was the only isolated yeast. The sensitivity of isolated microorganisms to natamycin was also tested. The growth of all species was completely inhibited at a tight contact with the PVC film on the agar medium (Fig. 6). Therefore, the insufficient antifungal efficiency of the PVC film during ripening of Blaťácké zlato could be caused by slight bag swelling and imperfectly tight contact of the film with the cheese surface.

Efficiency of antimicrobial film in wrapping and vacuum packaging of cheese

As wrapping of portioned cheese is widely used in Czech supermarkets and the guaranteed storage period of the wrapped cheeses is quite short (commonly 3–5 days), the antimicrobial activity of PVC film was also tested for re-packaging of portioned cheese Blaťácké zlato in cooperation with one of the retail chain. The initial mould growth appeared on the surface of control samples wrapped in PVC stretching film (thickness 12 μm) after 7 days, which is 2 days after durability. After 10 days, the fungal growth was clearly visible and the surface of the cheese was largely covered with mould after 3 weeks (Fig. 5B). Fungal growth did not occur on the surface of cheese packaged in PVC film releasing natamycin for this period. The occurrence of fungal growth was not apparent on the surface of samples even after 4 months, whereas guaranteed storage time of commercially marketed portioned Blaťácké zlato packaged in a modified atmosphere is 45 days. The total counts of bacteria increased from 10^4 CFU·g^{-1} to 10^6 CFU·g^{-1}, whereas the total counts of moulds and yeasts remained at the same level of 10^1 CFU·g^{-1} during the storage time of 4 months. The pH of the samples was 5.11 ± 0.05 and the values did not change during the experiment.

These results demonstrate that the tight contact with the packaged cheese is essential for the efficient migration. Fig. 4 indicates that the bag swelling in case of packaged rippening cheese could cause the lower natamycin migration and subsequent fungal growth, because the amount of released residual natamycin from the PVC film taken from the ripening cheese during storage was higher than in the case of wrapped cheese. Thus complete inhibition of fungal growth could be achieved by tight contact of the film with the wrapped cheese and presumable higher natamycin

Tab. 2. Identification of microorganisms isolated from cheese Blaťácké zlato during ripening in the dairy, their characteristics and inhibition zones formed by PVC film.

Isolated microorganisms	Inhibition zone [cm]	Common occurence	Toxinogenity
Debaryomyces hansenii	0.84 ± 0.07	Most common species of yeast found in all types of cheeses, common in dairies and in brine	Considered as non-pathogenic
Penicillium camembertii	0.81 ± 0.04	Widely used in the manufacture of soft cheeses, such as Camembert, Brie and Neufchatel	Capable of producing cyclopiazonic acid on synthetic media, absence of toxicity in cheeses cannot be taken for granted
Penicillium chrysogenum	0.94 ± 0.10	Ubiquitous fungus, occupies a very wide range of habitats	Produces roquefortine C, PR toxin and penicillin
Penicillium expansum	0.85 ± 0.06	Principal cause of spoilage of pome fruits, isolated also from cheese	Important producer of patulin and citrinin
Penicillium brevicompactum	1.05 ± 0.11	Widespread occurrence in dried foods also spoil refrigerated products, such as cheese	Produces mycophenolic acid and brevianamide A
Penicillium crustosum	0.90 ± 0.05	Ubiquitous spoilage fungus	Major producer of penitrem A (neurotoxin), produces also roquefortine C and terrestric acid
Penicillium commune	0.83 ± 0.06	Primary habitat in foods is cheese, is the wild type ancestor of *P. camembertii*	Produces cyclopiazonic acid
Cladosporium herbarum	1.65 ± 0.14	Contaminates fresh vegetables and fruits, causes also „black spot“ spoilage of cheese during ripening	Not known to produce mycotoxins; considered to be mycoalergen
Cladosporium cladosporioides	1.63 ± 0.14	Very wide variety of foods; causes also spoilage of refrigerated cheese	Not known to produce mycotoxins; considered to be a mycoallergen
Penicillium sp. (subg. *Furcatum*, series *Citrina*)	1.51 ± 0.07	Soil and contaminants of various foods	Capable of producing less significant toxic extrolites

Characterstics of microorganisms is given according to Pitt and Hocking [28] or Samson and Frisvad [29].

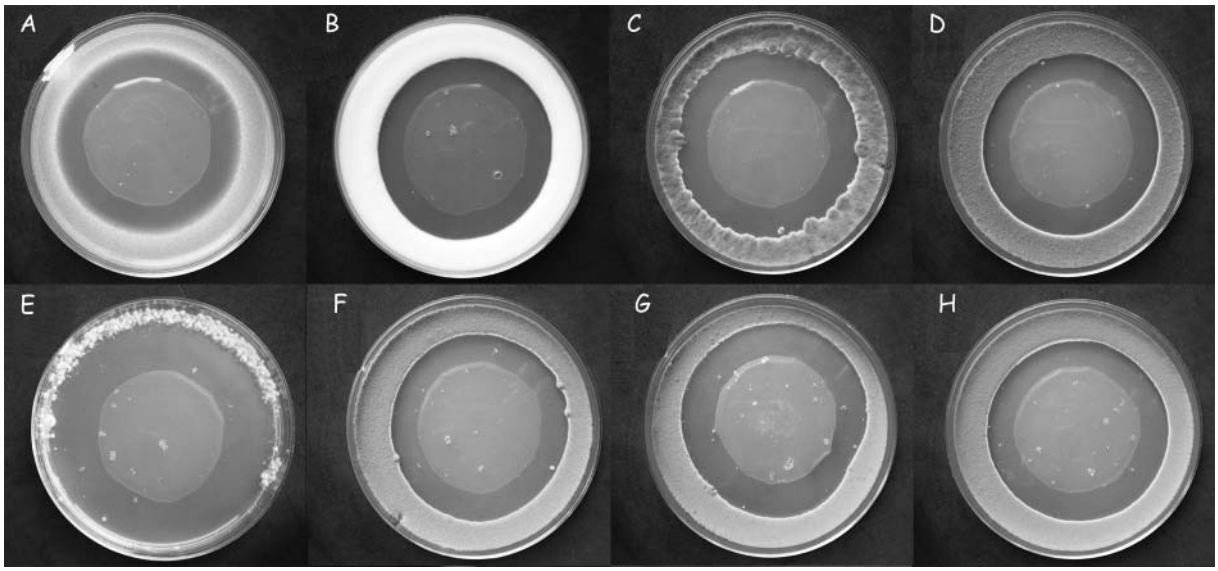

Fig. 6. The growth inhibition of yeasts and moulds isolated from the surface of Blaťácké zlato during ripening by the antimicrobial PVC film on agar media.

A – *Debaryomyces hansenii*, B – *Penicillium camembertii*, C – *Penicillium chrysogenum*, D – *Penicillium commune*, E – *Penicillium sp*. (subg. *Furcatum*), F – *Penicillium expansum*, G – *Penicillium brevicompactum*, H – *Penicillium crustosum*.

migration during storage, because lower levels of released residual natamycin were found.

Antimicrobial efficiency of PA/PE-1 film has already been studied by HANUŠOVÁ et al. [31], therefore only antimicrobial activity of PA/PE-2 film was assessed in this study. The PA/PE-2 film was tested for vacuum and modified atmosphere packaging of sliced semi-hard cheeses of Edam type, namely Eidamská cihla and Gaston Oregano, in cooperation with a Czech dairy. Vacuum packaging seems to be a promising field of application of antimicrobial films due to the tight contact of films with packaged foodstuffs.

The fungal growth was neither visually apparent on the surface of vacuum-packaged cheese Eidamská cihla in antimicrobial PA/PE-2 film, nor on the control samples vacuum-packaged in a commercial film during the storage time of 6 months. On the contrary, fungal growth appeared on the surface of Eidamská cihla packaged in the modified atmosphere and commercial film after 4 months. Mould spoilage developed on the surface of samples packaged in a modified atmosphere and antimicrobial film after 4 months, but only in the parts of cheese surface that were not in contact with the film.

Considering that herbs are potencial source of microbial contamination in foodstuffs, cheese Gaston Oregano containing 0.7% of oregano was spoiled in a shorter time compared to Eidamská cihla. The initial fungal growth was obvious on the surface of this cheese packaged in the modified atmosphere and antimicrobial film as well as on the control samples after 2 months. The moulds also developed on the vacuum-packaged control samples in commercial film after 75 days (Fig. 5C), whereas vacuum-packaged samples in antimicrobial PA/PE-2 film were without visible traces of fungal growth. The fungal growth became evident on these samples not sooner than on the fifth month of storage.

The area of PA/PE-2 film in tight contact with vacuum-packaged cheeses Eidamská cihla and Gaston Oregano was taken from the samples after a storage period of 5 months and natamycin migration from these areas of the film was determined to be at a level of 0.25 $mg \cdot dm^{-2} \pm 0.08$ $mg \cdot dm^{-2}$. This amount of released natamycin could be still sufficient to inhibit the most moulds considering minimum inhibitory concentration of natamycin, i.e. 0.5–6 $\mu g \cdot ml^{-1}$ [4]. The sufficient efficiency of natamycin for the control of mould growth in vacuum-packed hard cheeses was reported by BASÍLICO et al. [37]. DOS SANTOS PIRES et al. [44] reported that cellulose derivative polymer containing natamycin inhibited fungal growth on sliced Mozzarella cheese as well as chitosan containing natamycin studied by FAJARDO et al. [45] was effective against mould inoculated on portioned regional semi-hard Saloio cheese.

CONCLUSIONS

Results of our experiments demonstrate the influence of pH and temperature on the course of natamycin migration from the synthetic lacquers coated on polymer packaging films. The treatment of polymer films with lacquers containing the antimicrobial agent had only a minor effect on their mechanical properties as well as water vapour permeability. Conversely, lacquer coatings could create an additional barrier to oxygen as well as carbon dioxide permeation in polymer films with insufficient barrier properties.

The experiments indicated that microbiological stability of portioned vacuum-packaged cheeses in films with antimicrobial coatings was significantly better compared to those wrapped in a stretching film. The antimicrobial agents can serve as additional hurdle supporting microbiological stability (preventing microbial growth) in vacuum packaging of minimally processed food. The antifungal agents can be released from the lacquer coatings on polymer packaging films in amounts that can inhibit the fungal growth on the surface of the packaged foodstuffs during storage. With regard to more common and cheaper application of stretching films for wrapping in trade chains, the next research should be focused on development of these films with antimicrobial activity.

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