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Data on efficiency of developed packaging systems in semi plant (industrial) cheese production

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RE	Restricted to a group specified by the consortium (including the Commission Services)	
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Abstract

Two types of traditional Czech cheese (*Olomoucké tvarůžky* and *Blatácké zlato*) should have been packaged in polymer films releasing nisin as well as natamycin in pilot plant conditions in cooperation with two companies producing these types of cheese, i.e. *AW spol. S r.o. Loštice* and *Madeta a.s. Veselí nad Lužnicí*. As the laboratory scale experiments showed that above mentioned packaging system is not suitable for the cheese *Olomoucké tvarůžky* (deliverables D2B.4.3) the semi-pilot tests were carried out only in the company *Madeta a.s. Veselí nad Lužnicí* with the cheese *Blatácké zlato*.

Three types of cheese samples were prepared: (i) samples packaged in modified atmosphere (70 % N₂, 30 % CO₂), (ii) samples vacuum packaged in INVOS II (PA/PE film coated with lacquer releasing nisin and natamycin), (iii) samples wrapped in stretch film type PVC/PVdC.

During 30 day storage at 6 °C the significant inhibition of total bacteria, yeast and mould growth on the surface of samples packaged in film releasing nisin and natamycin compared with those on the samples in other packages was found. Favourable antimicrobial effect of packages made of INVOS II film was determined for aerobic spore forming bacteria, *Bacillus cereus* and *Lactobacillus* sp.

Even the sensory analyses indicated significant differences in smell as well as taste of cheese stored in active package and at modified atmosphere the inhibition of micro-organisms growth on the cheese surface makes this type of package as promising. The experiments carried out in semi-pilot plant in Madeta company confirmed suitability of films coated with lacquer releasing nisin and natamycin for packaging of cheese *Blatácké zlato*. But for the application in practice further development will be necessary.



Data on efficiency of developed packaging systems in semi plant (industrial) cheese production (D2B.4.5)

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1. Introduction

This report follows up with the reports on the results obtained within two years of Truefood project solution, i.e. the reports from the April 2007 and 2008. So far obtained results on packaging films coated with synthetic lacquer releasing nisin and natamycin could be characterized as follows:

Antimicrobial agent migration - films with nisin and natamycin:

1. Amount of released nisin: up to 3500 IU/dm² at 25 °C, i.e. less than 10 % of theoretical total amount of nisin added to coating.
2. Equilibrated nisin migration depended on coating thickness, but the correlation was not close at used experimental conditions.
3. Non linear relation between maximal nisin migration and Nisaplin[®] concentration in lacquer layer.
4. Diffusion coefficient of $2.37 \cdot 10^{-10} \text{ cm}^2/\text{s} \pm 0.82 \text{ cm}^2/\text{s}$ was determined for nisin transport in lacquer layer at 25 °C.
5. Amount of released natamycin up to 2.5 mg/dm² at 23 °C, i.e. less than 3 % of theoretical total amount of natamycin added to coating.
6. Diffusion coefficient of $1.03 \cdot 10^{-10} \text{ cm}^2/\text{s} \pm 0.17 \cdot 10^{-10} \text{ cm}^2/\text{s}$ was determined for natamycin transport in Kombilack L-1917 lacquer layer at 23 °C.

Antimicrobial activity of the film INVOS II

1. This film was able to release nisin up to level 400 IU/dm² and natamycin up to level 0.8 mg/dm².

2. The film provides significant inhibition of the growth of following indicator microorganisms on suitable agar media: *Lactobacillus helveticus* CH-1, *Penicilium expansum*, *Fusarium culmorum*, *Listeria ivanovii*.
3. The film provides significant inhibition of the growth of following indicator microorganisms on the surface of tested cheeses (*Olomoucké tvarůžky*, *Blaťácké zlato*): *Lactobacillu sp.*, total bacteria count, total yeast and mould count, aerobic spore forming bacteria, *Bacillus cereus*.
4. The film was not able to prevent growth of *Listeria monocytogenes* AW2007 strain, which was isolated directly from the cheese *Olomoucké tvarůžky* and which proved high nisin resistance.

Application of the film INVOS II for cheese packaging

1. Film INVOS II could be used for packaging of the cheese *Blaťácké zlato* after packaging process optimization.
2. Film INVOS II could not be used for packaging of the cheese *Olomoucké tvarůžky* due to two reasons:
 - a. Gas permeability of the packaging film was too low.
 - b. Preservative agents released by film had detrimental effect on cheese maturation.

The laboratory tests with the cheese *Olomoucké tvarůžky* packaged in INVOS II film were repeated with the same results as described in report from April 2008. Therefore the experiments with this cheese were stopped and semi-pilot essays were performed only with the cheese *Blaťácké zlato*. This report describes obtained results.

2. Experimental part

2.1 Materials and Methods

Packaging materials: packaging film (type LDPE/PA, thickness 80 µm, INVOS a.s., Svárov, Czech Republic), LDPE side of this film was treated with corona and then coated with lacquer based on polyvinylidene chloride (L-1471, ROTOFLEX, Switzerland) with addition of 16.7 % (w/w) of

commercial preparation Nisaplin[®] (Danisco, Brabrand, Denmark) containing 2.5 % (w/w) of pure bacteriocin nisin and 33.4 % (w/w) of commercial natamycin preparation Delvolid[®] (DSM Food Specialities, Netherland) containing 50 % (w/w) of pure antimycotic agent natamycin. The thickness of lacquer layer was about 4.5 µm. In this report this film is termed as INVOS II.

Cheese packaging: tested samples were packaged in the semi-pilot line in Madeta Řípec company. As the INVOS II film has parameters (i.e. saleability, mechanical characteristics, width, etc.) unsuitable for packaging machines the packaging in INVOS II film was carried out using manual vacuum sealing machine. Three types of samples (approx. 200 g) were prepared:

- (i) samples packaged in modified atmosphere on commercial producing line of Madeta company (thermoformed tray sealed with polymer film of PE/PA type, modified atmosphere composition 70 % N₂, 30 % CO₂),
- (ii) samples in INVOS II film,
- (iii) samples wrapped in stretch film simulated packaging commonly used by supermarket chains (stretched film type PVC/PVdC).

Tested cheese were taken from Madeta producing line and immediately packaged. Before testing the samples were stored at 6 °C for 30 days. Eighty samples of each type packaging were prepared.

Microbiological tests: The antibacterial efficiency of the film (i.e. nisin activity) as well as antimycotic efficacy (i.e. natamycin activity) were tested by procedures using *Lactobacillus helveticus* CH-1, *L. monocytogenes* AW2007, *L. ivanovii* CCM 5884 and *Fusarium proliferatum* M5689 or *Penicillium expansum* as indicator micro-organisms, respectively. The tests were done on agar plates and the details for both techniques were described in the report from April 2007.

During storage of packaged cheese the total count of bacteria (GTK medium, 37 °C, 48 hours), the total count of yeasts and moulds (PDA medium, 37 °C, 48 hours), the growth of aerobic spores forming bacteria *Bacillus sp.* (PCA medium, temperature 30 °C, 48 hours after inhibition of diluted sample at 85 °C for 10 min), the count of lactic acid bacteria (MRS medium, 37 °C, 48 hours), was followed on the surface of tested samples. A part of cheese was inoculated by *Bacillus cereus* DMF 2008 or *Penicillium expansum* before tests by dipping into a suspension of this micro-organism (density 10⁷ CFU.ml⁻¹).

Sensory evaluation: Sensory analyses of cheeses packaged in traditional and new films were carried out. The tests were performed by the ten members skilled panel, the triangle test was used for sample comparison. Each evaluator tested five sets of samples.

The mean value (\bar{x}) and the standard deviation (SD) were calculated for each of the tested parameters. The results in the following text are in the form $\bar{x} \pm \text{SD}$.

3 Results and discussion

3.1 Efficiency of antimicrobial packaging

The film INVOS II is shown in Figure 1. This film was prepared in the beginning of 2007. Therefore its efficiency against indicator microorganisms was tested. Due to storage in the dark at low temperature (below 4°C) the film retained antimicrobial activity, which was comparable with that in the year 2007.



Figure 1 Packaging film INVOS II (type LDPE/PA, 78 μm , produced by INVOS a.s.) with both nisin and natamycin in lacquer layer (3 μm of Kombilack L-1471 containing 16.7 % (w/w) of Nisaplin and 33.4 % (w/w) of Delvocid)

The Figures 2 and 3 document the INVOS II films efficiency to inhibit lactic bacteria as well as mould growth.

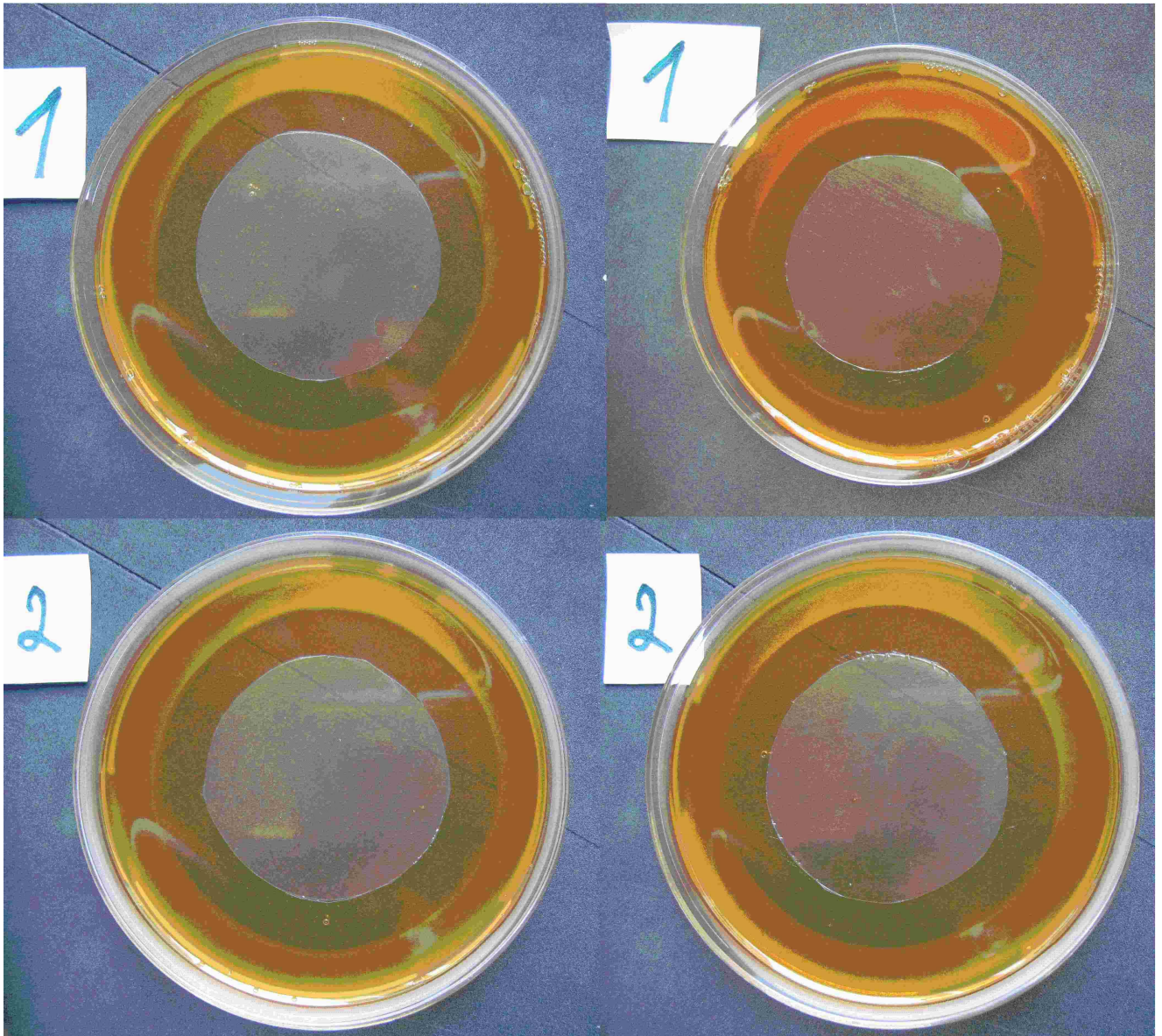


Figure 2 – Inhibition of *Lactobacillus helveticus* CH1 growth in contact INVOS II film (type LDPE/PA/LDPE, produced by INVOS a.s., 4.5 μm layer of Kombilack L-1917 containing 16.7 % (w/w) of Nisaplin). The samples numbered 1 were taken from the roll of film before the parts used for packaging tests, samples numbered 2 after them.

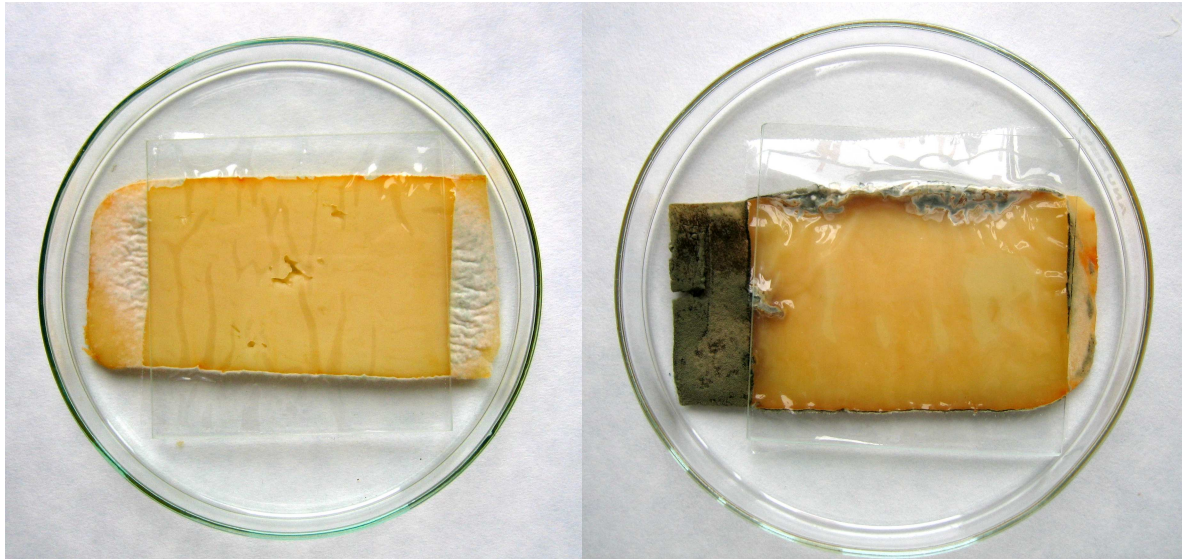


Figure 3 – Inhibition of *Penicilium expansum* growth on the surface of cheese *Blatácké zlato* inoculated with this mould in contact with INVOS II film after 3 days (left) and 18 days (right) at 23 °C. The black colour on the cheese after 18 days storage is caused by massive mould growth.

3.2 Testing of efficiency of polymer packaging films releasing nisin and natamycin in semi-pilot scale

In the Figures 4 - 6 the tested types of packages with cheese *Blatácké zlato* are shown.



Figure 4 – Packaging of cheese *Blaťácké zlato* in modified atmosphere (samples “MAP”)



Figure 5 – Packaging of cheese *Blaťácké zlato* in stretching film, tested samples (left) and samples from supermarket Globus (samples “wrapping”)



Figure 6 – Vacuum packaging of cheese *Blaťácké zlato* in INVOS II film (samples “INVOS II”)

The samples of traditional Czech cheese *Blaťácké zlato* in all three types of packaging were stored at 6 °C and after 5 days, 12 days, 18 days, 23 days and 30 days the micro-organism contamination on the samples surface was tested. For every test ten parallel samples of each type packaging were examined.

The results indicated that INVOS II film provided significant inhibition of all examined types of micro-organisms compared with both resting packaging systems. In Figure 7 the efficiency of film INVOS II in inhibition of aerobic and facultative anaerobic mesophylic bacteria capable to growth on the GTK media is demonstrated. It is obvious, that decrease of total bacteria count from 1 to 1.5 logarithmic cycle was observed during 30 day storage at 6 °C due to inhibition of nisin sensitive part of contamination.

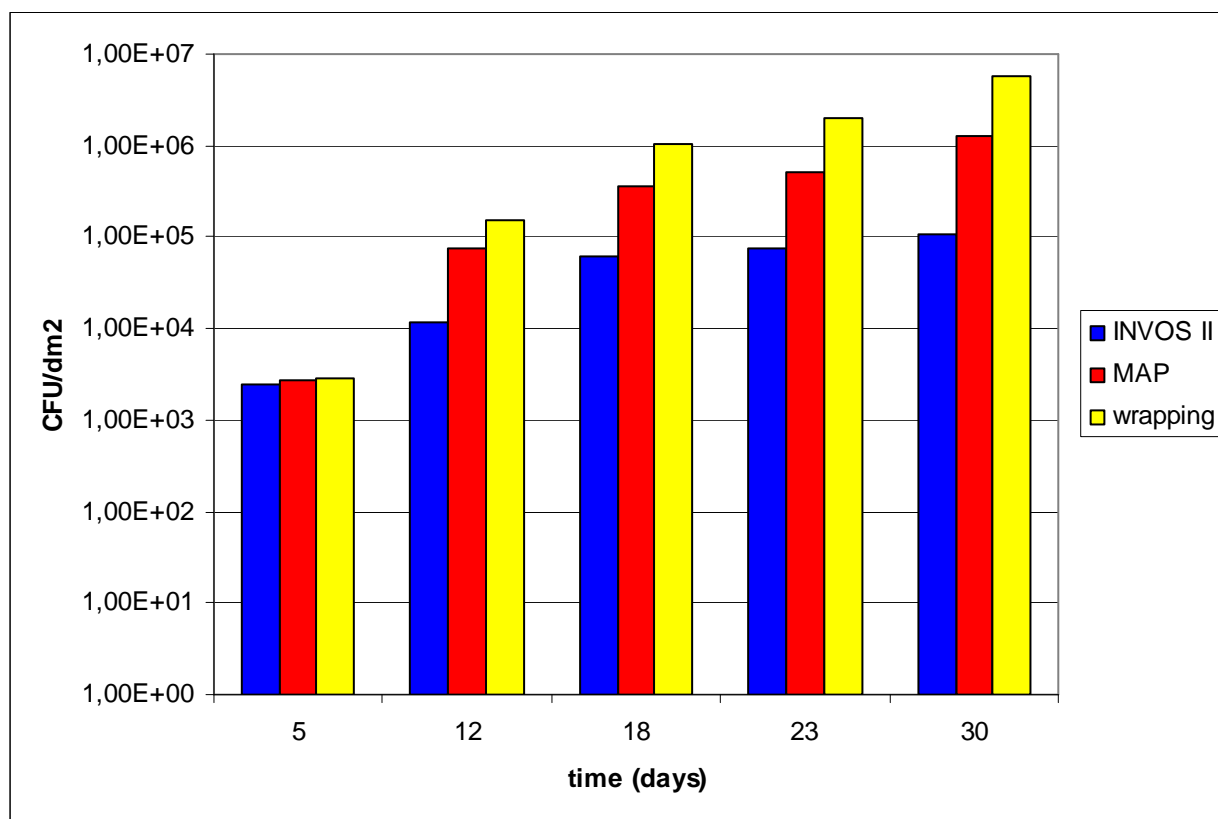


Figure 7 The influence of three packaging systems on the change of growth of aerobic and facultative anaerobic mesophylic bacteria capable to growth on the GTK media on the surface of the cheese *Blatácké zlato* during storage at 6 °C.

The antimycotic effect of the film INVOS II against yeast and moulds on the surface of the cheese *Blatácké zlato* during storage at 6 °C is shown in Figure 8. In this figure the growth of total fungal contamination capable to growth on PDA media is monitored. It is obvious that in this case the efficiency of INVOS II film was rather limited, i.e. the decrease of total yeast and mould count in the antimicrobial package was by less than one logarithmic cycle lower compared with both controls (MAP and wrapping). Such results were obtained in three experiment repetitions and they are in contrast with quite good inhibition of *Penicillium expansum* and/or *Fussarium proliferatum* during laboratory experiments with INVOS II film (see figure 3). Nevertheless, this limited efficiency could extend the stability of packaged cheese as increase of yeast and mould count by one logarithmic cycle corresponds approximately to one week storage at 6 °C.

Also in this case the growth of micro-organisms in INVOS II package was slower compared with MAP and/or wrapping samples. Similarly, from Figures 9, 10 and 11 the significant inhibitory effect

on the growth of aerobic spore forming bacteria (*Bacillus sp.*), *B. cereus* and *Lactobacillus sp.* on the surface of the cheese *Blatácké zlato* during storage at 6 °C is obvious. *Bacillus sp.*, *Bacillus cereus* and *Lactobacillus sp.* counts were decreased by two logarithmic cycles, three logarithmic cycles and two logarithmic cycles during 30 days compared with both controls (MAP and wrapping), respectively. The growth of micro-organisms of the tested type of cheese were also influenced by relatively low water activity (see table II).

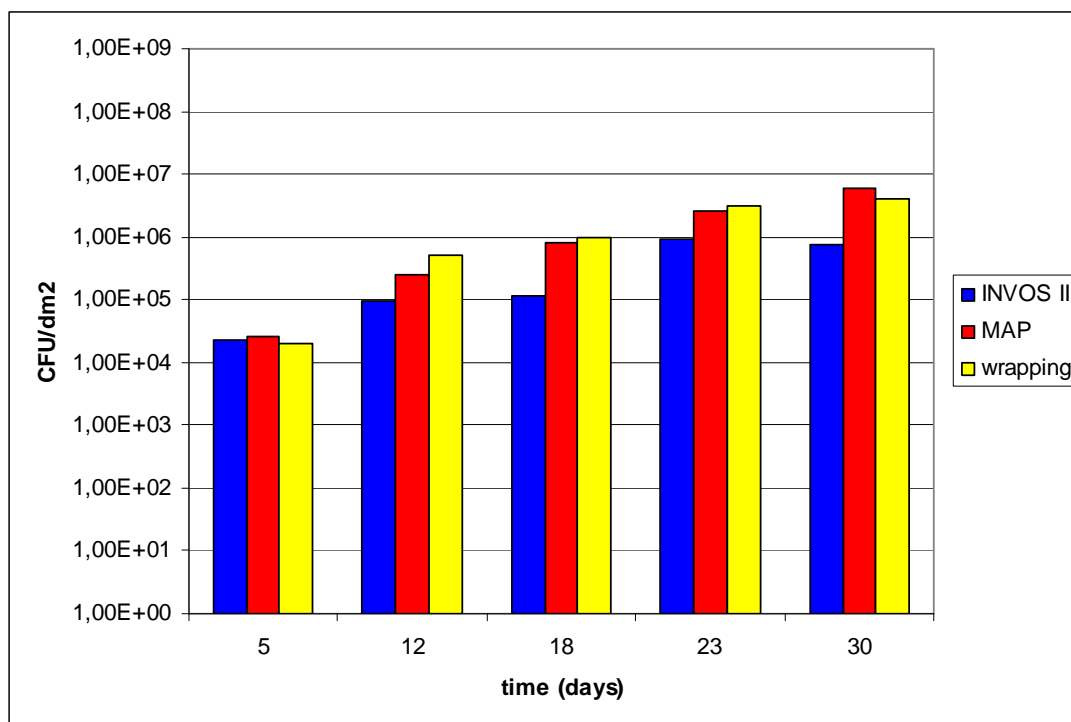


Figure 8 The influence of three packaging systems on the change of the total yeast and mould count (i.e. total fungal contamination capable to growth on PDA media) on the surface of the cheese *Blatácké zlato* during storage at 6 °C.

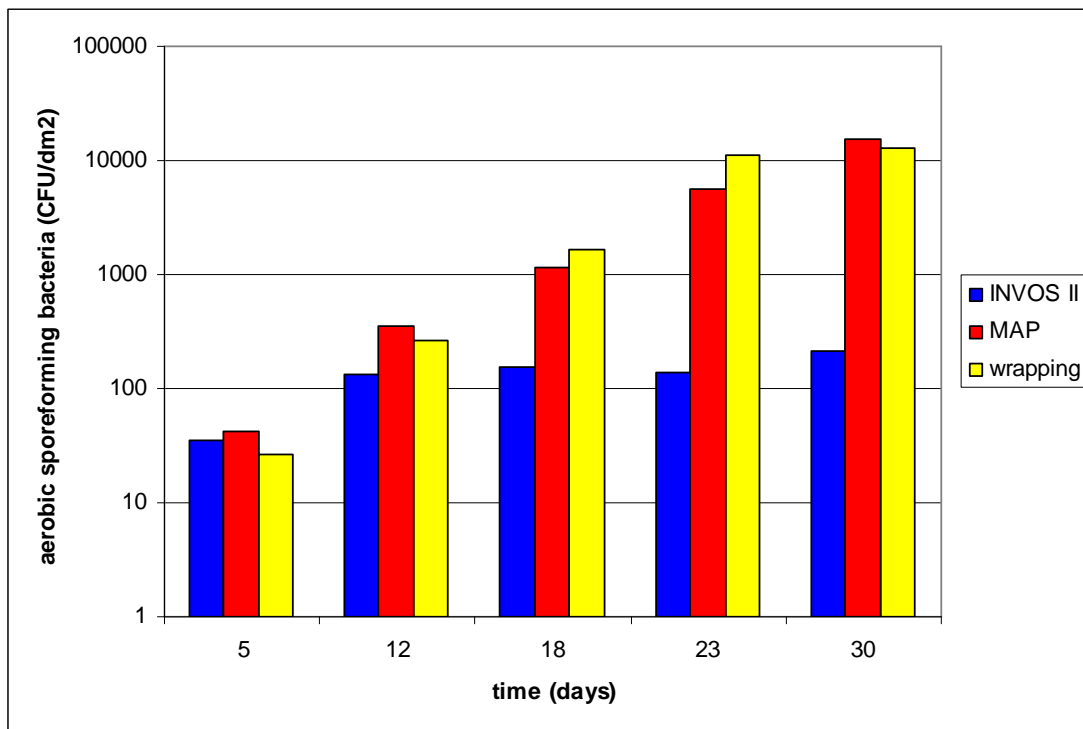


Figure 9 The influence of three packaging systems on the change of aerobic spore forming bacteria (*Bacillus sp.*) count on the surface of the cheese *Blaťácké zlato* during storage at 6 °C.

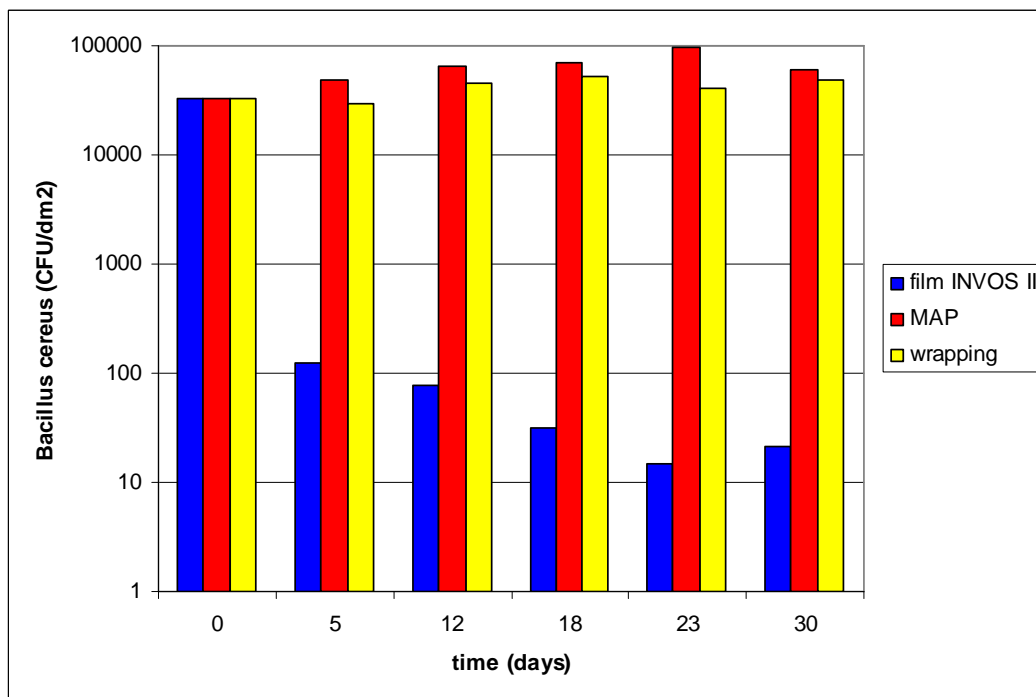


Figure 10 The influence of three packaging systems on the change of *Bacillus cereus* count on the surface of the cheese *Blaťácké zlato* during storage at 6 °C.

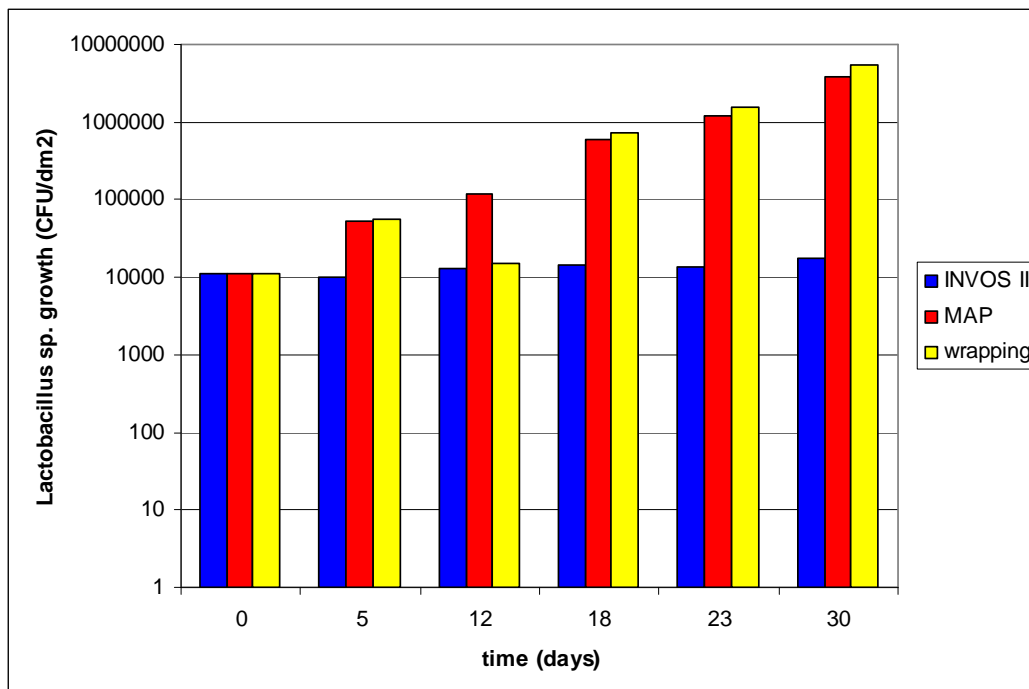


Figure 11 The influence of three packaging systems on the change of *Lactobacillus sp.* count on the surface of the cheese *Blatácké zlato* during storage at 6 °C.

It is obvious that INVOS II package inhibited micro-organisms growth on the cheese surface. EU hygienic legislation restricts the application of both nisin and natamycin in food. The maximal allowed concentration of nisin in food, i.e. 12.5 mg/kg, could not be achieved in done experiments, the limit for the content of natamycin in food is more strict, i.e. 1 mg/kg of food and no detectable agent deeper then 3 mm in food. Therefore the content of natamycin in cheese packaged in INVOS II film was determined both in outer 3 mm thick layer and in the layer from 3 mm to 5 mm deep the surface. Determination was carried out in three samples of cheese after 15 days storage as well as after 30 days storage at 6 °C. In both cases the concentrations lower than above mentioned limits were found, i.e. 0.65 mg/kg – 0.91 mg/kg in the outer part of cheese and concentration bellow the detection limit of used method (HPLC technique after extraction with acetone), i.e. 0.01 mg/kg.

Table I – Permeability parameters of the films used for *Blatácké zlato* packaging (oxygen permeability determined at 23 °C and RH=0 %, water vapour transmission rate was tested at 38 °C and RH = 85 %)

	INVOS II	MAP	WRAPPING
Oxygen permeability (ml (STP)/m ² .d.0.1 MPa)	4.7±1.2	6.2±1.2	115.3±3.2
WVTR (g/m ² .d)	1.25±0.28	2.9±0.5	5.1±1.1

Sensory evaluation of complex perception (taste, smell and appearance) of cheese stored in all tested types of package was also carried out after 15 and 30 days using triangle test. Difference between “INVOS II” and “MAP” samples as well as difference between “INVOS II” and “Wrapping” samples were tested. During each test ten panellists evaluated 50 triads of samples, i.e. each of them evaluated five triads. When tested “INVOS II” and “MAP” samples 24 and 34 correct responses were obtained after 15 day and 30 day storage, respectively. When tested “INVOS II” and “Wrapping” samples 20 and 24 correct responses were obtained at the same conditions. Twenty three correct responses is minimum number for statistically significant difference between tested samples on the level of significance $\alpha = 0.05$. It means that after 15 days the statistically significant difference was found between samples “INVOS II” and “MAP” while after 30 days between both “INVOS II” and “MAP” samples and “INVOS II” and “Wrapping” ones on mentioned level of significance. Even there were differences in smell and taste of product packaged in modified atmosphere and/or wrapped in stretching film and INVOS II film, product packaged in INVOS II film was acceptable for consumer. Even the barrier parameters of INVOS II film and film used for sealing of package containing the cheese in modified atmosphere were similar (see Table I), and the tested parameters of products in all packages types were similar (see Table II), the sensory panel was able in all cases to distinguish the tested cheeses. In this case it seems that the reason of the difference is in the way of manipulation during packaging operation.

Table II – Comparison of chemical and sensory parameters of the cheese *Blatácké zlato* packaged in common package and film INVOS II and stored for 40 days at 4 °C.

Parameter		MAP	INVOS II	WRAPPING
Water content (% w/w)		50.2±0.9	55.9±2.1	52.3±1.7
Fat content (% w/w)		26.1±3.3	24.5±2.3	26.0±1.9
Proteins content (% w/w)		22.3±3.6	19.2±1.3	20.9±2.0
Water activity		0.920±0.005	0.929±0.005	0.921±0.005
pH		5.1±0.2	5.2±0.2	5.0±0.3
Peroxide number of the fat (meq. O ₂ / kg)		5.3±0.6	5.3±1.1	5.3±0.6
Sensory difference (α = 0.05)	15 day storage	Not statistically significant		
	30 day storage	Statistically significant		
	15 day storage		Statistically significant	
	30 day storage		Statistically significant	

4. Conclusions

The experiments carried out in semi-pilot plant in Madeta company confirmed suitability of films coated with lacquer releasing nisin and natamycin for packaging of cheese *Blatácké zlato*. Even the sensory analyses indicated significant differences in smell as well as taste of cheese stored in active package and at modified atmosphere the inhibition of micro-organisms growth on the cheese surface makes this type of package as promising. The application in practice would require more time and longer development.

5. References

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