Suppression of Mould Growth on Dry Sausages

Petr PIPEK[1], Bo-Anne ROHLÍK[1], Anna LOJKOVÁ[1] and Ladislav STARUCH[2]

[1]Department of Food Preservation and Meat Technology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague, Prague, Czech Republic;
[2]Department of Food Science and Technology, Faculty of Chemistry and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic

Abstract

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The effects of two antifungal agents (natamycin, potassium sorbate) were compared on the mould growth on the surface of dry sausages (both heat treated and fermented). The mould growth was studied on fermented sausage Prešporska saláma and heat treated dry sausage Vysočina after the production and during the ripening and storage. Different samples were prepared: two natamycin treated (0.1% and 0.2%) and one potassium sorbate treated (2%), the control sample was not treated. All the samples were inoculated with spores of *Penicillium nalgiovense* and their germination and growth were monitored. An objective evaluation of the growth was carried out using video image analysis (NIS Element software 2.30). Natamycin proved to be the most efficient in the suppression of mould growth.

Keywords: sausage; moulds; natamycin; sorbate; video image analysis

Mould growth on the surface of sausages is generally undesired. Even the growth of cultural moulds (e.g. *Penicillium* from Hungarian sausages) on other dry sausages (not meant to be moulded) can be seen as a problem. Unsuitable storage conditions, such as high humidity and temperature, can affect the surface stability of the sausages and moulds spores can germinate and grow on them. The growth of moulds can be inhibited by air circulation and the presence of fungicide compounds in the smoking medium. Substances in the smoke medium such as carbonyls, phenols, carboxyl acids, act antimicrobiologically and affect the growth of moulds on the sausage surface (Jira 2004; Ulmer *et al*. 2005).

To increase the production security and prevent the growth of moulds, the sausage surface is often treated with various substances; most frequently is it sorbic acid, potassium sorbate, lactic acid, natamycin, and others. A modern method is the use of inside coated packaging materials (Hanušová *et al*. 2010). Another way to apply antifungal agents is to incorporate them into biopolymer films, especially natamycin with rosemary extract (Ture *et al*. 2008).

Salts of sorbic acid, most commonly potassium sorbate, are applied either by dipping or spray-drying; it is possible to apply sorbate even on the inside of the sausage casing (Stiebing *et al*. 2001). However, potassium sorbate has a lesser influence on the microbial growth rates than other preservatives, e.g. methyl *p*-hydroxybenzoate (Matos *et al*. 2007, 2008).

Natamycin is a natural antifungal agent produced during the fermentation of the bacterium *Strep-*

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tomyces natalensis (Zeuthen & Bøgh-Sørensen 2003). Natamycin is effective at very low levels and affects the growth of nearly all moulds and yeasts; it slows down their growth for up to six months but it cannot completely inhibit it (Zeuthen & Bøgh-Sørensen 2003). On the other hand, natamycin has no antibacterial activity (Delves-Broughton *et al*. 2006). This makes it useful for the application on such products which require bacterial processes, such as dry sausages (Stark 2004).

When applying a solution with a low concentration of natamycin on the surface of sausages, defects occur in the permeability of the cellular membrane of the fungi cells. Natamycin interacts with sterols, which are present in the cellular membrane of the fungi cells, and thus it destructs the selective permeable membrane (Jay 2000). First of all, potassium ions are forced out from the cell, followed by amino acids, when a solution of higher concentration of natamycin is used (Robert & Hui 2001).

Natamycin is applied by scattering or by dipping the sausages into the solution. There are different commercial preparations and the most popular one is Delvocid (Stiebing *et al*. 2001).

An old study on raw cured Italian dry salami showed that dipping or spraying the salami with natamycin (0.0002%) or sorbate (2.5%) did not successfully prevent the growth of surface moulds. Only at 10% concentration of potassium sorbate visual inhibition of the mould growth was observed (Holey 1986).

Natamycin can be applied for the surface treatment of sausages in different ways (dipping, spraying, pre-treatment of sausage casings, the use of the recently developed treatment gels) (Delves-Broughton *et al*. 2006).

Stiebing *et al.* (2001) applied natamycin and sorbate on raw sausages (Rohwurst) inoculated with *Penicillium nalgiovense*, *P. brevicompactum, Mucor racemosus*, and *Debaryomyces hansenii*. Natamycin preparations had a longer lasting antifungal effect than potassium sorbate. Xiaoguang *et al*. (2009) added a mixture of sodium lactate, nisin, and natamycin directly into the sausage and also showed a positive effect on the shelf life of the treated sausages.

The visual observation of the mould growth on the sausage surface is subjective and hard to quantify. More objective seems to be an evaluation using video image analysis (VIA) that enables to archive the images of sausages in different periods of storage and separately evaluate the number or the area of the measured objects. This method proved to be suitable for the evaluation of the colour and structure of meat and meat products (Pipek *et al*. 2007, 2008) as well as for the evaluation of meat histological samples. (Tremlová & Starha 2002, 2003).

The goal of this study was to evaluate the suitability of video image analysis for the evaluation of the mould growth on the sausages surfaces and to evaluate the efficacy of several agents in suppressing this growth with potassium sorbate and natamycin.

MATERIAL AND METHODS

Two types of dry shelf stable products were used – cooked salami Vysočina and fermented salami Prešporská saláma. Both meat products were produced under industrial conditions according to the traditional Czech and Slovak technology.

Preparation of samples. The dry/cooked sausage Vysočina was produced using lean beef and fatty pork. After mixing with 2.5% salt and spices, it was filled into collagenous casings, heat treated up to 70°C (including a short – 15 min – period of smoking) and dried 10 days to $a_w < 0.93$. Subsequently, the sausages were treated with three preparations containing either potassium sorbate or natamycin. Potassium sorbate was applied in spray as 2% solution and the concentration of the natamycin solution was 0.1% or 0.2%. We used the natamycin preparation from the company TRUMF International, Ltd. (Dolni Ujezd, Czech Republic). In all the experiments, non-treated sausages served as control samples.

The fermented salami Prešporská saláma was produced using lean beef, lean pork, and fatty pork mixed with 2.2% salt and spices and starter cultures Bessastart. The mixture was filled into collagenous casings (55 mm) and slightly smoked (using cold smoke at 20–25°C). After the fermentation process, the salami was dried up to $a_w < 0.93$. The samples of the fermented salami Prešporská saláma were treated with 2% solution of potassium sorbate and with 0.1% or 0.2% solution of natamycin.

All samples were inoculated with the spores of the cultural mould *Penicillium nalgiovense*; a commercial started mould culture from the mould salami production was used. To accelerate and facilitate the moulds growth, the sausages were

intentionally kept under elevated air humidity (90–95%). The storage time means the time period from the inoculation.

Methods. The dynamics of the moulds growth on the surface was evaluated using video image analysis, software NIS-Elements 2.20 (Laboratory Imaging Prague, Czech Republic). The pictures of the surfaces of the exposed sausages were shot using a Nikon Coolpix 800 under identical orientation during all the experiments.

One of our tasks was to find a suitable criterion to evaluate the mould growth. We measured the number of colonies, the whole relative surface area, which was covered by moulds, and finally the diameter of the mould colonies.

A more complicated method was tested simultaneously; in this, a digital camcorder took pictures of the rotating sausages and the videosequences were then automatically evaluated by precise video image analysis in LabVIEW according to Korbářová *et al*. (2007).

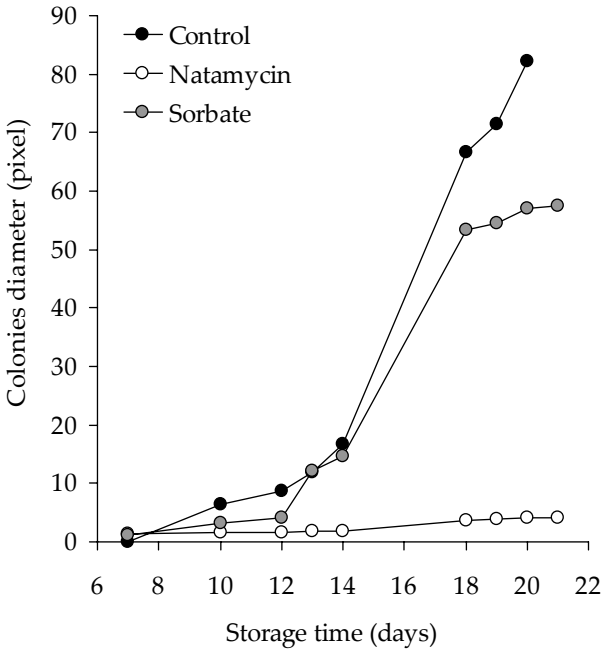

Figure 2. The growth of the moulds on cooked/dry sausage – evaluated by colonies diameter

RESULTS AND DISCUSSION

Video image analysis (VIA) seems to be a suitable method to evaluate the mould growth on the surface of dry sausages. As mentioned above, we tested the number of colonies, the whole relative surface area that was covered by moulds, and finally the diameter of moulds colonies. Each method provided different information and can be advantageous for different purposes.

The enumeration of the colonies numbers was suitable in the first period of the evaluation up to the moment when the growing colonies began to merge together. On the other hand, the number was also influenced by the inhomogeneous contamination of the surface by other spores. Maybe the colonies numbers can be used as a criterion of spores germination (Figure 1).

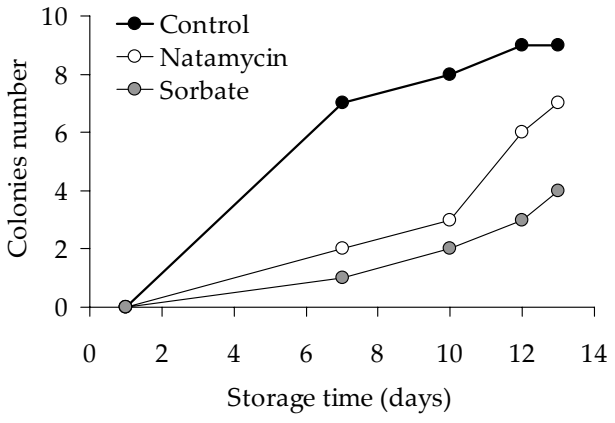

Figure 1. The growth of the moulds on cooked/dry sausage – evaluated by colonies number

The colonies diameter seems to be the most objective data as it reflects real dynamics of the mould growth. However, this method is limited by the moment when the colonies begin to grow into an undividable object. Video image analysis surely enables to separate two or several colonies, but only up to a certain dimension (Figure 2).

The common area seems to be the most simple and reliable way to evaluate the dynamics of the moulds growth. It is not influenced by the merging of mould colonies (Figure 3). On the other hand, it must be taken into consideration that this criterion can be influenced by inhomogeneous contamination (different initial numbers of moulds spores).

The results of the camcorder method are demonstrated in Figure 4. It is evident that it offers exact values. It shows very precisely the inhibition effects of all preparations tested. The most efficient preparation is natamycin; sorbate has a small effect on the mould growth. The disadvantage of this method resides in it requiring a more complicated apparatuses and software for the evaluation. For this reason, we prefer a very simple method that uses a normal digital camera while the images can

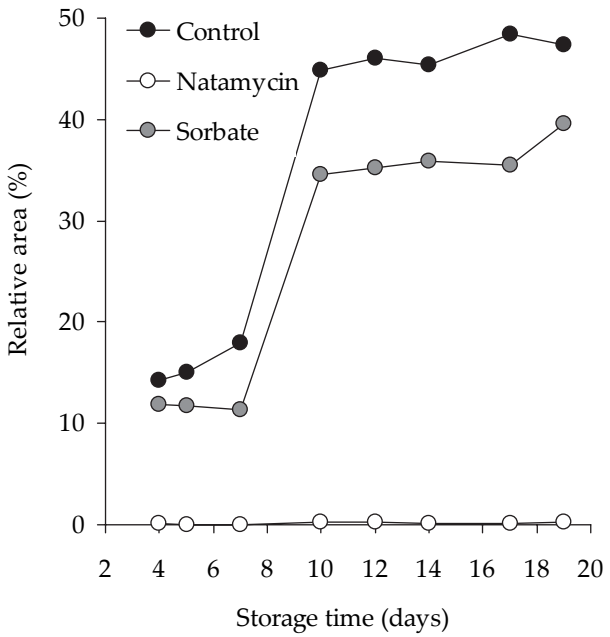

Figure 3. The growth of the moulds on cooked/dry sausage – evaluated by relative area covered by moulds

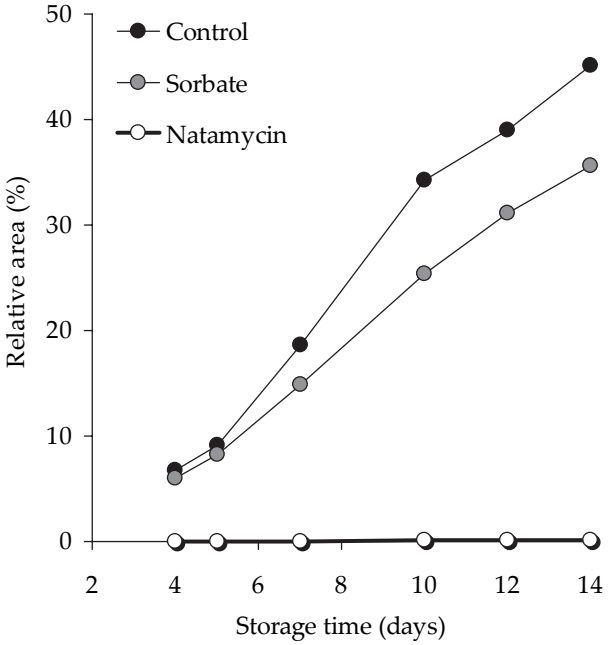

Figure 4. The growth of the moulds on cooked/dry sausage – the results of camcorder method

be collected and evaluated later. The preciseness of the simpler method is sufficient, even though the round surface of the intact sausage can somehow deform the picture. However, when the sausages are fixed in the identical position, the deformation is the same in all pictures and no systematic error should take place.

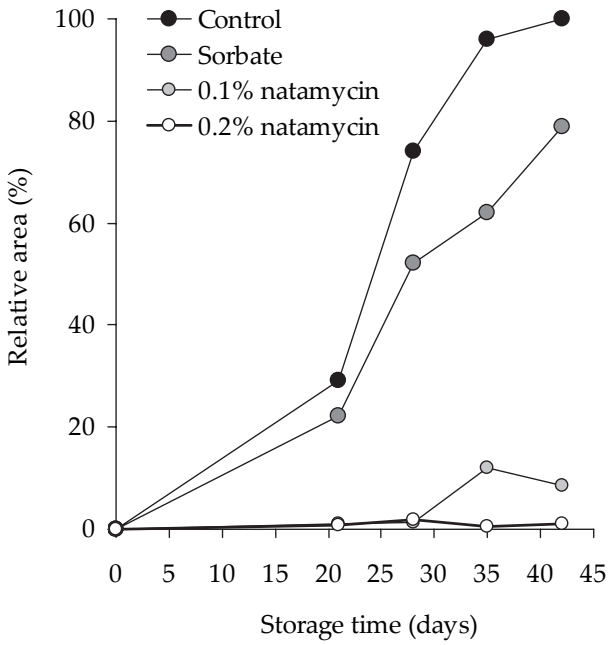

Figure 5. The growth of the moulds on fermented sausage – evaluated by relative area covered by moulds

Efficacy of preparations used

All preparations used in our experiments were effective in suppressing the mould growth on the surfaces of both types of durable sausages cooked and fermented in various ways. Natamycin proved to be the most effective one (preparation). Although Stiebing *et al.* (2001) report that *Penicillium nalgiovense* should be sensitive to sorbate, in this experiment this treatment was not satisfactory at the concentration used.

Some differences between the two types of tested sausages can be expected in the initial spores/moulds counts, as the cooked sausages are decontaminated through pasteurisation (smoking).

The suppression of the spores of the cultural moulds *Penicillium nalgiovense* is important. Both molded and non-molded sausages can often occur in the same places and the spores of these moulds can contaminate the non-molded sausages during common storage or distribution. The growth of these moulds on the surfaces of the sausages makes them unsightly and suspicious-looking.

The effects of antifungal preparations on the microbial growth on the surface of heat treated dry sausages (cooked salami) are evident from the above discussed experiment (Figures 1–4). In a repeated experiment, we received similar results. Natamycin can be seen as the most suit-

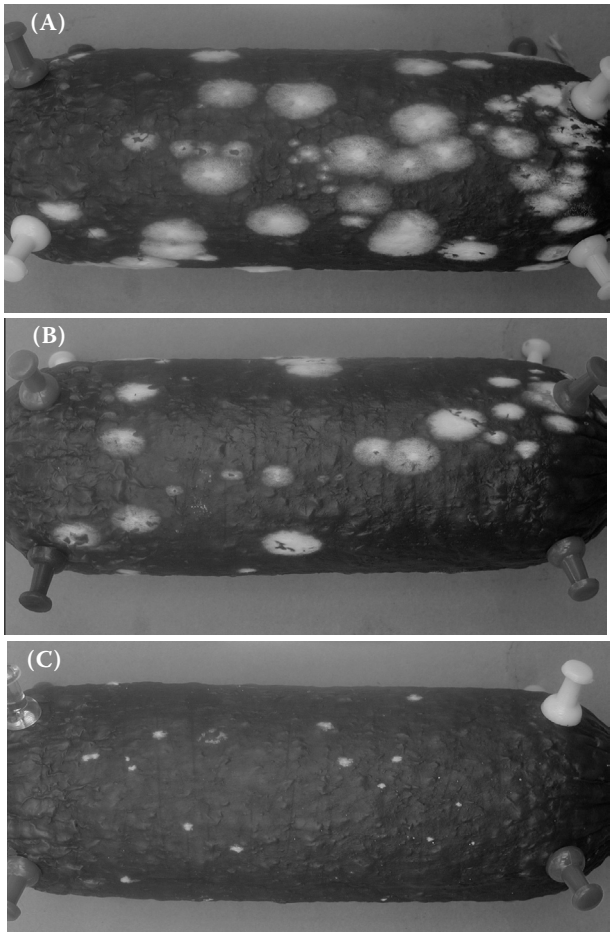

Figure 6. Picture of mould grown on cooked/dry sausage Vysočina after 20 days – (A) control; (B) sorban; (C) natamycin

able preparation to suppress moulds. Although it slows down their growth, it inhibits it fully. The same fact is mentioned also by Zeuthen and Bøgh-Sørensen (2003).

The suppression of the mould growth on the surface of fermented sausage (Prešporská saláma) was similar as in the case of the heat treated sausages (Figure 5). Two different concentrations of natamycin solution (0.1% and 0.2%) were used; the difference between them in the mould suppression was minimal. For this reason, the dipping of sausages into a natamycin solution (concentration of 0.1%) seems to be sufficient. The use of natamycin is advantageous for such sausages as it can not interfere with the starter bacteria as it has no antibacterial activity (Stark 2004). Potassium sorbate showed a smaller inhibitory effect than natamycin. Similarly, a smaller effect of sorbate has been found by Matos *et al.* (2007) with Portuguese fermented sausages chorizo.

CONCLUSIONS

The addition of all three agents under study showed a positive effect on the suppression of the mould growth on the surface of dry sausages. Natamycin proved to be very effective in suppressing the mould growth in both types of sausages – cooked and fermented, whereas sorbate, that is generally used to suppress the mould growth on the dry sausages, was less effective than natamycin.

Video image analysis seems to be a very suitable method to observe the growth of moulds on the surface of dry sausages. The simplified method of using only a digital camera to take pictures of the sausage proved to be sufficient to assess the dynamics of the moulds growth.

References

Delves-Broughton J., Thomas L.V., Williams G. (2006): Natamycin as an antimycotic preservative on cheese and fermented sausages. Food Australia, 58: 19–21.

Hanušová K., Šťastná M., Votavová L., Klaudisová K., Dobiáš J., Voldřich M., Marek M. (2010): Polymer films releasing nisin and/or natamycin from polyvinyldichloride lacquer coating: Nisin and natamycin migration, efficiency in cheese packaging. Journal of Food Engineering, **99**: 491–496.

Holey R.A. (1986): Effect of sorbate and pimaricin on surface mold and ripening of Italian dry salami. Lebensmittel-Wissenschaft und -Technologie, **19**: 59–65.

Jay J.M. (2000): Modern Food Microbiology. 6th Ed. Springer-Verlag, Berlin: 272–273.

Jira W. (2004): Chemische Vorgänge beim Pökeln und Räuchern. Fleischwirtschaft, **84**(6), 107–108.

Korbářová A., Jirák E., Pipek P., Bělková B-A. (2007): Obrazová analýza v potravinářství – měření plochy plísní na salámech. Automa, **13**(12): 9–10.

Matos T.J.S., Jensen B.B., Bernardo F.M.A., Barreto A.S.F.H., Hojberg O. (2007): Mycoflora of two types of Portuguese dry smoked sausages and inhibitory effect of sodium benzoate, potassium sorbate and methyl *p*-hydroxybenzoate on mould growth rate. Journal of Food Protection, **70**: 1468–1474.

Matos TJ.S., Bruno-Soares B., Jensen B.B., Barreto, A.S., Hojberg O. (2008): Growth inhibition of bacterial isolates recovered from two types of Portuguese dry smoked sausages (chouriço). Meat Science, **80**: 1352–1358.

Pipek P., Lojková A., Bělková B-A., Staruch L.: (2007): Analýza obrazu při hodnocení kvality masa a masných výrobků. In: Proceedings XVI. Medzinárodnej konferencie LABORALIM 2007. Banská Bystrica 7.–8. 2. 2007. STU, Bratislava: 16–21.

Pipek P., Staruch L., Izumimoto M. (2008): Stabilization of minced meat colour by carbon monoxide. Czech Journal of Food Sciences, **26**: 333–338.

Robert S.I., Hui Y.H. (2001): Dictionary of Food Ingredients. 4th Ed. Springer-Verlag, New York: 350–354.

Stark J. (2004): Permitted preservatives – natamycin. In: Robinson K.K., Batt C.A.: Encyclopedia of Food Microbiology. Academic Press, London: 1776–12781.

Stiebing A., Oberhaus T., Baumgart J (2001): Natamycin – prevention of mould growth in raw sausages. Fleischwirtschaft, **81**: 97–100.

Tremlová B., Starha P. (2002): Histometric evaluation of meat products – determination of size and number of objects. Czech Journal of Food Sciences, **20**: 175–180.

Tremlová B., Starha P. (2003): Histometric evaluation of meat products – determination of area and comparison of results obtained by histology and chemistry. Czech Journal of Food Sciences, **21**: 101–106.

Ture H., Ergolu E., Soyer F., Ozen B. (2008): Antifungal activity of biopolymers containing natamycin and rosemary extract against *Aspergillus niger* and *Penicillium roquefortii*. International Journal of Food Science and Technology, **43**: 2026–2032.

Ulmer K., Berner S., Leutz U., Herrmann K., Fischer A. (2005): Reifezeitverkürzung bei schnittfestem Rohwurst. Fleischwirtschaft, **85**: 102.

Xiaoguang G., Kunsheng Z., Jingwei W., Yunxia R. (2009): Studies on the effect of compound natural preservatives used in emulsion-type sausage. Journal of Chinese Institute of Food Science & Technology, 9: 133–137.

Zeuthen P.., Bøgh-Sørensen L. (eds) (2003): Food Preservation Techniques. Woodhead Publishing, Cambridge: 15–16.

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Corresponding author:

Prof. Ing. Petr Pipek, CSc., Vysoká škola chemicko-technologická v Praze. Fakulta potravinářské a biochemické technologie, Ústav konzervace potravin a technologie masa, Technická 5, 160 28 Praha 6, Česká republika
tel.: +420 220 443 198, e-mail: petr.pipek@vscht.cz