THE PRESENCE OF UNDESIRABLE MOULD SPECIES ON THE SURFACE OF DRY SAUSAGES*

ABSTRACT: Transition from manufacture to the industrial way of meat production and processing, as well as contemporary concept of food quality and safety, have led to the application of starter cultures. Their application leads towards the streamlining of the production process in the desired direction, quality improvement and its harmonization, and thereby to its standardization. Application of moulds in the meat industry is based on positive effects of their proteolytic and lipolytic egzoenzymes which, as a consequence, leads to the creation of characteristic sensory properties (“flavor”) of fermented products. Penicillium nalgiovense is a typical representative of moulds used in the production of fermented sausages-salamis from our region.

Samples of “zimskia salama” (dry sausage), produced with Penicillium nalgiovense, were evaluated as hygienically unacceptable. Their sensory properties changed due to contamination of this mould during the ripening process. Micological analysis discovered the presence of Penicillium aurantiogriseum, which is a frequent mould contaminant in the meat industry. At the same time, thin layer chromatography revealed no possibility of metabolic activity of this mould in the creation of mycotoxins. However, the presence of this mould on the surface of “zimskia salama” is considered as undesirable due to formation of “off flavor” in products. Such product is considered as hygienically unacceptable and cannot be used for the human consumption.

KEYWORDS: dry sausage, mould, mycotoxin, off-flavor, Penicillium nalgiovense, P. aurantiogriseum

INTRODUCTION

Initial usage of moulds in the production of fermented sausages is connected with Italy and the year 1730, while in Hungary, their application was first introduced by two Italian butchers in 1835 (Leistner, 1986). Today, the application of moulds in meat and dairy industry is common in most of the

* The paper was presented at the first scientific meeting MYCOLOGY, MYCOTOXICOLOGY AND MYCOSES held from 18—20. April 2007. in Novi Sad.
European countries. Meat industry in southern-European countries, especially in Italy, Spain, France, Hungary and South Germany, uses positive properties of certain mould species during the fermentation of some fermented salamis (Sunesen & Stahnke, 2003). Their controlled application is ensured by the application of starter cultures.

Positive effects of moulds, such as the creation of desirable sensory characteristics of fermented sausages, so called “flavor”, are the result of their influence on the proteolytic processes, lactate oxidation, amino acid degradation, lipolysis, β-oxidation, occurring during the maturing process (Gratia at al., 1986; Leistner, 1984; Lücke, 1997; Cook, 1995; Lücke, 1998). No less significance is attributed to the protective effect of moulds, colonized on the surface of the sausage, against other undesirable species of yeasts, moulds and bacteria (Lücke & Hechelmann, 1987). The positive effect on the colour stabilization process, as well as the process of postponing the product’s rancidity, was also described. These effects are the result of emphasized enzymatic — catalase activity of added moulds, oxygen consumption and protection from light (Bacus, 1986; Lücke & Hechelmann, 1987). Furthermore, white and grayish mycelia of colonized moulds on the surface of the sausage, decreases the possibility of additional drying and the occurrence of the so called “gray edge” (Lücke, 1997), simultaneously forming smooth surface and uniform appearance of the product (Grazia, 1986; Sunesen & Stahnke, 2003).

Besides the fact that many of the meat industries are using native mould cultures for the production of certain fermented sausages, the usage of starter cultures with already determined desired functional properties, is more and more in practice. This approach eliminates the risk of obtaining a product with undesirable sensory properties, as well as poisoning caused by mycotoxins (Cook, 1995).

As a result of inadequate, unprofessional application of moulds in the meat industry, the economic losses can be extremely high, especially if the mistakes are connected to the maturing process involving large part of production lot.

Penicillium nalgiovense (Figure 1) represents the typical mould used in the European meat industry (Ludemann, 2004). Numerous researches dedicated to the examination of the *P. nalgiovense* application in the production of raw sausages, have shown its exquisite potential and superiority compared to other mould species from genus *Penicillium*. Today, many authorities in this area approve its application in commercial starter cultures (Sunesen & Stahnke, 2003; Garcia et al., 2001).

In our country, *P. nalgiovense* is used for the production of “zimska salama” (Radetić, 1997). The revitalized culture, in the concentration of cca $10^6$ cfu/ml water, is sprayed on the surface of the sausages during certain maturing phases. Guided by the principles of good laboratory practice, and eliminating the risk of a consequent contamination, it is also possible to use broth mould culture made at meat industry plant laboratories.
MATERIALS AND METHODS

Due to the presence of the spoilage elements three months after the production the samples of “zimska salama” were subjected to the sensory analysis, as well as detailed mycological analysis, aiming at determination of colonized mould species, occurring on the surface of the product. At the same time, myco-toxicological analysis was conducted to establish mycotoxin production ability of the isolated mould.

Mycological analysis

Mould isolation was conducted on Sabouraud — 4 Maltose Agar, (Merck) (European Pharmacopeia II) having the following composition: peptone 10,0 g, D(+) glucose 40,0 g, agar-agar 15,0 g. Identification of the mould of different age (7, 14, 21. and 28. days) isolated and cultivated under laboratory conditions, was conducted according to the key described by R. A. S a m s o n et al. (2004).

Myco-toxicological analysis

The isolated mould culture, during its growth under laboratory conditions (temperature 4° and 25°C, growth period 14 and 21 days), was examined with the aim of determining the potential production of extra cellular metabolites — mycotoxines. Determination of ability for mycotoxin synthesis was done by thin layer chromatography, as described by G i e m e n o et. al. (1983). Toxin identification was achieved by various developing solvents, spray reagents, and chemical reactions, and then quantified. The minimum detectable concentration of the examined mycotoxin (ochratoxin A) was 10 µg/kg.

The obtained results were evaluated according to the enforced regulative of our country (The Official Gazette of FRY no. 5/92).

RESULTS AND DISCUSSION

The isolated mould culture, P. aurantiogriseum (synonym P. verrucosum var. cyclopium) is a frequent contaminant of the P. nalgiovense starter culture. The isolated mould culture (Figure 2) plays a significant role in contamination of fermented meat products (H o r v a t - S k e n d e r o v i ć & Š k r i n j a r, 1990). Source of contamination can be attributed mostly to the disrespect of basic principles of good hygienic practice, such as inadequate plant hygiene, that is, hygiene of the working plateaus in direct contact with raw materials and end-products, as well as air contamination and, of course, human factor that can be of decisive influence (Š k r i n j a r & D i m i ć, 1996). Of equal influence are the mycological profiles of raw materials, additives and spices (especially natural, thermally untreated), that can be significant sources of moulds (H o r v a t - S k e n d e r o v i ć, 1989).
*P. aurantiogriseum*, according to the literature data is potentially toxic mould that can produce neurotoxic — verrucosidin (Schueer, 1995), or nephrotoxic, carcinogen and immunotoxic — ochratoxin A (Pestk a, 1995). The obtained results showed that the isolated and examined strain of *P. aurantiogriseum*, did not show the ability for mycotoxin-ochratoxin production. This
result was quite convenient from the consumers’ point of view since this product could have come to the final user-human.

The sensory results implied that the samples of “zimska salama” were hygienically unacceptable, and due to the changed sensory properties, could not be used for human consumption (The Off. G. FRY no. 53/91 and annexes — The Off. G. FRY no. 24/94 and 28/96). Visible changes were especially emphasized on the surface of the casing and in the outer part of the filling, right beneath the casing. At these spots, the presence of moulds was recorded. Moulds were unevenly growing on the surface of salami, creating grayish layers in the form of stamps of different sizes. The aroma was adverse with expressed admixture note of mould, bitter and hot.

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**ПРИСУСТВО НЕПОЖЕЛЕНХХ ВРСТА ПЛЕСНИ НА ПОВРШИНИ СИРОВИХ КОБАСИЦА**

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Резиме

Прелазак са занатске производње и прераде меса на индустријски начин, као и савремени концепт обезбећења квалитета и безбедности хране, услови су употребу стартер-култура. Њихова примена доводи до усмеравања процеса зрења у жељеном правцу, побољшавању и уједначавању квалитета а томе и стандардизацији производње. Истовремено, постиже се хигијенска сигурност у току производње као и добијање здравствено-безбедног производа. Употреба пlesenи у industriji меса заснива се на позитивним ефектима њихових протеолитичких и липолитичких егзоензима, што следичко доводи до настанка карактеристичних сензорских својстава (flavour) сирових производа. Незaborilazan je i заштитни ефекат, као и утицај на стабилизацију боје и успоравање настанка ранидите-та производа. Док је употреба бактерија и квасаца везана за деловање у унутрашњости надева кобасице, дотле је примена селекционисаних пlesenи везана за
површинску „контаминацију“. Карактеристичан изглед (сиво-бела површина), типична арома производа као и заштитни ефекат, које остварују селекционисане и намерно додате, по површини, одређене врсте нетоксигених плесни, представљају основе њихове примене у индустрији mesa.

*Penicillium nalgiovense* представља типичну врсту плесни која се користи на нашим просторима у производњи ферментисаних кобасица — salama.

У овом раду анализирани су узорци „зимске саламе“, у чијој производњи је била употребљена култура *Penicillium nalgiovense* и који су органолептичком анализом оцењени као хигијенски неисправни. Узорци су имали изменена сензорска својства, настала као резултат процесне или постпроцесне контаминације у току процеса производње или, пак, неадекватног или неусловног складиштења. Миколошком анализом утврђено је присуство *Penicillium aurantiogriseum*, који је у литератури и прaksi познат као чест контаминант плесни. Истовремено, методом танкослојне хроматографије, у лабораторијским условима гајења, није утврђена могућност њене метаболичке активности у правцу стварања микотоксина. Међутим, присуство ове врсте плесни, на површini „зимске саламе”, сматра се непожељним, због настанка „of flavour-a” производа. Такав производ је хигијенски неисправан и неупотребљив за људску исхрану.