OCCURENCE OF OCHRATOXIN A-PRODUCING MOULDS AND OCHRATOXIN A IN DRY SAUSAGE

KRINJAR M.* and DANEV M.**

* Faculty of Technology, University of Novi Sad, Novi Sad, Yugoslavia ** Veterinary Institute, Skopje, Macedonia

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SUMMARY

Since the ochratoxin A-producing moulds are very frequent contaminants of food and feed in our country, the aim of this study was to investigate their occurence as follows> a) in raw materials for dry sausage production (18 samples - ground pork, ground beef, mixture of spices, additives), b) on sausage surface during the processing (72 samples - after filling and smoking, on 10th and 20th day of the ripening, 5th, 10th and 15th day of the storage), c) on the surface of sausages taken from the market (30 samples) and d) in air in meat processing plant. All the tested samples were analyzed on ochratoxin A (OA) presence, too. Moulds were isolated from the raw materials by the standard Koch method, from the sausage surface by the smear method and from the air by the exposition of Petri dishes with isolating medium for 10 min. Qualitative and quantitative determination of OA was performed by using the TLC method.

Following OA-producing fungal species were isolated from the raw materials and sausage surfaces> <u>Aspergillus ochraceus</u> Wilhelm, <u>Penicillium aurantiogriseum</u> Dierckx, <u>P. chrysogenum</u> Thom and <u>P. commune</u> Thom. <u>P. aurantiogriseum</u> was the most frequent fungus. About 17% of sausage samples after filling and smoking, then 67% of sausages during the storage and 45% of the sausages taken from the market were contaminated with <u>P. aurantiogriseum</u>. At the same time, this species was a dominant fungus in the mycopopulations isolated from the air in the meat processing plant.

About 13% of sausages during the storage and 4% of those taken from the market were contaminated with OA (40.0 μ g/kg). This toxin was not found in raw materials and in sausages during the processing.

Introduction

Ochratoxin-producing moulds (<u>Aspergillus</u> and <u>Penicillium</u> spp.) are widespread contaminants of various environments. In our country, especially <u>Penicillium</u> species were very frequent and occured as the most dominant fungi in mycopopulations isolated from cereals (Pepeljnjak and Cvetni', 1985), forages and grain feeds ({krinjar et al., 1992}, some cheeses ({krinjar et al., 1991}) and from meat and meat products ({krinjar, 1992}).

Occurence of ochratoxin A-producing fungi and ochratoxin A is of great significance having in mind a hypothesis that ochratoxin A is associated with Balkan endemic nephropathy (Krogh et al., 1977< Petkova-Bocharova and Castegnaro, 1985< Macgeorge and Mantle, 1990).

The purpose of this study was to examine the occurence of ochratoxin A-producing moulds and ochratoxin A in a) raw materials used for dry sausage production, b) sausages during the processing and storage and c) dry sausages taken from the market. The frequency of fungi possible producers of ochratoxin A in air in meat plant was also investigated.

Material and methods

In this study the occurence of ochratoxin A-producing moulds was investigated as follows> a) in raw materials for dry sausages production (18 samples - ground pork, ground beef, mixture of spices, additives), b) on surface of sausages during the processing (72 samples - after filling and smoking, on 10th and 20th day of the ripening, 5th, 10th and 15th day of the storage), c) on the surface of sausages randomly taken from the market (30 samples) and

d) in air in meat processing plant.

All of raw material and sausage samples tested were analyzed on ochratoxin A (OA) finding, too.

<u>Mycological analysis</u>. Moulds were isolated from the raw materials by the standard KochÆs method, from the sausage surface by the smear method, and from the air by the exposition of Petri dishes with sterile isolation medium for 10 min. Sabouraud maltose agar with streptomycin (0.05%) was used as an isolation medium. Incubation was carried out at 25_C for seven days. Identification of ochratoxin A-producing species was performed according to Samson and van Reenen-Hoekstra (1988).

<u>OA analysis.</u> The method described by Balzer et al. (1978) was slightly modified and used for the determination of OA as follows> 25 g of ground sample was mixed with 5 g of silica gel (0.08 mm) and 5 g of anhydrous Na2SO4< the sample was extracted with 90 ml of acetonitrile and 10 of tap water, agitated with a mixer (3000 rpm) for 15 min, and then filtered< filtrate (50 ml) was defatted with n-hexane (3 x 25 ml). The detection of OA was performed by thin-layer chromatography (TLC). Concentrations of OA were determined visually. Pure OA from <u>Aspergillus ochraceus</u> was supplied by Fluka Biochemika 7411, Switzerland.

Results and discussion

<u>Mycological analysis</u>. Occurence of ochratoxin A-producing moulds in raw materials (ground pork, ground beef, miture of spices, additives) used for dry sausage production is given in Table 1. Two <u>Penicillium</u> species, known as possible ochratoxin A-producers (Leistner and Eckardt, 1979< Smith and Hacking, 1983), were isolated as follows> <u>P. aurantiogriseum</u> Dierckx from additive 1 and 2 and <u>P. chrysogenum</u> Thom, from ground pork and from additive 2, too.

<u>Penicillium</u> species were found to be dominant in mycopopulations isolated from the sausage surface during the processing and storage. The most frequent fungus was <u>P. aurantiogriseum</u>. It was isolated from about 17% of sausages after filling and smoking and even from 67% of sausages during the storage. Beside <u>P. aurantiogriseum</u>, <u>Aspergillus ochraceus</u> Wilhelm (10th day of the ripening), <u>Pz chrysogenum</u> (5th and 10th day of storage), and <u>P. commune</u> Thom (after filling) were also present on the same samples tested (Table 2).

The same <u>Penicillium</u> spp. were observed as contaminants of the air in meat processing plant (Table 3). These results pointed out to the fact that microclimatic conditions in the meat processing plant were optimal for the occurrence of <u>Penicillium</u>. Evidently, fungi which occurred as sausage surface contaminants, originated from the air.

<u>P. aurantiogriseum</u>, <u>P. chrysogenum</u> and <u>P. commune</u> were also isolated from the surface of sausages taken from the market.

It was found out, that 45% of these sausages were contaminated with <u>P. aurantiogriseum</u>, but only 3% with <u>P. chrysogenum</u> and <u>P. commune</u>.

<u>Mycotoxicological analysis</u>. About 13% of sausages during the storage (5th, 10th and 15th day) and 4% of those taken from the market were contaminated with OA at concentration of 40.0 μ g/kg. At the same time, <u>P</u>. <u>aurantiogriseum</u> and <u>P</u>. <u>chrysogenum</u> isolated from OA-positive sausages originated from meat processing plant. Two OA-positive samples, taken from the market, were contaminated with <u>P</u>. <u>aurantiogriseum</u> and one with <u>P</u>. <u>sommune</u>.

OA was not found in raw materials and sausages during the processing.

Conclusion

From the raw materials used for dry sausage production and from sausage surface, which were obtained from the meat processing plant, the following OA-producing moulds were isolated> <u>Aspergillus ochraceus</u> Wilhelm, <u>Penicillum aurantiogriseum</u> Dierckx, <u>P. chrysogenum</u> Thom and <u>P. commune</u> Thom. <u>P. aurantiogriseum</u> was the most frequent fungus. About 17% of sausage samples after the filling and smoking, then 67% sausages during the

storage and 45% of sausages taken from the market were contaminated with P. aurantiogriseum.

At the same time, this species was a dominant fungus in mycopopulations isolated from the air in meat processing plant. About 13% of sausages during the storage and 4% of those taken from the market were contaminated with OA ($40.0 \,\mu$ g/kg). This toxin was not found in raw materials and in sausages during the ripening.

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