

OCHRATOXIN A PRODUCTION IN DRY SAUSAGE BY PENICILLIUM VERRUCOSUM VAR. CYCLOPIUM STRAINS

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SUMMARY

The objective of present study was to examine the possibility of ochratoxin A (OA) production in „tea sausage“, a type of dry sausage, by four P. verrucosum var. cyclopium strains.

Penicillium strains tested were isolated from the air in smoke-house and from the surface of a dry sausage. In preliminary experiments this fungal strains were analyzed on OA production in laboratory conditions growing on ground and sterile wheat grains at 26 to 28°C for 20 days. In further experiments, the possibility of OA production in dry sausage during the ripening and storage periods by toxigenic P. verrucosum var. cyclopium strains were investigated.

After filling, sausage surface was inoculated with a suspension of fungal conidia (10^6 /mL). Sausage was dipped into a spore suspension for 10 to 15 min, and than strained and smoked for 2 to 3 days. Mycotoxicological analysis were done on the 10th and 20th day of the ripening and on the 10th and 15th day of the storage period. Experiments were carried out in meat processing plant. All tests were done in triplicates. Determination of OA was performed by using a method according to Balzer et al. (1978), which was slightly modified.

The results obtained indicated that dry sausage was a good substrate for fungal growth and for toxin production. All of the fungal strains tested produced OA already after ten days of the ripening (12.0 to 20.0 µg/kg). The highest concentration of OA (50.0 µg/kg) was found at the end of ripening period synthesized by P. verrucosum var. cyclopium, strain L II 14A.

INTRODUCTION

The investigations which were carried out in Yugoslavia last few years, pointed to a high frequency of P. verrucosum var. cyclopium. It was found out that this Penicillium species was dominant in mycopopulations isolated from feed- and foodstuffs (SKRINJAR and ŽAKULA, 1985; SKRINJAR et al., 1992).

Since P. verrucosum var. cyclopium can produce toxic metabolites, such as ochratoxin A, citrinin, penicillic acid etc. (SKRINJAR, 1985; PITT, 1987), it is classified as an undesirable fungus.

Ochratoxin A (OA), a nephrotoxic fungal secondary metabolite, is quite frequent in our country (PEPELJNJAK and CVETNIC, 1985, CVETNIC and PEPELJNJAK 1990; SKRINJAR, 1992, SKRINJAR et al., 1992). The appearance of endemic nephropathy in humans in the Balkans is probably connected with the distribution of this toxin.

The aim of this study was: a) to isolate P. verrucosum var. cyclopium strains from raw materials used for dry sausage production, as well as from the air in meat processing plant, b) to test the possibility of OA production by some strains in laboratory conditions and c) to inoculate the sausage surface with toxic P. verrucosum var. cyclopium strains and to investigate the possibility of OA production in dry sausages.

MATERIALS AND METHODS

Isolation and determination of Penicillium verrucosum var. cyclopium strains. P. verrucosum var. cyclopium strains were isolated from the air in meat processing plant, smoke-house, ripening-house, store-house, from the surface of dry sausage during the ripening and storage periods and from additives used for dry sausage production.

The moulds were isolated from the air by the method of the exposition of Petri dishes with sterile medium for 10 min, from the sausage surface by the method of Svab and from additives by using the standard Koch's method.

Sabouraud dextrose medium with streptomycin (0.01–0.02%) was used as an isolation medium. Incubation was carried out at 25°C for five to seven days. Determination of isolated mould strains was performed according to SAMSON et al. (1976).

Ochratoxin A production by *P. verrucosum* var. *cyclopium* strains growing on sterile crushed wheat. The possibility of OA production by 22 strains of *P. verrucosum* var. *cyclopium* isolated from various environments in meat plant was tested. For that purpose, ground and sterile wheat grains (50 g) were inoculated with 5 ml of fungal inoculum (10^6 conidia/ml). Sterilized Erlenmeyer flasks (500 ml) with the inoculated medium were incubated for 20 days at 26 to 28°C. During the second and third day of cultivation 5 ml of destillated water was added into the medium.

Production of ochratoxin A by toxigenic *P. verrucosum* var. *cyclopium* strains in „tea sausage“. In further experiments the possibility of OA production in „tea sausage“, during the ripening and storage periods, by four ochratoxigenic *P. verrucosum* var. *cyclopium* strains (L II 14A, L II 14E, L 22 and L 15) was investigated.

After filling, „tea sausage“ surface was inoculated with suspension of fungal conidia (10^6 /ml). Sausage was dipped into a spore suspension for 10 to 15 min, and than strained and smoked for 2 to 3 days.

Mycotoxicological analysis were done after 10 and 20 days of the ripening and after 10 and 15 days of the storage period.

All tests were done in triplicates.

Qualitative and quantitative determination of ochratoxin A. The method described by BALZER et al. was slightly modified and used for the determination of OA as follows: 25 g of sample was mixed with 5 g of silica gel (0.08 mm) and 5 g of anhydrous Na_2SO_4 . The sample was extracted with 90 ml of acetonitrile and 10 ml of tap water, agitated with a mixer (3000 rpm) for 15 min, and then filtered. Filtrate (50 ml) was defatted with *n*-hexane (3x25 ml). Detection of OA was carried out by thin-layer chromatography (TLC). Concentrations of OA were determined visually. Data are presented as average values. Pure OA from *Aspergillus ochraceus* was supplied by Fluka Biochemika 7411, Switzerland.

RESULTS AND DISCUSSION

A large number of *P. verrucosum* var. *cyclopium* was isolated from additives, used for „tea sausage“ production, from the sausage surface after filling, during the ripening and storage periods and from the air in meat processing plant, smoke-house, ripening-house and from the air in the store-house.

It was found out that eight *P. verrucosum* var. *cyclopium* strains (36%) produced OA growing on sterile crushed wheat for 20 days. Concentrations were approximately the same and they were from 40.00 to 65.00 $\mu\text{g}/\text{kg}$. The highest concentration of the toxin (65.00 $\mu\text{g}/\text{kg}$) was produced by *P. verrucosum* var. *cyclopium*, strain L II 14A, isolated from the air in smoke-house.

In Table 1 the results of the investigations of the ability of OA production by four *P. verrucosum* var. *cyclopium* strains (L II 14A, L II 14E, L 22 and L 15) in „tea sausage“ are given. The results obtained indicated that „tea sausage“ was a good medium for fungal growth and for toxin production. All of the fungal strains tested produced OA, although at concentrations somewhat lower than in crushed wheat. The highest concentration of OA (35.90 $\mu\text{g}/\text{kg}$) was synthesized by *P. verrucosum* var. *cyclopium* L II 14A again.

Table 1. Ochratoxin A production by toxic *P. verrucosum* var. *cyclopium* strains growing on „tea sausage“ surface

Sausage	Strain number	Conc. of OA ($\mu\text{g}/\text{kg}$)
10th day of ripening	L II 14A	20.00
	L II 14E	12.00
	L 22	12.00
	L 15	15.00
20th day of ripening	L II 14A	50.00
	L II 14E	32.00
	L 22	28.25
	L 15	24.25
10th day of storage	L II 14A	21.10
	L II 14E	10.00
	L 22	20.20
	L 15	10.00
15th day of storage	L II 14A	21.00
	L II 14E	9.00
	L 22	15.00
	L 15	3.00

Since *P. verrucosum* var. *cyclopium* is one of the most widespread *Penicillium* species, the percentage of ochratoxigenic *Penicillium* strains, found in these experiments, is worrying. Especially, because it is known that *P. verrucosum* var. *cyclopium* can produce various toxic metabolites, other than ochratoxins, such as citrinin, cyclopiazonic and penicillic acid and penitrem A (PITT, 1987).

Dry sausage constitutes a substrate in which toxin producing fungi, such as *Aspergillus* and *Penicillium* spp., may develop. In our earlier investigations (ŠKRINJAR and HORVAT-SKENDEROVIĆ, 1989) *Penicillium* species were dominant in mycopopulations isolated from dry sausages taken from the market. About 5.5% of sausage samples were contaminated with OA at concentration of 40.0 $\mu\text{g}/\text{kg}$. From OA-contaminated sausages *P. verrucosum* var. *cyclopium*, *P. commune* and *P. chrysogenum*, the OA-producing moulds, were isolated from the same time.

LABIE and TACHE (1979) reported that during the first days of sausage fabrication, when the substrate moisture level was high and the environmental conditions were favourable (temperature above 20°C, relative humidity 80-100%), OA was produced by *A. ochraceus* at concentrations from 80 to 120.0 $\mu\text{g}/\text{kg}$.

CVETNIC and PEPELJNJAK (1990) found out that about 37% of strains of *A. ochraceus* group, isolated from dry sausage, bacon and ham, collected from individual households in the nephropathic areas in Yugoslavia, produced OA under laboratory conditions at concentrations between 0.07 and 240.0 $\mu\text{g}/\text{kg}$.

CONCLUSION

The results obtained indicated that „tea sausage“ was a good substrate for the growth of *P. verrucosum* var. *cyclopium* strains tested and for OA production.

All of the Penicillium strains investigated, produced OA after 10 days of the sausage ripening at concentrations from 12.0 to 20.0 µg/kg. The highest concentration of this toxin (50.0 µg/kg) was detected at the end of ripening synthesized by P. verrucosum var. cyclopium L II 14A.

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