Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 121, 115—124, 2011

UDC 582.28:663
DOI:10.2298/ZMSPN1121115S

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AIR MYCOPOPULATIONS IN *PETROVSKÁ KLOBÁSA* PRODUCING FACILITY*

ABSTRACT: Different types of filamentous fungi periodically cause problems in small-scale facilities for traditional dry fermented sausages, such as *Petrovská klobása* from Vojvodina province (Serbia). Mould contamination can be observed during processing, ripening, and storage. Sausages may become spoiled due to visible mould colonies on the surface and off-flavours they produce. The most important – if mycotoxin production occurs it might promote a number of health disorders. Knowledge and control of filamentous fungi are, therefore, essential to produce sausages that satisfy the criteria of hygienic quality, sensorial characteristics, and food safety. The aim of this study was to survey mycoflora of a small-scale facility producing traditional dry fermented sausage – *Petrovská klobása*. The mould contamination of the air in processing unit and ripening chambers was investigated, in order to determine the important fungi in terms of spoilage of the products and ability to produce mycotoxins.

The mould contamination of air in processing unit and ripening chambers examined was in range 0.22 – 1.89 log CFU/P.d. Isolated moulds belong to 6 genera: *Aspergillus* (3 species), *Cladosporium* (1 species), *Eurotium* (2 species), *Fusarium* (1 species), *Penicillium* (12 species) and *Scopulariopsis* (1 species). The most abundant were species of *Penicillium* genus, many of which are capable for mycotoxin production.

The level and diversity of fungal contamination of air varied between samples, influenced by the general hygiene, the buildings, the airflow, the outdoor environments, and the time of year. This knowledge is crucial for the improvement of hygiene control systems in small-scale processing units.

KEY WORDS: mycopopulations, processing unit, air

INTRODUCTION

Traditional dry fermented sausage production has increased overall Europe since the 1980's. The development is due mainly to the consumer's request for natural and authentical products that are made in non-industrial environ-

* The paper was presented at the fourth international scientific meeting *Mycology, mycotoxiocology, and mycoses*, which was organized in *Matica srpska, Department for natural sciences* from April 20-22, 2011.

ment, characterized by small-scale batch production with a limited degree of mechanization, and strongly identified with a place or region of origin (L e b - e r t et al., 2007). One of them is *Petrovská klobása*, produced in the area of Bački Petrovac (Vojvodina Province, Serbia), in the traditional way, without additives and starter cultures. Because of its specific and distinctive quality, *Petrovská klobása* has been protected with Designation of Origin (PDO) according to Serbian legislation (P e t r o v i ć et al., 2007).

The traditional procedure relies on indigenous microflora whose origins could result from the raw material used or from the environment. Each processing facility has a specific house flora composed of useful microorganisms for the fermentation and flavour of sausages, as well as spoilage and pathogenic flora. Thus, the characterization of this house-flora is crucial because safety (pathogenic flora), acceptability (spoilage flora), and sensorial quality (technological flora) of the products depend totally on it (C h e v a l - l i e r et al., 2006). This study dealt with spoilage microflora, particularly filamentous fungi. Moulds periodically cause problems in small-scale facilities for traditional dry fermented sausages production. Mould growth can be observed during processing, ripening, and storage and can be a quality problem. Sausages may become spoiled due to visible mould colonies on the surface and the off-flavors they produce. Besides, mould growth may also represent a health risk because of the possibility of mycotoxin production by several mould species (P a p a g i a n n i et al., 2007, V e s k o v i ć - M o r a č a n i n et al., 2009). Consumption of contaminated sausages might promote a number of health disorders. Knowledge and control of filamentous fungi are, therefore, essential to produce sausages that satisfy the criteria of hygienic quality, organoleptic characteristics and food safety (Š k r i n j a r et al., 2010).

Only few studies have focused on mycobiota in the processing areas of meat processing plants (I s m a i l et al., 1995; A n d e r s e n , 1995; B a t t i - l a n i et al., 2007). Therefore, the aim of this study was to survey the typical house-flora, particularly mycoflora, of a small-scale facility producing traditional dry fermented sausage – *Petrovská klobása.* The mould contamination of the air in processing unit and ripening chambers was investigated, in order to determine the important fungi in terms of spoilage of the products and ability to produce mycotoxins.

MATERIALS AND METHODS

The processing unit investigated in this research was located in Bački Petrovac (Vojvodina Province, Serbia). Sampling of the air was carried out during the production of *Petrovská klobása* in December 2009. The first sampling was done in the unit where sausages were prepared and stuffed on the day of preparation (day 1). Produced sausages were left to ripen in chambers in Bački Petrovac (days 1-11), so the sampling of the air in ripening chamber was done on days 2, 6, and 9 of ripening. On day 11, the sausages were moved to Kucura for further ripening, so the sampling of the air in Kucura's cham-

bers was carried out on days 11, 14, 34, and 65 after sausage production. On day 65, sausages were moved to an industrial facility in Novi Sad to finish ripening process there, and the air sampling in industrial ripening chambers was done on days 65, 90, 120, and 217 of ripening (Table 1).

Tab. 1 – Air samples

Room	Day of sampling	Sign
Processing unit	1	A-1
Ripening chamber Bački Petrovac	2	A-2
	6	A-3
	9	A-4
Ripening chamber Kucura	11	A-5
	14	A-6
	34	A-7
	65	A-8
Ripening chamber An industrial facility in Novi Sad	65	A-9
	90	A-10
	120	A-11
	217	A-12

Occurrence (presence/absence) of moulds and identification of species isolated from collected samples were then carried out. Air in the sausage processing rooms was examined by gravity sedimentation onto 9 cm Petri dishes containing 15 ml of Sabouraud-maltose agar with 2% of chloramphenicol, for 20 minutes, at five different locations. After 7 days incubation at 25°C, the Petri dishes were inspected and the colonies were sub-cultured onto agar plates according to S a m s o n et al. (2004), as follows: *Penicillium* and *Aspergillus* species were plated onto Czapek agar (CzA), *Fusarium* species onto potato dextrose agar (PDA) and others onto Sabouraud-maltose agar (SMA). Agar plates were incubated for 7 days at 25°C. The isolates were then identified by their morphological characteristics, following the methods of E l l i s (1971), N e l s o n et al. (1983), S a m s o n and F r i s v a d (2004) and S a m s o n et al. (2004).

RESULTS AND DISCUSSION

Results for total mould counts per Petri dish, isolated from air in *Petrovská klobása* processing unit and ripening chambers, expressed as logCFU/Petri dish, are presented in Table 2.

Tab. 2 – Total mould counts per Petri dish (TMC/P.d.), isolated from air in *Petrovská klobása* processing unit and ripening chambers, (log CFU/P.d)

Sample	Room	Day of sampling	TMC/P.d.
A-1	Processing unit	1	0.40 ± 0.14*
A-2	Ripening chamber Bački Petrovac	2	1.46 ± 0.08
A-3		6	1.31 ± 0.03
A-4		9	0.22 ± 0.2
A-5	Ripening chamber Kucura	11	1.68 ± 0.04
A-6		14	1.34 ± 0.06
A-7		34	1.53 ± 0.05
A-8		65	0.37 ± 0.09
A-9	Ripening chamber An industrial facility in Novi Sad	65	1.89 ± 0.02
A-10		90	0.94 ± 0.14
A-11		120	1.84 ± 0.04
A-12		217	0.15

* – Mean ± standard deviation from 5 measurements

The mould contamination of air in processing unit and ripening chambers examined was in range 0.22 – 1.89 logCFU/P.d. Similar results were obtained by S ø r e n s e n et al. (2008) who investigated air contamination of some fermented sausage processing area. Their results for air contamination by filamentous fungi are in range 0.23 – 1.2 logCFU/P.d.

Table 3 presents the results obtained by identification of moulds isolated from air in processing unit and ripening chambers. Table also present the moiety of species in mycopopulations isolated from specific sample.

Isolated moulds belong to 6 genera and 20 species. Genera are: *Aspergillus, Cladosporium, Eurotium*, *Fusarium*, *Penicillium* and *Scopulariopsis*. Species are: *A. caespitosus* Raper & Thom, *A. fumigatus* Fres, *A. versicolor* (Vuill.) Tiraboschi, *C. oxysporum* Berk. & Curt, *E. chevalieri* Mangin, *E. herbariorum* (Wiggers), *Fusarium sporotrichioides* Sherb, *P. aurantiogriseum* Dierckx, *P. brevicompactum* Dierckx, *P. camemberti* Thom, *P. chrysogenum* Thom, *P. corylophilum* Dierckx, *P. glabrum* (Wehmer) Westling, *P. griseofulvum* Dierckx, *P. janthinellum* Biourge, *P. neoechinulatum*, *P. velutinum* van Beyma, *P. olsonii* Bainier & Sartory, *P. solitum* Westling and *S. brevicaulis* (Sacc.) Bain. The most abundant were species of *Penicillium* genus; all air samples tested were contaminated with at least one *Penicillium* species. It was followed by *Aspergillus*, whose species were found in 6 (50%) of tested samples, then *Eurotium*, that contaminated 5 (41.67%) samples, while species of *Scopulariopsis*, *Fusarium* and *Cladosporium* genera were isolated from one air sample each (Fig. 1).

Tab. 3 – Mycopopulation of air in *Petrovská klobása* processing unit and ripening chambers

Sample	Room	Day of sampling	Species	Isolated (%)
A-1	Processing unit	1	*Aspergillus fumigatus* *Penicillium chrysogenum* *Scopulariopsis brevicaulis* *Penicillium olsonii*	50 26 14 10
A-2	Ripening chamber Bački Petrovac	2	*Penicillium aurantiogriseum* *Penicillium neoechinulatum* *Penicillium glabrum*	69 20 11
A-3		6	*Penicillium aurantiogriseum* *Eurotium chevalieri* *Eurotium herbariorum* *Aspergillus caespitosus* *Fusarium sporotrichioides* *Penicillium olsonii*	39 26 21 10 3 1
A-4		9	*Penicillium janthinellum*	100
A-5	Ripening chamber Kucura	11	*Penicillium aurantiogriseum* *Penicillium chrysogenum*	75 25
A-6		14	*Penicillium aurantiogriseum* *Penicillium chrysogenum* *Penicillium velutinum*	65 22 13
A-7		34	*Eurotium herbariorum* *Aspergillus versicolor* *Penicillium griseofulvum* *Aspergillus caespitosus*	70 20 6 4
A-8		65	*Eurotium herbariorum* *Penicillium aurantiogriseum* *Aspergillus versicolor* *Penicillium glabrum*	37 27 20 16
A-9	Ripening chamber An industrial facility in Novi Sad	65	*Eurotium herbariorum* *Cladosporium oxysporum* *Penicillium brevicompactum* *Penicillium solitum* *Penicillium olsonii* *Penicillium chrysogenum* *Aspergillus caespitosus*	23 23 18 15 13 5 3
A-10		90	*Penicillium chrysogenum* *Penicillium camemberti* *Aspergillus versicolor* *Penicillium corylophilum*	43 39 16 2
A-11		120	*Penicillium aurantiogriseum* *Penicillium chrysogenum*	58 42
A-12		217	*Penicillium aurantiogriseum* *Penicillium chrysogenum* *Eurotium herbariorum*	42 33 25

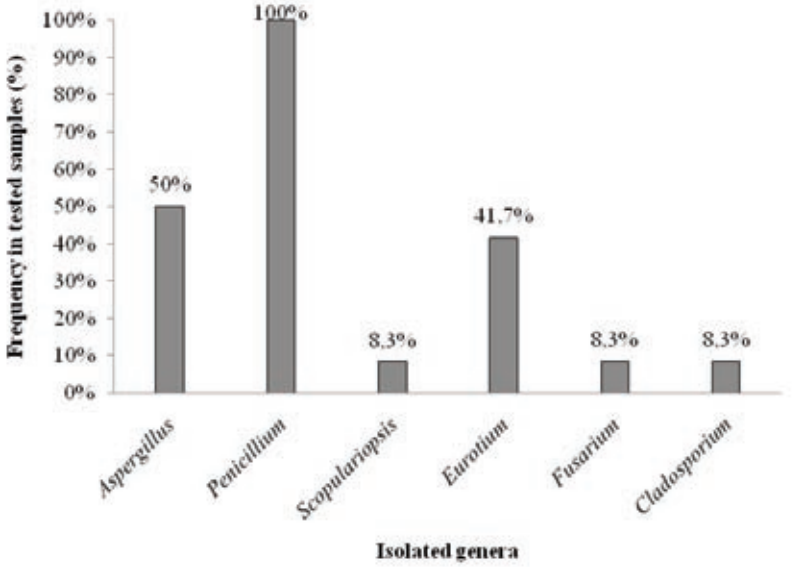

Fig. 1 – Frequency of isolated genera in samples of air in ripening chambers

The highest number of different mould species (7), which makes 35% of all identified, was isolated from ripening chamber in industrial facility in Novi Sad, on the 65th day of ripening (A-9). From sample A-3, six different species were isolated (30%), while the majority of samples (A-1, A-7, A-8 and A-10) were contaminated with four different species (20% of all identified). Samples A-2, A-6 and A-12 were contaminated with three different species (15%), samples A-5 and A-11 with two (10%), while from the sample A-4 just one species was isolated (5%), Fig. 2.

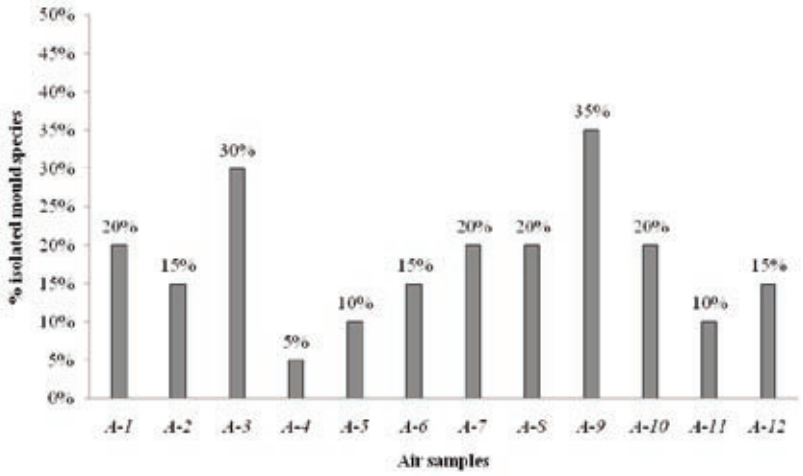

Fig. 2 – Presence of different mould species in mycopopulations isolated from air in *Petrovská klobása* processing unit and ripening chambers

Penicillium species were isolated frequently from air in the processing areas and ripening chambers and it was the most frequent genus found. Among the *Penicillium* isolates, 12 species were identified. The most abundant were *P. aurantiogriseum* and *P.chrysogenum*, which were isolated from 7 samples each (58.3%). They were followed by *P.olsonii,* which was isolated from three samples (25%), and *P.glabrum*, that was isolated from two samples (16.7%). All of 8 species remain were isolated from just one sample (8.3%).

Aspergillus genus was found with quite low occurrence in the air. It was presented with three different species. The most abundant was *A. versicolor*, isolated from three air samples (25%), followed by *A. caespitosus* (16.67%) and *A. fumigatus* (8.33%). *Eurotium*, the perfect state of the *Aspergillus glaucus* group, was found relatively frequently. *Eurotium* generally grows well on substrates with low water activity. It was presented with two species – *E. herbariorum* was isolated from five air samples, which makes 41.67% of all tested, and *E. chevalieri* was isolated from one sample (8.33%). Genera *Scopulariopsis*, *Fusarium* and *Cladosporium* were found in one air sample each and all three genera were presented with one species – *S. brevicaulis*, *F. sporotrichioides* and *C. oxysporum*.

This work surveyed the mould contamination of air in *Petrovská klobása* processing unit and ripening chambers, and contributed to knowledge of the mycological ecosystems of food environments.

The level of fungal contamination of air in processing unit and ripening chambers varied between the individual rooms and the time of sampling, probably influenced by the general hygiene, the buildings, the airflow, the outdoor environments and the time of the year. The diversity of fungi isolated from air was relatively high. This probably reflects that fungal conidia are air-borne and are therefore easily spread. Important reservoirs can be humans, soil, dust, raw materials, drains, equipment surfaces, and ventilation ducts (S c h o l t e et al., 2002). Many of the mould species were isolated rarely, only once or twice during the survey, which mean that they were most likely isolated by chance and were not representatives of a consistent house biota. Fungi present in more than five samples during the survey were considered as present in significant number. In that mean, the important genera were: *Penicillium, Aspergillus* and *Eurotium*.

Many *Penicillium* species can produce mycotoxins. At least half of the *Penicillium* species identified in this study are potentially able to produce toxic metabolites, according to F r i s v a d and T h r a n e (2002). Some species of *Aspergillus* and *Eurotium* also produce toxic metabolites. Little is known about the toxigenic potential of *Cladosporium* species. In this study, the most frequent toxigenic *Penicillium* species were *P. aurantiogriseum* and *P. chrysogenum*. Important toxic metabolites known to be produced by these species are: ochratoxin A, citrinin, penicilic acid, roquefortine C, and other (S a m s o n and F r i s v a d , 2004; M a r a s a s et al., 1984), (Table 4).

Tab. 4 – Production of mycotoxin by fungi isolated from air in *Petrovská klobása* processing unit and ripening chambers

Species	Toxins
Eurotium chevalieri	Emodin, gliotoxin, physicon, xantocillin X
Eurotium herbariorum	Sterigmatocystin (traces)
Aspergillus fumigatus	Fumigalclavines, fumigallin, fumigatin, fumitoxins, fumitremorgins, gliotoxin, spinulosin, verruculogen
Aspergillus versicolor	Nidulotoxin, sterigmatocystin
Fusarium sporotrichioides	Trichothecenes A, butenolide, zearalenone
Penicillium aurantiogriseum	Penicilic acid, xanthomegnin, viomellein, viridicatin, ochratoxin A
Penicillium brevicompactum	Brevianamides, mycophenolic acid, botryodiploidin
Penicillium camemberti	Cyclopiazonic acid
Penicillium chrysogenum	Roqufortine C, PR-toxin, xanthocillin X, penicillin, ochratoxin A
Penicillium glabrum	Citromycetin
Penicillium griseofulvum	Patulin, cyclopiazonic acid, roqufortine C, griseofulvin
Penicillium solitum	Viridicatin

CONCLUSION

This study targeted fungal ecosystems of food processing environments, which are not usually surveyed. *Petrovská klobása* processing unit and ripening chambers showed a high variability of microbial levels in their environments, some of them with excessive levels of toxigenic species. The different cleaning, disinfecting, and manufacturing practices of the small-scale processing units could be responsible for this variability. Also, mycobiota in the processing plants showed a high diversity of fungal species, mainly belonging to the genera *Penicillium*, *Aspergillus*, *Eurotium*, etc. Many toxigenic mould species were isolated from the processing areas, which poses a high risk for human health. This knowledge is crucial for the improvement of hygiene control systems in small-scale processing units, in order to provide high level of product stability and safety.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Science and Technological Development of Republic of Serbia, Project No. TR31032.

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ПЛЕСНИ У ВАЗДУХУ ПРОСТОРИЈА ЗА ПРОИЗВОДЊУ И ЗРЕЊЕ *ПЕТРОВАЧКЕ КОБАСИЦЕ*

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Резиме

Различите врсте филаментозних гљива периодично изазивају проблеме у малим погонима за производњу традиционалних сувих ферментисаних кобасица, као што је *Petrovská klobása* из Војводине, Србија. До контаминације плеснима може доћи током производње, зрења и складиштења. Квар кобасица се може манифестовати у виду раста колонија на површини или промене укуса. Оно што је много важније – уколико дође до продукције микотоксина, може доћи до читавог низа здравствених поремећаја. Познавање и контрола филаментозних гљива су, стога, од великог значаја при производњи кобасица које задовољавају критеријуме хигијенског квалитета, сензорних карактеристика и безбедности хране. Циљ овог рада је био да се испита типична микофлора малог погона за производњу суве ферментисане кобасице *Petrovská klobása*. Испитана је контаминација ваздуха у производној јединици и коморама за зрење, у циљу детерминације гљива значајних са аспекта кварења производа и способности продукције микотоксина.

Контаминација ваздуха плеснима у испитаној производној јединици и коморама за зрење кретала се од 0.22 до 1.89 log CFU/P.d. Изоловане плесни сврстане су шест родова: *Aspergillus* (3 врсте), *Cladosporium* (1 врста), *Eurotium* (2 врсте), *Fusarium* (1 врста), *Penicillium* (12 врста) и *Scopulariopsis* (1 врста). Најзаступљеније биле су врсте рода *Penicillium*, од којих су многе способне да продукују микотоксине.

Ниво и разноврсност контаминације ваздуха варирали су између узорака, под утицајем опште хигијене, градње, протока ваздуха, спољашње средине и годишњег доба. Ова сазнања су од великог значаја за побољшање система контроле хигијене у малим производним погонима.