CHANGES IN THE MYCOFLORA DURING RIPENING OF NATURALLY MOULD-FERMENTED SAUSAGES.

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Changes in the composition of the mycoflora on naturally mould-fermented sausages have been examined. The moulds belonged to the houseflora which colonizes the surface spontaneously. The samples were collected from small-scale production plants in Northern Italy. Initially in the ripening process, yeast was the predominating microorganism, constituting more than 95% of the mycoflora. However, moulds influenced the yeast growth and after two weeks' ripening, yeast and moulds were present in equal quantities. The moulds continued to increase in numbers resulting in a more than 95% dominance at the end of processing.

At the end of the ripening process 96% of the mycoflora belonged to the genus Penicillium. Penicillium nalgiovense, a species frequently used as starter culture, constituted 50% of the moulds. Species such as e.g. P.chrysogenum, P.verrucosum and P.commune comprised 10%, 5% and 3% of the mycoflora, respectively. Furthermore, the three species, P.olsonii, P.spathulatum and P.capsulatum not hitherto isolated from this environment comprised 15%, 5% and about 1% of the mycoflora.

The ability to produce mycotoxins after growth on synthetic agar media was tested by chromatographic methods for sausage isolates of P.nalgiovense, P.verrucosum and P.commune. No known mycotoxins was detected in 53 strains of P.nalgiovense examined. Six out of nine strains of P.verrucosum produced ochratoxin A and B, and one strain produced citrinin. One strain of P.commune was examined and it produced cyclopiazonic acid. Especially, the growth of ochratoxin A producing P.verrucosum on sausages is a serious problem. The reason is that ochratoxin A production can take place in the sausage in which the toxin is stable.

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INTRODUCTION

Traditionally, mould-fermented sausages are produced by spontaneous colonization of the houseflora. Normally, the houseflora is dominated by Penicilium species but also Aspergillus species are found (Leistner & Eckardt, 1979; Grazia et al., 1986). However, the composition and development of the mycoflora is dependent on the nature of the product, processing time and the ripening conditions (Casado et al., 1991; Leistner, 1986).

The production of mycotoxins in mould-fermented meat products represents a potential health hazard. Thus, Leistner & Eckardt (1979) found that 40% to 60% of the Penicillium strains isolated from Italian and Hungarian sausages produced mycotoxins. The importance of these matters is stressed by the fact that mycotoxin production has also been demonstrated in meat products. For instance, the following mycotoxins have been found in either dry sausages or ham after inoculation with toxicogenic moulds: aflatoxins, brevianamide A, citreoviridine, citrinine, cyclopiazonic acid, fumitremorgen B, griseofulvin, ochratoxin A, rugulosin, sterigmatocystine and verrucologen TR_1 (Leistner, 1986). Patulin and penicillic acid were not found, which may be explained by their ability to react with amino acids in the meat (Leistner & Pitt, 1977; Hofman et al., 1971).

Today, there is a growing interest in using well-characterized starter cultures for surface treatment of meat products. The intention is to avoid

undesirable moulds which may produce mycotoxins, result in off-flavour or cause discoloration. Further, a more uniform product in respect of taste, flavour and colour can be obtained.

MATERIALS AND METHODS

Isolation and identification of the mycoflora

Sampling : Sampling took place at 7 small-scale production plants in Northern Italy. Sausage samples were taken in the production rooms and transferred into sterile plastic bags. At each production plant, samples were taken during the first week of ripening and, additionally, at two more advanced stages of the drying process (max. 8 weeks). Air-samples were collected by leaving open petridishes with dichloran 18% glycerol agar (DG18) for 10-15 minutes in the production rooms.

Isolation : The mycoflora was isolated from the sausages by aseptical removal of the casings by use of sterile scalpel and tweezers. The casings were transferred into sterile plastic bags, 150 ml dilution-medium was added and the sample was homogenized for 2 minutes on Stomacher (Colworth, 400). Suitable dilutions were made before plating.

Identification : The mycoflora was identified according to Samson & Reenen-Hoekstra (1988), Pitt (1979) and Frisvad & Filtenborg (1989).

Media and growth conditions : The mycoflora was isolated from DG18. For identification, the moulds were inoculated on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), yeast extract-sucrose agar (YES), Czapek yeast extract sucrose 20% agar (CY20S) and creatine-sucrose agar (Crea). For formulations of the media see Samson & Reenen-Hoekstra (1988) and Samson & Pitt (1990). The fungi were incubated at 25°C for 7 days.

Chromatographic examination of mycotoxin production

Fungi : 53 strains of Penicillium nalgiovense, 9 strains of P.verrucosum and 1 strain of P.commune isolated from sausages.

Media and growth conditions : CYA, MEA, YES (YES was prepared with yeast extract from Difco and Sigma, respectively) and Oat meal agar (OA). For formulations see Samson & Reenen-Hoekstra (1988) and Samson & Pitt (1990). The fungi were incubated at 25°C for 14 days.

Analyses for mycotoxins : Isolates were examined for mycotoxin production by use of the agar plug methods of Filtenborg & Frisvad (1980), Filtenborg & Svendsen (1983) and Frisvad & Thrane (1987) based on thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Frisvad & Thrane, 1987).

RESULTS AND DISCUSSION

The compositional changes in the mycoflora during processing are illustrated in Figure 1. Initially in the processing, yeast was the predominating microorganism constituting more than 95% of the mycoflora. At this stage moulds were only present in limited numbers. As the ripening process proceeded, moulds became competitive and after two weeks' ripening, the number of yeast and moulds balanced. Later in the drying process, i.e. after a production time of 4-8 weeks' the moulds dominated completely and constituted more than 95% of the mycoflora. The change in composition of the mycoflora is probably caused by alterations in the environmental conditions as moulds, in general, are more tolerant to low water activities than yeast. Also, the growth of yeast may be influenced by secondary metabolites produced by moulds.

The air-samples collected in the production rooms represent the houseflora. The mycoflora on the surface of the sausages constitutes those species in the houseflora that are well adapted to the sausage environment (substrate, water activity, temperature etc.). A very good agreement was observed between air-samples and the mycoflora isolated from the sausages. However, three species i.e. Penicillium expansum, Aspergillus ochraceus and A.niger were only found in air-samples. These species have previously been isolated from mould-fermented sausages (Dragoni & Cantoni, 1987; Leistner & Eckardt, 1979). No yeast was demonstrated in air-samples.

The composition of the mycoflora at the end of the processing is given in Table 1. The predominating genus was Penicillium constituting 96% of the mycoflora. The remaining 4% belonged to the genera Aspergillus, Eurotium, Mucor, Cladosporium, Wallemia and yeast. P.nalgiovense was the predominating species comprising 50% of the mycoflora. The P.nalgiovense strains isolated belonged to different types of this species that produce white, green or turquoise conidia, on synthetic agar media. As P.nalgiovense comprised 50% of the mycoflora, it must be well adapted to the growth conditions and will play an important role in the development of the characteristic sausage flavour and aroma.

Three species, P.olsonii, P.spathulatum and P.capsulatum, which never have been isolated from this environment, comprised 15%, 5% and about 1% of the mycoflora, respectively (Table 1). Like P.nalgiovense these species, especially P.olsonii, appeared well adapted to the environment and their enzymatic activity could well influence the final product. P.olsonii may originate from the content of spices as it has previously been isolated from parsley and other herbs (Frisvad & Filtenborg, 1989). The species P.chrysogenum, P.verrucosum, P.oxalicum and P.commune constituted 10%, 5%, 3% and 3% of the mycoflora, respectively (Table 1). Together with other species present, they are potential producers of mycotoxins (Frisvad & Filtenborg, 1989 and Frisvad, 1988). The species P.nalgiovense, P.olsonii, P.spathulatum and P.capsulatum do not produce any known mycotoxins. Still, more than 20% of the isolated houseflora are potential producers of mycotoxins (Table 1).

The ability to produce mycotoxins was tested for sausage isolates belonging to the species P.nalgiovense, P.verrucosum and P.commune. None out of 53 tested P.nalgiovense strains produced known mycotoxins. Nine strains of P.verrucosum were examined. One strain produced citrinin and six strains produced ochratoxin A and B. In particular ochratoxin A, which is known to have pathological effects on kidney and liver, was detected in high quantities. The only strain of P.commune examined produced cyclopiazonic acid.

The mycotoxins produced by the isolated strains of P.verrucosum and P.commune may also be produced in sausages. Consequently, the presence of these mycotoxin producing moulds plus other potential mycotoxin producers in the houseflora represent a health hazard as mycotoxin production may take place under the given environmental conditions.

CONCLUSION

The results show distinct compositional changes in the mycoflora during ripening of mould-fermented sausages. The mycoflora changed from a yeast dominated to a mould dominated flora. The numbers of yeast and moulds are balanced after two weeks' ripening.

P.nalgiovense comprised 50% of the mycoflora at the end of the processing. The three species P.olsonii, P.spathulatum and P.capsulatum which has hitherto not been isolated from this environment comprised 15%, 5% and about 1% of the mycoflora, respectively.

The ability to produce mycotoxins was tested on synthetic agar media for sausage isolates belonging to the species P.nalgiovense, P.verrucosum and P.commune. None of the examined P.nalgiovense strains produced any known mycotoxins, whereas the only strain of

P.commune examined produced cyclopiazonic acid. Six out of nine P.verrucosum strains produced ochratoxin A and B, and one strain produced citrinin.

The consumption of mycotoxins is regarded a health hazard. In particular, the growth of the ochratoxin A producing species P.verrucosum on sausages is a serious problem. The reason is that ochratoxin A production can take place in the sausage in which the toxin is stable.

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REFERENCES

Betina, V. 1989. "Mycotoxins, chemical, biological and environmental aspects", Bioactive molecules, Vol. 9. Elsevier, Amsterdam.

Casado, M.-J.M, M.-A.D. Borrás and R.V. Aguilar. 1991. Fungal flora present on the surface of cured spanish ham. Fleischwirtschaft, 71 (11) : 1300-1302.

Dragoni, I. and C. Cantoni. 1987. "Muffe e Alimenti". Cleasav, Milano.

Filtenborg, O. and J.C. Frisvad. 1980. A simple screening method for toxigenic moulds in pure cultures. Lebensm. Wiss. Technol. 13 : 128-130.

Filtenborg, O. and J.A. Svendsen. 1983. Simple screening method for molds producing intracellular mycotoxins in pure cultures. Appl. Environ. Microbiol. 45 : 581-585.

Frisvad, J.C. and U. Thrane. 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography, 404 : 195-214.

Frisvad, J.C., 1988. Fungal species and their specific production of mycotoxins. In "Introduction to food-borne fungi" (ed. R.A. Samson and E.S. van Reenen-Hoekstra), pp.239-249.

Frisvad, J.C. and O. Filtenborg. 1989. Terverticillate Penicillia : Chemotaxonomy and mycotoxin production. Mycologia, 81 (6) : 837-861.

Grazia, L., P. Romano, A. Bagni, D. Roggiani and G. Guglielmi. 1986. The role of moulds in the ripening process of salami. Food Microbiology, 3 : 19-25.

Hofmann, K., H.-J. Mintzlaff, I. Alperden and L. Leistner. 1971. Untersuchung über die Inaktivierung des Mykotoxins Patulin durch Sulfhydrylgruppen. Fleischwirtschaft, 51 (10) : 1534-1536 + 1539.

Leistner, L. and J.I. Pitt. 1977. Miscellaneous Penicillium toxins. In "Mycotoxins in human and animal health". (ed. J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman), pp.639-653.

Leistner, L. and C. Eckardt. 1979. Vorkommen toxinogener Penicillien bei Fleischerzeugnissen. Fleischwirtschaft, 59 (12) : 1892-1896.

Leistner, L., 1986. Mould-ripened foods. Fleischwirtschaft, 66 (9) : 1385-1388.

Pitt, J.I., 1979. "The genus Penicillium and its telemorphic states, Eupenicillium and Talaromyces". Academic Press, London.

Samson, R.A. and E.S. van Reenen-Hoekstra. 1988. "Introduction to food-borne fungi". Centraalbureau voor schimmelcultures, Baarn.

Samson, R.A. and J.I. Pitt. 1990. "Modern concepts in Penicillium and Aspergillus classification". Plenum Press, New York.

Wood, G.M., P.J. Mann, D.F. Lewis, W.J. Reid and M.O. Moss. 1990. Studies on a toxic metabolite from the mould Wallemia. Food additives and contaminants, 7 (1) : 69-77.

FIGURE 1. Compositional changes in the mycoflora during processing.

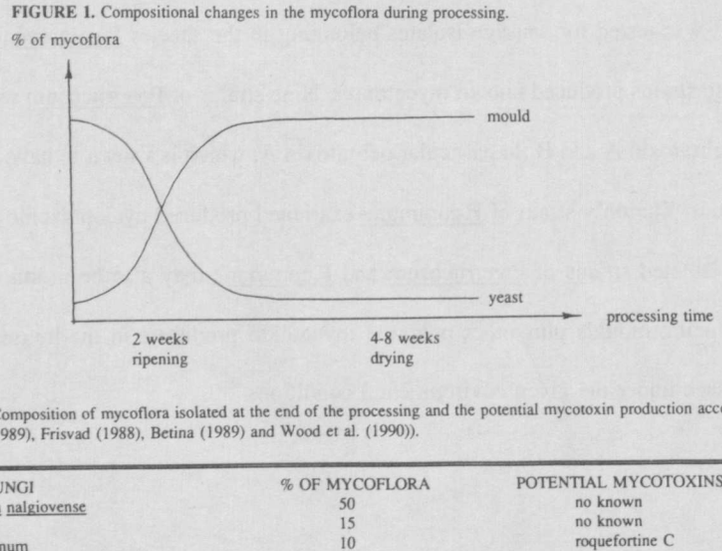

TABLE 1. Composition of mycoflora isolated at the end of the processing and the potential mycotoxin production according to Frisvad & Filtenborg (1989), Frisvad (1988), Betina (1989) and Wood et al. (1990)).

FUNGI	% OF MYCOFLORA	POTENTIAL MYCOTOXINS
Penicillium nalgiovense	50	no known
P.olsonii	15	no known
P.chrysogenum	10	roquefortine C
P.verrucosum (Chemotype 1 and 2)	5	ochratoxin A (ch.1+2), citrinin (ch.2)
P.spathulatum	3	no known
P.solitum	3	no known
P.oxalicum	3	secalonic acid D, roquefortine C
P.commune	3	cyclopiazonic acid, rugulovasine
P.brevicompactum		mycophenolic acid, botryodiploidin
P.aurantiogriseum var. viridicatum		penicillic acid, xanthomegnin, viomellein
P.aurantiogriseum var. polonicum	4	penicillic acid, verrucosidin
P.capsulatum		no known
P.crustosum		penitrem A, roquefortine C
Aspergillus candidus		xanthoascin, terphenyllin
Aspergillus spp.		
Eurotium repens		physcion
Eur.rubrum	4	physcion
Mucor spp.		
Cladosporium spp.		
Wallemia sebi		walleminol A
Yeast		no known