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ORIGINAL PAPER

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Pumpkin seeds – the mycobiota and potential mycotoxins

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Abstract 75 surface disinfected and 75 non-disinfected Austrian pumpkin seeds were plated directly on to each of the three media, DG-18, DRBC, and MSA 5%, to determine the quantitative as well as qualitative mycobiota. Overall, 902 moulds belonging 25 different species and 17 different genera were isolated. The species were divided as follows: ascomycota, 2; zygomycota, 3; mitosporic fungi, 12. The mycobiota were dominated by *Penicillium* spp. (263 isolations), *Eurotium* spp. (139 isolations), and *Cladosporium* spp. (99 isolations). *Rhizopus stolonifer* was the predominating fungal species (113 isolations). 15 potential mycotoxigenic mould species contributed to the mycotiota of pumpkin seeds.

Keywords Pumpkin seeds · Mycobiota, moulds · Mycotoxins

Introduction

The mycobiota of different kinds of foodstuff have been described in various papers [e.g., 1–4]. These investigations mainly concentrated on cereals and nuts and their products since these foods are frequently contaminated with moulds and mycotoxins [5–9]. However, up to now no detailed information concerning the mycobiota and potential mycotoxigenic fungi of pumpkin seeds is available. Based on this situation, the following study was carried out to obtain particular information about the microbial quality of pumpkin seeds.

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Material and methods

According to Samson et al. [10], 75 surface disinfected and 75 non-disinfected Austrian pumpkin seeds were plated directly on to each of the three media, DG-18 [11], DRBC [12], and MSA 5% [3, 13, 14], to determine the quantitative as well as qualitative mycobiota [15, 16]. Disinfection was carried out by shaking the seeds in NaOCl (0.4%) for 2 min. Afterwards, the kernels were rinsed in sterile distiller water three times. Three kernels were transferred to one dish. The dishes were incubated at 25 °C in the dark.

Colonies of developing fungi were transferred to malt extract-agar from day 5 (unsterilized kernels) and day 7 (sterilized kernels) onwards. Identification of *Aspergillus* and *Penicillium* was carried out on recommended media according to the methods of [17] and [18], respectively.

For determination of the remaining moulds, standard taxonomic schemes were used [19–23].

Results and discussion

Significant differences in fungal counts were observed between disinfected and non-disinfected kernels (DG-18:24/437, DRBC: 11/229, MSA: 38/163). In accordance to this, only minor numbers of different species could be isolated from surface disinfected compared to non-disinfected seeds (DG-18:5/16, DRBC: 1/11, MSA: 4/16) (Table 1).

Overall, 25 different species belonging to 17 different genera contribute to the mycobiota of pumpkin seeds. The species were divided as follows: ascomycota, 2; zygomycota, 3; mitosporic fungi, 12. Species variation was highest in the case of *Aspergillus* (4), *Eurotium* and *Cladosporium* (both 3). The mycobiota were dominated by *Penicillium* spp. (263 isolations), *Eurotium* spp. (139 isolations), and *Cladosporium* spp. (99 isolations). *R. stolonifer* was the predominating fungal species (113 isolations). In total, 15 potential mycotoxigenic mould species contribute to the mycobiota of pumpkin seeds (Table 1).

Members of the genus *Alternaria* are common on cereal grains [24–26] and different kinds of nuts [4, 27,

Table 1 Number of moulds isolated from pumpkin seeds

Mould species	DG18		DRBC		MSA 5%	
	nsd[a]	sd[b]	nsd	sd	nsd	sd
Absidia spp.					1	
Alternaria alternata (Fr.) Keissler[c]	1					2
Alternaria spp.		6				3
Aspergillus flavus Link ex Gray[c]					1	
A. niger v. Tieghem[c]					2	
A. ochraceus gr.[c]	12				1	
A. terreus Thom[c]					3	
Aspergillus spp.	22		7			
Aureobasidium pullulans (de Bary) Arnaud	26		19			
Chaetomium sp.[c]			2			
Chrysosporium spp.		3				
Cladosporium cladosporioides (Fres.) de Vries	17	2			8	9
C. herbarum (Pers.) Link[c]	1		2			2
C. sphaerospermum Penzig	10	3	27		3	
Cladosporium spp.	8		3		1	3
Epicoccum purpurascens Ehrenb.[c]					1	
Eurotium amstelodami Mangin[c]	9				4	
E. herbariorum de Bary[c]	17					
E. rubrum (Koenig et al.)					1	
Eurotium spp.	49		24		34	1
Fusarium oxysporum Schlecht.: Fr.[c]	3					
Mucor plumbeus Bon.			1			
Mucor spp.	19		23		4	
Phoma spp.	9		1		3	
Penicillium chrysogenum Thom[c]	29		11		4	
P. griseofulvum Dierckx[c]					8	
Penicillium spp.[d]	105		35		37	
Sg. Aspergilloides					1	
Sg. Biverticillium					1	
Sg. Furcatum	1		1		1	
Sg. Penicillium	29					
Rhizopus stolonifer (Ehrenb.) Lind.[c]	45	2	35	3	28	
Scopulariopsis fusca Zach	1				1	
Trichoderma harzianum Rifai	2					
Wallemia sebi (Fr.) von Arx[c]	1					
sterile mycelium	10	6	19	8	6	13
n. i.[e]	11	2	19		9	5
Total number of fungi	437	24	229	11	163	38
Number of different species	16	5	11	1	16	4

[a] Not surface disinfected
[b] Surface disinfected
[c] Potential mycotoxin producing isolates
[d] Detailed identification not possible because these *Penicillium* isolates were overgrown by *R. stolonifer* on each medium
[e] Not identified

28]. Although *Alternaria* spp. are limited on pumpkin seeds, their detection is noteworthy since several members of this genus, e.g., *A. alternata*, produce mycotoxins such as alternariols, altenuens, altertoxins, and tenuazonic acid [29]. Some of these mycotoxins have already been found in food [e.g., 30–35].

Aspergillus spp. are found in different kinds of foodstuff [27, 36–38] but their occurrence on nuts and spices is most critical since they are frequently contaminated with mycotoxins [39–43]. In the present study at least four toxigenic *Aspergillus* species were isolated, from which *A. flavus* Link (aflatoxins) and *A. ochraceus* (ochratoxin A) are most important (Table 1). In addition, aflatoxins have already been isolated from pumpkin seeds [7].

Fungi of the genus *Cladosporium* occurred in high numbers (99 isolations) on pumpkin seeds. They are of ubiquitous distribution and frequently found on wide variety of foodstuff [6, 27, 36, 44]. *C. herbarum* produces epi- and fagicladosporic acid but their natural occurrence in food has not yet been reported [7].

Eurotium spp. frequently occurred on pumpkin seeds (Table 1). They tolerate low a_w values ($0.62 = $ *E. echinulatum*) and therefore belong to the primary colonizers of stored seeds [38, 45]. They synthesize different mycotoxins, e.g., ochratoxin A and sterigmatocystin, but usually not in dangerous concentrations [46]. The occurrence of *Eurotium* spp. is very important since seed moisture is increased due to their metabolic water, enabling the growth of more important toxigenic fungi [25].

Phoma spp. are a contaminant of different kinds of nuts and also common on pumpkin seeds (Table 1) [3, 27, 28, 36]. The detection of *Phoma* spp. is noteworthy since these fungi, together with *Alternaria* spp., are well known for the production of tenuazonic acid [47, 48]. This mycotoxin should be involved in onyalai, a mycotoxicosis which may be due to the consumption of contaminated sorghum and millet [49]. Up to now, tenuazonic acid has been detected in different kinds of food and feedstuff but the contamination of pumpkin seeds remains to be clarified [32, 35, 48, 50, 51].

Ubiquitous species like *P. chrysogenum* and *P. griseofulvum* contribute to the *Penicillium* spectrum of pumpkin seeds. This genus bears a high degree of mycotoxigenic moulds [46]. The isolated *Penicillium* species (Table 1) are known to produce mycotoxins such as cyclopiazonic acid, patulin, and roquefortine C but as yet they had not been detected in pumpkin seeds [7].

Concerning the number of isolated fungi, DG-18 (total: 461 isolations) was superior to the remaining media, DRBC (total: 240 isolations) and MSA (total: 201 isolations). This is in contrast with previous results on DRBC and DG-18 where MSA enabled the highest fungal counts [6, 52]. However, a similar high number of different fungal species (16) could be isolated from pumpkin seeds plated on DG-18 and MSA while DRBC enabled the isolation of only 11 different species (Table 1). DG-18 and MSA showed an almost similar effectiveness when the numbers of isolated species were compared [44, 52].

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