



## MICROBIAL CONTAMINATION OF SPICES USED IN PRODUCTION OF MEAT PRODUCTS

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### ABSTRACT

There was investigated microbial quality of spices used in production of meat products (black pepper, allspice, coriander, juniper, cumin, cinnamon, badian, mustard, bay leaf, paprika, rosemary, garlic, ginger, thyme, cardamom). The spices were analysed on the presence of total count of mesophilic, thermoresistant and coliforming microorganisms, *Staphylococcus aureus*, methicilin resistant *S. aureus* (MRSA), *Escherichia coli*, *Salmonella* spp., *Bacillus cereus*, *Bacillus licheniformis* and moulds. For the detection of fungal contamination was used agar with glucose, yeast extract and oxytetracyclin and dichloran-glycerol agar. The cultivation was performed at  $25 \pm 1^\circ\text{C}$  for 5 – 7 days. The microscopic method was used for species identification. The aflatoxin presence was confirmed by ELISA test in all of tested spices and was performed in ppb (pars per billion =  $\mu\text{g}/\text{kg}$ ). TCM ranged from 200 to 5600000 cfu/g, TRM from 20 to 90000 cfu/g and coliforming bacteria from 30 to 3200 cfu/g. *B. cereus* was present in juniper, mustard, bay leaf, thyme and cardamom (32%), while *B. licheniformis* was confirmed in 58% of cases (allspice, pepper, ground juniper, badian, bay leaf, paprika, garlic, thyme and cardamom). *S. aureus* was detected in whole coriander, cinnamon, badian and mustard but only in law number (30, 40, 20 and 10 cfu/g respectively). No strains *S. aureus* was identified as MRSA. The presence of *Salmonella* spp. and *E. coli* was not confirmed. The fungal contamination was found in 14 spices and their count varied from 0 to 1550 cfu/g. There were confirmed the presence of *Aspergillus flavus* (allspice whole and ground, black pepper whole and ground, whole coriander, ground cumin, ground bay leaf), *Aspergillus niger* (allspice whole and ground, black pepper ground, ground juniper, cumin ground, bay leaf ground, ground rosemary, ground thyme), *Penicillium glaucum* (allspice whole and ground, whole juniper, whole cinnamon), *Penicillium claviforme* (whole black pepper, whole coriander, cardamom ground), *Alternaria alternata* (cumin ground, rosemary ground, thyme ground), *Mucor* (whole and ground coriander and thyme) and *Phoma* (ground cumin). The aflatoxin presence was confirmed in 11 of samples (57.9%) and the value ranged from 0 to 4 ppb (ground allspice, whole and ground pepper, whole juniper, cumin, cinnamon, badian, bay leaf, paprika, rosemary, thyme).

**Keywords:** spices; meat; bacterial species; moulds; aflatoxin

### INTRODUCTION

The consumption of spices is connected with the human diet much sooner than later meat diet or the use of spices in combination with other types of food. Spices have not only the beneficial effects on the human body, but also improve and enhance the gustatory pleasures from food and promote the health. Srinivasan et al. (2004) and Srinivasan (2005a, b) confirmed the beneficial influence of turmeric/curcumin, red pepper/capsaicin, and garlic on lipid metabolism, especially anti-hypercholesterolemic effect of these spices, then anti-lithogenic effect of curcumin and capsaicin, then antioxidant effects of curcumin, capsaicin and eugenol (of clove), in his study with animals. Srinivasan (2007) also dealt with health benefits of black pepper/piperine and demonstrated in vitro studies its protective properties against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Platel and Srinivasan (2004) found the positive influences of some spices on terminal enzymes

during digestive proces. They documented that dietary curcumin, capsaicin, piperine and ginger enhanced intestinal lipase activity and also the sucrase and maltase, and dietary cumin, fenugreek, mustard and asafoetida decreased the level of phosphatases and sucrase.

Some spices can also have antibacterial properties. Arora and Kaur (1999) described the antimicrobial activity of garlic and clove to *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Tajkarimi et al. (2010) found out the antimicrobial activity of herbs and spices (basil, oregano, allspice, cinnamon, clove, thyme, rosemary) containing essential oils against foodborne pathogens, such as *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*. Mandal et al. (2011) confirmed also antimicrobial effect against methicilin-resistant *Staphylococcus aureus* (MRSA) of three indian spices (cinnamon, cumin and clove). Some authors described also

antifungal activities of spices and herbs essential oils. **Omidbeygi et al. (2007)** identified antifungal activity of three essential oils (thyme, summer savory and clove) against *Aspergillus flavus*, and **Atanda et al. (2007)** described antifungal properties of sweet basil, cassia, coriander and bay leaf against *Aspergillus parasiticus* CFR 23 and aflatoxin production. **Škrinjar and Nemet (2009)** published the review of antimicrobial activity of essential oils used spices (garlic, mustard, cinnamon, cumin, clove, bay leaf, thyme, basil, oregano, pepper, ginger, sage, rosemary etc.) against common bacteria and fungi such as *Listeria* spp., *Staphylococcus* spp., *Salmonella* spp., *Escherichia* spp., *Pseudomonas* spp., *Aspergillus* spp., *Cladosporium* spp. and many others.

Although many spices show the antifungal properties, they are themselves extensively contaminated. In case of bacterial contamination, there are mostly sporeforming bacteria (genus *Bacillus*, *Clostridium*), then *Salmonella* spp. and the others such as enterobacteria, pseudomonades and aeromonades as well as lactobacilli and enterococci (**Kneifel and Berger, 1994**). *Salmonella enterica* subspecies *enterica* and *Bacillus* spp. were the most common causative agents of foodborne illness outbreaks identified in spices (**Van Doren et al., 2013**). Next to the bacterial contamination, the fungal contamination is also very common. **Mandeeel (2005)** detected 665 fungal isolates in total, representing 14 species (mostly *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium* and *Trichoderma*), which were recovered and identified from 17 dried and ground spice samples. The most heavily contaminated spice was observed in red chili and black pepper (1580 and 1120 cfu/g, respectively). Fungal contamination represents serious risk for human health primarily in case of *Aspergillus flavus* and *Aspergillus parasiticus* occurrence. These are able to produce aflatoxins in food and feedstuffs, which are known to be potent hepatocarcinogens in animals and humans (**Šarić and Škrinjar, 2008**).

The aim of this work was to find out the level of bacterial and fungal contamination of selected spices and to detect the aflatoxin presence in spices.

## MATERIAL AND METHODOLOGY

**Spices selection.** There were tested spices which were used during technological process for meat products production (canned meat and pastes): black pepper, allspice, coriander, juniper, cumin, cinnamon, badian, white mustard, bay leaf, sweet paprika, rosemary, garlic, ginger, thyme, cardamom – Table 1. The mentioned spices were not treated with ionizing irradiation and originated from different countries. Their origin is included in Table 1.

**Microbiological analyses.** The total count of mesophilic (TCM) and thermoresistant (TRM) microorganisms and coliforms (COLI), then *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Salmonella* spp., *Bacillus cereus* and *Bacillus licheniformis* were determined in cfu in 1 g of prepared spice samples. The starting samples were prepared as follows: 5 g of spices (milled or powder) was dissolved in 45 g of sterile distilled water (the whole spices were

crushed in the bowl before). The solution was filtered and the supernatant was used for analyses.

TCM were cultivated using GTK-M Agar at 30 °C / 72 hours (according to standard ČSN EN ISO 4833) and COLI on VRLB Agar (Milcom, Tábor, CR) at 37 °C / 24 hours by standard ČSN ISO 4832. The samples for enumeration of TRM were inactivated at 85 °C during 10 minutes and then were cultivated on GTK Agar (Milcom, Tábor, CR) at 30 °C / 72 hours (ČSN EN ISO 4833).

Baird Parker Agar (HiMedia, India) was used for detection of *S. aureus* (ČSN EN ISO 6888-1). Plates were incubated at 37 ±1°C for 48 hours and colonies with zones of precipitation were submitted to the tube for free coagulase test for confirmation. STAPHYtest and the identification program TNW pro.7.5 were used for species identification (Erba Lachema, s.r.o., Brno, Czech Republic). The isolates identified as *S. aureus* were confirmed by the multiplex PCR method for the detection of the species specific fragment SA442 (**Martineau et al., 1998**). MRSA and MR-CNS (methicillin resistant coagulase-negative staphylococci) were screened for the presence of *mecA* gene, which encodes the resistance to methicillin (**Bosgelmez-Tinaz et al., 2006**).

The test for *Salmonella* spp. occurrence was performed according to norm ČSN EN ISO 6579. The samples were multiplied in buffered pepton water (Oxoid, Basingstoke, UK), cultivated at 37 ±1°C for 18 ±2 hours and then they were cultivated in selective medium RVS and MkTTn (Oxoid, Basingstoke, UK) at 37 ±1°C for 24 ±3 hours. This suspension was inoculated on the surface of Rambach medium (Merck, Germany) and XLD (Oxoid, Basingstoke, UK).

For the detection of fungal contamination was used agar with glucose, yeast extract and oxytetracycline (GKCH, HiMedia; India) and dichloran-glycerol agar (DG18, HiMedia; India). The cultivation was performed at 25 ±1°C for 5 – 7 days according to Czech norm ČSN ISO 21257-2. The microscopic method was used for species identification.

**Aflatoxin detection.** The aflatoxin presence was confirmed by ELISA test in all of tested spices. Aflatoxin was determined using commercial assay kit Veratox for Aflatoxin (Neogen, USA). Veratox is a competitive direct ELISA test that provides a quantitative analysis of aflatoxin. Lower limit of detection is 2 ppb, range of quantitation is 5 – 50 ppb (parts per billion = µg/kg).

## RESULTS AND DISCUSSION

**Microbial contamination.** The results of bacterial and fungal contamination are shown in the Table 2. From our results is evident that the count of mesophilic microorganisms ranged from 200 to 5600000 cfu/g. The highest TCM (>1 mil cfu/g) was found in cumin, bay leaf and ground coriander (5600000, 4800000 and 4000000 cfu/g, respectively). The lowest TCM was confirmed in case of whole juniper, cinnamon, badian and white mustard (TCM <1000 cfu/g). TCM <10000 cfu/g was found in whole coriander, rosemary and ginger. In the other spices, TCM varied between 20000 and 400000 cfu/g. The similar results in 55 different spices and herbs are indicated by **Kneifel and Berger (1994)**.

Table 1 Spices origin.

species	form	name	origin
allspice	whole	<i>Pimenta dioica</i>	Mexico
allspice	ground		Mexico
black pepper	whole	<i>Piper nigrum</i>	Vietnam
black pepper	ground		Vietnam
coriander	whole	<i>Coriandrum sativum</i>	Ukraine
coriander	ground		Ukraine
juniper	whole	<i>Juniperus communis</i>	Bosna-Hercegovina
juniper	ground		Macedonia
cumin	ground	<i>Carum carvi</i>	Czech Republic
cinnamon	whole	<i>Cinnamomum verum</i>	Czech Republic
badian	whole	<i>Illicium verum</i>	Vietnam
white mustard	whole	<i>Sinapis alba</i>	India
bay leaf	ground	<i>Laurus nobilis</i>	Turkey
sweet paprika	ground	<i>Capsicum annum</i>	Hungary
rosemary	ground	<i>Rosmarinus officinalis</i>	Morocco
garlic	powder	<i>Allium sativum</i>	China
ginger	ground	<i>Zingiber officinale</i>	Nigeria
thyme	ground	<i>Thymus vulgaris</i>	Poland
cardamom	ground	<i>Elettaria cardamomum</i>	Guatemala

Table 2 Results of bacterial contamination (in cfu/g).

species	TCM	TRM	COLI	<i>E.coli</i>	<i>B.cereus</i>	<i>B. licheniformis</i>	<i>S. aureus</i>	MRSA	<i>Salmonella</i> spp.
allspice whole	60000	6000	–	–	–	100	–		–
allspice ground	20000	14000	1000	–	–	150	–		–
black pepper whole	160000	15000	880	–	–	200	–		–
black pepper ground	320000	90000	3200	–	–	220	–		–
coriander whole	8000	50	600	–	–	–	30	neg.	–
coriander ground	4000000	24000	3000	–	–	–	–		–
juniper whole	200	100	–	–	10	–	–		–
juniper ground	60000	8000	100	–	120	150	–		–
cumin ground	5600000	600	2000	–	–	–	–		–
cinnamon whole	400	50	–	–	–	–	40	neg.	–
badian whole	800	140	–	–	–	10	20	neg.	–
white mustard whole	500	80	–	–	10	–	10	neg.	–
bay leaf ground	4800000	4000	480	–	80	120	–		–
sweet paprika ground	400000	9000	–	–	–	150	–		–
rosemary ground	5000	30	220	–	–	–	–		–
garlic powder	1700000	16000	–	–	–	220	–		–
ginger ground	3000	20	–	–	–	–	–		–
thyme ground	180000	6000	30	–	70	100	–		–
cardamom ground	60000	7000	–	–	80	80	–		–

cfu/g = colony forming units per gram; TCM = total count of mesophilic microorganisms; COLI = coliforming bacteria; RM = termoresistant bacteria; MRSA = methicilin resistant *S. aureus*

**Table 3** Results of fungal contamination (in cfu/g).

species	total count				
allspice whole	1550	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium glaucum</i>	
allspice ground	140	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium glaucum</i>	
black pepper whole	30	<i>Aspergillus flavus</i>	<i>Penicillium claviforme</i>		
black pepper ground	20	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>		
coriander whole	140	<i>Aspergillus flavus</i>	<i>Penicillium claviforme</i>	<i>Mucor</i>	
coriander ground	40	<i>Mucor</i>			
juniper whole	180	<i>Penicillium glaucum</i>			
juniper ground	10	<i>Aspergillus niger</i>			
cumin ground	80	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Phoma</i>	<i>Alternaria alternata</i>
cinnamon whole	10	<i>Penicillium glaucum</i>			
badian whole	0				
white mustard whole	0				
bay leaf ground	340	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>		
sweet paprika ground	0				
rosemary ground	10	<i>Aspergillus niger</i>	<i>Alternaria alternata</i>		
garlic powder	0				
ginger ground	0				
thyme ground	10	<i>Aspergillus niger</i>	<i>Mucor</i>	<i>Alternaria alternata</i>	
cardamom ground	10	<i>Penicillium claviforme</i>			

**Table 4** Results of aflatoxin detection (in ppb).

spice	ppb	<i>Aspergillus flavus</i>
allspice whole	0	+
allspice ground	4	+
black pepper whole	3	+
black pepper ground	4	+
coriander whole	0	+
coriander ground	0	
juniper whole	2	+
juniper ground	0	
cumin ground	2	+
cinnamon whole	4	
badian whole	4	
white mustard whole	0	
bay leaf ground	4	+
sweet paprika ground	3	
rosemary ground	3	
garlic powder	0	
ginger ground	0	
thyme ground	3	
cardamom ground	0	

More than 50% of samples contained TCM between  $10^4$  and  $10^6$  cfu/g. Some of them (chilli, black pepper and Chine spice) obtained TCM  $>10^7$  cfu/g. Schwab et al.

(1982) confirmed higher count of TCM ( $<100 - 3.1 \times 10^8$  cfu/g), but geometric means of cinnamon, ginger, paprika, pepper, rosemary and thyme were 39000, 21000, 100000, 820000, 7200 and 2100 cfu/g, respectively.

The count of TRM ranged from 20 to 90000 cfu/g. The highest count was confirmed in ground black pepper, ground coriander and garlic (90000, 24000 and 16000 cfu/g, respectively). The lowest count was detected in ginger, rosemary, cinnamon, whole coriander and mustard (20, 30, 50, 50 and 80 cfu/g, respectively). Compared to Kneifel and Berger (1994) that detected bacilli  $> 10^5$  cfu/g in almost 40% of the samples, our results were much lower. *B. cereus* was present in juniper, mustard, bay leaf, thyme and cardamom (32%), while *B. licheniformis* was confirmed in 58% of cases (allspice, pepper, ground juniper, badian, bay leaf, paprika, garlic, thyme and cardamom). The results show that *B. licheniformis* represents the higher risk than *B. cereus* which is considered as food pathogen. This finding confirms also our previous results in case of other kind of food (Vyletřlová et al., 2001, 2002).

The count of coliforming bacteria ranged from 30 to 3200 cfu/g and their presence was found out in all of spices except for whole allspice, whole juniper, cinnamon, badian, mustard, paprika, garlic, ginger and cardamom. The similar results are described by Schwab et al. (1982). They analysed cca 1500 samples of each spices and found out that the count of coliforms ranged from  $<3$  to  $1.1 \times 10^6$  cfu/g. The geometric mean of coliform bacteria was in cinnamon, ginger, paprika, pepper, rosemary and thyme (19, 12,  $<3$ , 9, 3.6 and 5 cfu/g, respectively).

*E. coli* was not detected. Sporadic occurrence of *E. coli* (geometric mean = <3 cfu/g) in some spices is confirmed also by Schwab et al. (1982). Shamsuddeen (2009) described *E. coli* presence in one mixture spice composed of ginger, cloves, black pepper, groundnut, salt and seasoning. This result confirms the finding of some authors that the spices have had antimicrobial effect against *E. coli* (Arora and Kaur, 1999, Tajkarimi et al., 2010, Sağdıç, 2003).

*S. aureus* was detected in whole coriander, cinnamon, badian and mustard but only in low number (30, 40, 20 and 10 cfu/g, respectively). No strains *S. aureus* was identified as MRSA. Shamsuddeen (2009) found out the high *Staphylococci* occurrence in the above mentioned mixture spice. The geometric mean was  $1.73 \times 10^9$  cfu/g but they didn't specify the staphylococcal species.

The presence of *Salmonella* spp. was not confirmed. Shamsuddeen (2009) detected *Salmonella* presence only in one sample of mixture spice (0.05%), Kneifel and Berger (1994) found *Salmonella arizonae* in one sample of black pepper from all of 160 tested spices, as well as.

**Fungal contamination.** Results of fungal contamination are summarized in Table 3. There were confirmed the presence of *Aspergillus flavus* (allspice whole and ground, black pepper whole and ground, whole coriander, ground cumin, ground bay leaf), *Aspergillus niger* (allspice whole and ground, black pepper ground, ground juniper, cumin ground, bay leaf ground, ground rosemary, ground thyme), *Penicillium glaucum* (allspice whole and ground, whole juniper, whole cinnamon), *Penicillium claviforme* (whole black pepper, whole coriander, cardamom ground), *Alternaria alternata* (cumin ground, rosemary ground, thyme ground), *Mucor* (whole and ground coriander and thyme) and *Phoma* (ground cumin). The fungal count varied from 0 to 1550 cfu/g, where the most occurrence was in whole allspice. The count from 10 to 340 cfu/g was observed in case of ground allspice, black pepper whole and ground, coriander whole and ground, juniper whole and ground, ground cumin, cinnamon whole, ground bay leaf, ground rosemary, ground thyme and cardamom. The moulds were not detected in the rest of spices (badian, mustard, garlic and ginger). Schwab et al. (1982) stated the fungal count from <5 to 300000, while geometric mean was for cinnamon, ginger, paprika 290, 7 and 14 cfu/g respectively. They don't specify the fungal species in this work. The similar species representation (*Penicillium*, *Aspergillus*, *Mucor*) were described by Imandel and Adibnia (2000) in turmeric, black pepper and sumac. Mandeel (2005) writes that the most predominant fungal genera encountered were *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium* and *Trichoderma* and the most contaminated spice samples examined were observed in red chili and black pepper in order of magnitude of 1580 and 1120 cfu/g, respectively.

**Aflatoxin presence.** Aflatoxin detection is presented in Table 4. From obtained results is evident that the aflatoxin presence was confirmed in 11 of samples (57.9%). The presence of aflatoxin corresponds to the presence of *A. flavus* only in ground allspice, whole and ground pepper, whole juniper, ground cumin and ground bay leaf. In case of whole allspice and whole coriander there wasn't the aflatoxin occurrence detected even there were confirmed

presence of *A. flavus*. This difference can be explained by sensitivity of ELISA test or the identified *A. flavus* strain produced no aflatoxins in under this condition, which was described also by (Schindler et al., 1967, Ritter et al., 2011). In the other spices (whole cinnamon, whole badian, sweet paprika ground, ground rosemary and ground thyme) was aflatoxin determined even the *A. flavus* occurrence was negative. This difference can be explained by the used method (e.g. sample preparation - dilution) or by the presence only aflatoxins in spices.

Recorded results of aflatoxin (0 – 4 ppb) meet the requirements for limit of aflatoxin occurrence in spices according to Commission Regulation (EU) No. 165/2010 which defines the maximal limits of aflatoxin B1 (5 ppb) and total aflatoxin B1 + G1 + B2 + G2 (10 ppb) in some spices.

## CONCLUSION

There are no legislative requirements for microbiological quality of spices in CR. The results can be evaluated by other regulations relating to food, which the spices are used for (e.g. Commission Regulation (ES) No. 2073/2005). According to this regulation and from the viewpoint of bacterial contamination and food pathogens (*S. aureus*, *E. coli*, *Salmonella*) it is possible to state that tested spices did not exceed allowed limits occurrence. The results fulfil also the limit value for microbiological quality according to previous Czech Regulation No. 132/2004.

The microbial contamination (TCM) in spices suggests the need for a thorough control of spices, used in food processing in general (heat treated food, semi food, food for direct consumption). Mainly food for direct consumption (including spices or herbs) represents a higher risk. The use of radiation treated with ionizing irradiation is one option to reduce or eliminate this risk. Maximum permissible overall average absorbed radiation dose which is allowed in the CR is 10.0 kGy (gray) according to Decree of Ministry of Health (133/2004).

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