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ORIGINAL ARTICLE

Microbiological quality and aflatoxin B1 content of some spices and additives used in meat

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Abstract

Introduction Being a product of agricultural practice, spices can carry high numbers of microorganisms as well as mycotoxins, especially aflatoxin B1. Thus, effective control of these parameters is a prerequisite for their utilization in the food sector. *Objectives* The objective of this study was to assess the microbiological quality and the aflatoxin B1 content of 15 additives, 13 spices and 38 spice mixtures used in the meat industry. *Methods* Three series of samples were taken for analyses. Standard and established methods were used for both microbiological analyses and aflatoxin B1 detection. *Results and Conclusion* *Staphylococcus aureus*, enterococci, *Bacillus* sp., *Bacillus cereus*, sulphur-reducing clostridia and *Escherichia coli* were below detection limit in all samples examined. This was also the case regarding the total aerobic mesophilic count, *Enterobacteriaceae*, coliforms and yeasts/moulds counts of additives. On the other hand, 12 out of 13 spices and 20 out of 38 spice mixtures were found to be contaminated with some of them being of unacceptable microbiological quality according to Recommendation 2004/24/EC. Analyses for aflatoxin B1 content revealed absence or, at least, presence of this contaminant below the detection limit of 1.0 p.p.b. in all samples. The absence of bacterial pathogens and aflatoxin B1 is an important finding regarding the safety of additive, spice and spice mixtures in the meat industry. However, the presence of members of the *Enterobacteriaceae* family, raise questions regarding the hygienic status of their handling.

Introduction

Herbs and spices are among the most diverse and extensively used ingredients in food preparation, in both household and industrial scale, contributing to flavour, aroma and even colour. However, being a product of agricultural practice, they can carry high numbers of microorganisms, including pathogenic bacteria, moulds and yeasts. Indeed, most spices and herbs contain a very high number of bacteria, thus contributing to the spoilage of food products (De Boer *et al*., 1985). The presence of *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, as well as moulds of the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Cunninghamella* and *Trichoderma* have been reported (Satchell *et al*., 1989; Garcia *et al*., 2001; Banerjee & Sarkar, 2003; Mandeel, 2005; Sagoo *et al*., 2009). Moreover, several spices, including turmeric, paprika, aniseed and pepper have been linked with salmonellosis outbreaks (Gustavsen & Breen, 1984; Lehmacher *et al*., 1995; Little *et al*., 2003; Koch *et al*., 2005). Additionally, the presence of moulds creates a potential risk for human health due to mycotoxin production. Among the mycotoxins, aflatoxins are the most dangerous to human health; especially aflatoxin B1, which is considered to be the most potent naturally occurring hepatocarcinogen known. The presence of aflatoxin B1 in many herbs and spices, including various pepper types, ginger, paprika, cumin, curry powder, cayenne, chilli powder, coriander and turmeric has been reported (Fazekas *et al*., 2005; Colak *et al*., 2006; Zinedine *et al*., 2006; Ardic

et al., 2008; Cho *et al*., 2008; O'Riordan & Wilkinson, 2008), the content of which was found, in many cases, to be above the upper limit that has been set [European Commission (EC), 2006].

Herbs and spices are extensively used in the meat industry. They usually take up 0.5–2% of the formula, may be used either singly or in strictly defined mixtures and significantly contribute to the organoleptic properties of the final product. Despite the fact that the occurrence of both pathogenic bacteria and aflatoxin B1 has been generally exhibited, there is a lack in the literature concerning the spices specifically used in the meat industry.

In the present study, the microbiological quality as well as the aflatoxin B1 content of several additives, spices and spice mixtures used in the meat industry is assessed.

Materials and methods

Sampling

A total of 66 spices and additives (Table 1) used in the meat industry were collected from Southern Greece. The spices after purchase were kept in a dry storage room at 15–20 °C. Approximately 300 g were aseptically collected, transferred to the laboratory and analysed the same day. The whole procedure was performed in triplicate.

Microbiological analyses

Samples (25 g) were homogenized with 225 mL of sterile saline containing 0.1% (w/v) peptone (Merck, Darmstadt, Germany) and 0.85% (w/v) NaCl (Merck) using a stomacher apparatus (Seward Medical, London, UK). Serial dilutions were performed in sterile Ringer's solution (Merck). Pour plating technique was performed by mixing 1 mL of the appropriately diluted sample with molten media. A surface spreading technique was performed by spreading 0.1 mL of the appropriately diluted sample to the surface of the media. In all cases, duplicate plates were prepared. The total aerobic mesophilic count (TAMC), was estimated by spreading on Plate Count agar (Merck), and incubating at 30 °C for 48 h. *S. aureus* determination was carried out by spreading 0.1 mL on Baird-Parker selective agar (Merck) and incubation at 35 °C for 24–48 h. Yeasts and moulds were determined by spreading on Yeast Glucose Chloraphenicol agar and incubating at 25 °C for 48 h, while enterococci, *Bacillus* sp. and *B. cereus* estimation took place by spreading on Kanamycin Aesculin Azide agar (Merck), Dextrose Casein-Peptone agar (Merck) and Cereus Selective agar (Merck) and incubating at 35, 30 and 32 °C for 72, 72 and 48 h, respectively. Sulphur-reducing clostridia were determined by pouring 10 mL aliquots in 20 mL of molten Sulphite Polymyxin Sulfadiazine agar (Merck). After solidification, the agar was overlaid with 5 mL of sterile paraffin (Merck). Incubation was carried out at 35 °C for 24 h. The determination of members of the *Enterobacteriaceae* family was carried out by pouring 1 mL in Violet Red Bile Dextrose agar (Merck) and incubating at 35 °C for 24 h. Coliforms and *E. coli* were estimated by pouring in Chromocult® coliform agar (Merck) and incubation at 35 °C for 24 h. The qualitative determination of *Listeria monocytogenes* and *Salmonella* sp. was performed as follows: in the first case a pre-enrichment in Fraser broth (Merck) at 35 °C for 48 h was performed and then inoculation on PALCAM agar (Biolife, Milano, Italy) and incubation at 37 °C for 48 h. In the second case, a pre-enrichment step in Salmosyst® broth (Merck) at 35 °C for 24 h was followed by inoculation on Xylose Lysine Desoxycholate agar (Merck) and incubated at 35 °C for 48 h, according to the manufacturer's instructions.

Aflatoxin B1 determination

Aflatoxin B1 determination took place using a Veratox® HS quantitative aflatoxin B1 high-sensitivity test (Neogen Corporation, Lansing, MI, USA) according to the manufacturer's instructions, with a limit of detection of 1.0 p.p.b.

Results and discussion

The microbiological quality of the additives, spices and spice mixtures analysed is shown in Table 1. Generally, *S. aureus*, enterococci, *Bacillus* sp., *B. cereus*, sulphur-reducing clostridia and *E. coli* were below detection limits. Moreover, *Listeria* spp. and *Salmonella* spp. were absent in 25 g of product. This was also the case regarding the TAMC, *Enterobacteriaceae*, coliforms and yeasts/moulds counts of additives. On the other hand, spices and spice mixtures were microbiologically contaminated. In the first case, all but garlic were found to contain at least a TAMC of 4.14 log CFU g^{-1}. The most heavily loaded was whole pepper with TAMC of 7.36 log CFU g^{-1}, 4.46 log CFU g^{-1} *Enterobacteriaceae* count and 2.61 and 6.72 log CFU g^{-1} coliforms and yeasts/moulds count, respectively. As far as the microbiological quality of spice mixtures was concerned, 20 out of 38 contained microorganisms above the detection limits. The highest TAMC and yeasts/mould count of 6.66 and 5.71 log CFU g^{-1}, respectively, were determined in 'texas no. 7' mixture whereas the highest *Enterobacteriaceae* and coliform counts of 3.50 and 2.30 log CFU g^{-1}, respectively, were observed in 'dry salami' mixture.

Table 1 Microbiological quality of additives, spices and spice mixtures used in the meat industry

Sample	TAMC[1]	*Enterobacteriaceae*	Coliforms	Yeasts/moulds
Additives				
Albumin	< 1.0	< 1.0	< 1.0	< 2.0
Carbohydrate mixture	< 1.0	< 1.0	< 1.0	< 2.0
Egg	< 1.0	< 1.0	< 1.0	< 2.0
Gelatin	< 1.0	< 1.0	< 1.0	< 2.0
Maltodextrine	< 1.0	< 1.0	< 1.0	< 2.0
NaCl/nitrate mixture	< 1.0	< 1.0	< 1.0	< 2.0
Phosphates	< 1.0	< 1.0	< 1.0	< 2.0
Protein mixture E32	< 1.0	< 1.0	< 1.0	< 2.0
Protein mixture supro s95	< 1.0	< 1.0	< 1.0	< 2.0
Sodium ascorbate	< 1.0	< 1.0	< 1.0	< 2.0
Sodium caseinate	< 1.0	< 1.0	< 1.0	< 2.0
Sodium glutamate	< 1.0	< 1.0	< 1.0	< 2.0
Soy protein	< 1.0	< 1.0	< 1.0	< 2.0
Wheat starch	< 1.0	< 1.0	< 1.0	< 2.0
Whey	< 1.0	< 1.0	< 1.0	< 2.0
Spices				
Coriander	4.53 (0.15)	2.20 (0.18)	< 1.0	4.38 (0.42)
Cumin	4.38 (0.24)	2.36 (0.28)	< 1.0	4.59 (0.27)
Garlic (flakes)	< 1.0	< 1.0	< 1.0	< 2.0
Leek	4.20 (0.19)	< 1.0	< 1.0	< 2.0
Mustard seeds	5.59 (0.42)	< 1.0	< 1.0	3.47 (0.25)
Nutmeg	4.79 (0.39)	2.71 (0.31)	< 1.0	< 2.0
Onion	4.72 (0.25)	2.85 (0.16)	< 1.0	< 2.0
Oregano	4.91 (0.47)	2.56 (0.31)	< 1.0	4.44 (0.24)
Paprika	5.80 (0.28)	3.61 (0.29)	< 1.0	5.80 (0.19)
Pepper	6.59 (0.17)	4.04 (0.41)	2.32 (0.21)	5.98 (0.27)
Pepper (whole)	7.36 (0.41)	4.46 (0.27)	2.61 (0.15)	6.72 (0.31)
Savory	4.56 (0.09)	3.20 (0.38)	< 1.0	4.51 (0.42)
White pepper	5.39 (0.13)	2.47 (0.24)	< 1.0	3.36 (0.16)
Spice mixtures				
Aromazia	< 1.0	< 1.0	< 1.0	< 2.0
Beef aroma	< 1.0	< 1.0	< 1.0	< 2.0
Bierschinken	6.17 (0.27)	< 1.0	< 1.0	< 2.0
Bier wurst	5.94 (0.31)	< 1.0	< 1.0	5.00 (0.31)
Brat wurst	4.54 (0.42)	< 1.0	< 1.0	3.20 (0.14)
Chopped ham	< 1.0	< 1.0	< 1.0	< 2.0
Dry salami	6.44 (.14)	3.50 (0.20)	2.30 (0.20)	4.27 (0.23)
Fluewu	< 1.0	< 1.0	< 1.0	< 2.0
Frankfurter	4.04 (0.23)	< 1.0	< 1.0	3.60 (0.14)
Frankfurter continental	4.14 (0.37)	< 1.0	< 1.0	< 2.0
Ham 82 plus	< 1.0	< 1.0	< 1.0	< 2.0
Ham N	4.42 (0.24)	< 1.0	< 1.0	< 2.0
Ham Niagara	4.94 (0.12)	2.54 (0.12)	< 1.0	< 2.0
Italian mortadella	< 1.0	< 1.0	< 1.0	< 2.0
Kozaken wurst	5.22 (0.32)	3.40 (0.24)	< 1.0	4.14 (0.16)
Krainer natur	5.96 (0.18)	< 1.0	< 1.0	< 2.0
Lakin brine	< 1.0	< 1.0	< 1.0	< 2.0
Local mixture[2] 1	4.64 (0.08)	< 1.0	< 1.0	< 2.0
Local mixture 2	4.82 (0.10)	2.20 (0.10)	< 1.0	3.76 (0.22)
Local mixture 3	3.24 (0.13)	< 1.0	< 1.0	< 2.0
Local mixture 4	4.46 (0.15)	< 1.0	< 1.0	< 2.0
Luncheon meat	< 1.0	< 1.0	< 1.0	< 2.0
Meat ham	< 1.0	< 1.0	< 1.0	< 2.0

Table 1 Continued

Sample	TAMC[1]	*Enterobacteriaceae*	Coliforms	Yeasts/moulds
Mortadella Milano	3.95 (0.32)	2.66 (0.20)	< 1.0	3.73 (0.43)
Mortadella strong	< 1.0	< 1.0	< 1.0	< 2.0
Nosti ham	< 1.0	< 1.0	< 1.0	< 2.0
Pariser	4.07 (0.12)	< 1.0	< 1.0	< 2.0
Pariser extra	4.44 (0.18)	< 1.0	< 1.0	< 2.0
Peperone	5.63 (0.29)	2.47 (0.12)	< 1.0	4.77 (0.13)
Poultry seasoning	< 1.0	< 1.0	< 1.0	< 2.0
Roh wurst	< 1.0	< 1.0	< 1.0	< 2.0
Schinken wurst	5.23 (0.16)	2.06 (0.06)	< 1.0	< 2.0
Super ham spice	< 1.0	< 1.0	< 1.0	< 2.0
Taroma beef	< 1.0	< 1.0	< 1.0	< 2.0
Taroma pikant smoky	< 1.0	< 1.0	< 1.0	< 2.0
Texas no7	6.66 (0.13)	< 1.0	< 1.0	5.71 (0.21)
Toast ham	< 1.0	< 1.0	< 1.0	< 2.0
Zamek 8018	< 1.0	< 1.0	< 1.0	< 2.0

Microbial populations are given in log CFU g^{-1}. All determinations were performed in triplicate. The standard deviation is given in parentheses.
Staphylococcus aureus, enterococci, *Bacillus* sp., *Bacillus cereus*, sulphur-reducing clostridia, *Escherichia coli* below detection limit.
Listeria spp., *Salmonella* spp. absent in 25 g of product.
[1]Total aerobic mesophilic count.
[2]Spice mixture made specially for local products.

Analyses for aflatoxin B1 content revealed the absence or, at least, presence of this contaminant below the detection limit of 1.0 p.p.b. in all samples.

Herbs and spices have delivered a variety of functions from antiquity until today. Their widespread use in the food industry along with several studies reporting presence of pathogenic bacteria and their implication in food-borne salmonellosis has rightfully raised concerns regarding the microbiological quality and safety of the end product. In considering the meat industry, the absence of spore-forming or pathogenic bacteria is of vital importance, especially for products that are not subjected to any kind of thermal treatment. Although *Salmonella* spp., *B. cereus*, *C. perfringens*, *S. aureus* and *E. coli* have been found in a wide range of herbs and spices (Schwab *et al*., 1982; De Boer *et al*., 1985; McKee, 1995; Garcia *et al*., 2001; Banerjee & Sarkar, 2003; Sagoo *et al*., 2009), they were not detected in any of the samples examined in the present study.

Regarding the additives that were examined in the present study, the absence of any microbial count can be attributed to the nature of those products; although some of them could possibly support microbial growth, the level of treatment that they have undergone along with good hygiene practices during distribution and storage has resulted in a high microbiological quality and safety.

As far as the spices were concerned, garlic, leek and mustard seeds were found to be of satisfactory microbiological quality according to Recommendation 2004/24/EC (EC, 2004). The rest were found to be of unacceptable quality due to the presence of *Enterobacteriaceae*. Given the sensitivity of the members of this family to irradiation, which is commonly applied as a decontamination method for spice production (Kume *et al*., 2009), the high *Enterobacteriaceae* population may result either from the absence of sufficient decontamination or deviations from good hygiene practices applied during production, distribution and storage in the meat industry.

Regarding the microbiological quality of the spice mixtures that were examined, assuming that Recommendation 2004/24/EC applies to these products as well, the presence of *Enterobacteriaceae* renders seven mixtures, namely dry salami, ham Niagara, kozaken wurst, local mixture 2, mortadella Milano, pepperone and schinken wurst, microbiologically unacceptable. As little information is available concerning the composition of these mixtures, it can only be assumed that the microbial load may be due to inadequate decontamination or improper distribution and storage conditions of either the mixture itself or of its constituents.

Although in many of the samples significant mould populations were present, the absence of aflatoxin B1 contamination was evident, most probably due to unfavourable conditions for its development during production, product storage and distribution.

Conclusions

The purpose of the present study was to assess the microbiological quality as well as the aflatoxin B1 content of additives, spices and spice mixtures used in the meat industry. The absence of bacterial pathogens and aflatoxin B1 was an important finding with respect to the safety of additive, spice and spice mixtures in the meat industry. In particular, absence of important indicators of faecal contamination, such as *E. coli* and *C. perfringens*, was of significant importance. However, presence of members of the *Enterobacteriaceae* family, raise questions regarding the hygienic status of their handling and moreover renders many of them microbiologically unacceptable according to Recommendation 2004/24/EC.

References

Ardic M., Karakaya Y., Atasever M., Durmaz H. (2008) Determination of aflatoxin B_1 levels in deep-red ground pepper (isot) using immunoaffinity column combined with ELISA. *Food and Chemical Toxicology*, **46**, 1596–1599.

Banerjee M., Sarkar P.K. (2003) Microbiological quality of some retail spices in India. *Food Research International*, **36**, 469–474.

Cho S.-H., Lee C.-H., Jang M.-R., Son Y.-W., Lee S.-M., Choi I.-S., Kim S.-H., Kim D.-B. (2008) Aflatoxins contamination in spices and processed spice products commercialized in Korea. *Food Chemistry*, **107**, 1283–1288.

Colak H., Bingol E.B., Hampikyan H., Nazli B. (2006) Determination of aflatoxin contamination in red-scaled, red and black pepper by ELISA and HPLC. *Journal of Food and Drug Analysis*, **14**, 292–296.

De Boer E., Spiegelenberg W.M., Janssen F.W. (1985) Microbiology of spices and herbs. *Antonie van Leeuwenhoek*, **51**, 435–438.

European Commission (EC) (2002) Commission Regulation No. 472/2002 of 12 March 2002. *Official Journal of European Communities*, **L 75**, 18.

European Commission (EC) (2004) Commission Recommendation of 19 December 2003 concerning a coordinated programme for the official control of food stuffs for 2004 (2004/24/EC). *Official Journal of European Union*, **L6**, 29–37.

European Commission (EC) (2006) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, **L 364**, 5–24.

Fazekas B., Tar A., Kovacs M. (2005) Aflatoxin and ochratoxin A content of spices in Hungary. *Food Additives and Contaminants*, **22**, 856–863.

Garcia S., Iracheta F., Galvan F., Heredia N. (2001) Microbiological survey of retail herbs and spices from Mexican markets. *Journal of Food Protection*, **64**, 99–103.

Gustavsen S., Breen O. (1984) Investigation of an outbreak of *Salmonella* Oranienburg infections in Norway, caused by contaminated black pepper. *American Journal of Epidemiology*, **119**, 806–812.

Koch J., Schrauder A., Alpers K., Weber D., Frank C., Prager R., Rabsch W., Broll S., Feil F., Roggentine P., Bockemuhl J., Tschape H., Ammon A., Stark K. (2005) *Salmonella* Agona outbreak from contaminated aniseed, Germany. *Emerging Infectious Diseases*, **11**, 1126–1127.

Kume T., Furuta M., Todoriki S., Uenoyama N., Kobayashi Y. (2009) Status of food irradiation in the world. *Radiation Physics and Chemistry*, **78**, 222–226.

Lehmacher A., Bockemuhl J., Aleksic S. (1995) Nationwide outbreak of salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiology and Infection*, **115**, 501–511.

Little C.L., Omotoye R., Mitchell R.T. (2003) The microbiological quality of ready-to-eat foods with added spices. *International Journal of Environment and Health*, **13**, 31–42.

Mandeel Q.A. (2005) Fungal contamination of some imported spices. *Mycopathologia*, **159**, 291–298.

McKee L.H. (1995) Microbial contamination of spices and herbs: a review. *Lebensmittel Wissenschaft und Technologie*, **28**, 1–11.

O'Riordan M.J., Wilkinson M.G. (2008) A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market. *Food Chemistry*, **107**, 1429–1435.

Sagoo S.K., Little C.L., Greenwood M., Mithani V., Grant K.A., McLauchlin J., de Pinna E., Threlfall E.J. (2009) Assessment of the microbiological safety of dried spices and herbs from production and retail premises in the United Kingdom. *Food Microbiology*, **26**, 39–43.

Satchell F.B., Bruce V.R., Allen G., Andrews W.H., Gerber H.R. (1989) Microbiological survey of selected imported spices and associated fecal pellet specimens. *Journal of the Association of Official Analytical Chemists*, **72**, 632–637.

Schwab A.H., Harpestad A.D., Swartzentruber A., Lanier J.M., Wentz B.A., Duran A.P., Barnard R.J., Read R.B. Jr. (1982) Microbiological quality of some spices and herbs in retail markets. *Applied and Environmental Microbiology*, **44**, 627–630.

Zinedine A., Brera C., Elakhdari S., Catano C., Debegnach F., Angelini S., De Santis B., Faid M., Benlemlih M., Minardi V., Miraglia M. (2006) Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, **17**, 868–874.

