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Assessment of the Microbiological Quality and Mycotoxin Contamination of Iranian Red Pepper Spice

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ABSTRACT

The objective of this study was to assess the microbial contamination of Razavi Khorasan (Iran) hot red pepper. The natural occurrence of aflatoxins and ochratoxin A in those samples was also investigated. For this purpose, 36 samples of this kind of pepper were collected from a farm and sun-dried. Standard and established methods were used for both microbiological analyses and mycotoxins identification. Total aerobic mesophilic counts of samples varied from 10^2 to 4×10^6 cfu g^{-1}. Coliforms were present at high levels in all samples ranging from 1.9×10^2 to 3.52×10^6 cfu g^{-1}that may indicate inappropriate hygienic quality of samples. 42% of the samples were of unsatisfactory quality due to the presence of *E. coli*. In all samples examined, sulphite-reducing clostridia (SRC) was below detection limit and *Salmonella* spp. was not detected. Fungi were found in all of the collected samples. Mold and yeast were generally high ranging from 2.4×10^3 to 4.6×10^6 cfu $g^{-1}$and the most predominant fungal genera were *Aspergillus* spp., *Penicillium* spp and *Rhizopus spp.* Considering the results obtained, the samples analyzed contain a high level of microorganisms and only two samples (6%) had acceptable levels for all microbial factors according to EU Commission Recommendation (directive2004/24/EC). 69% and 17% of samples were found contaminated with total aflatoxins and ochratoxin A, respectively, that might contribute to health hazards for humans. Overall, The Razavi Khorasan hot red pepper samples collected for this study were contaminated with microorganisms and mycotoxins, which suggests that hygiene practice pre- and post-harvesting must be improved if the region is to exploit fully the potential for this valuable product.

Keywords: Aflatoxins, Microbiological quality, Ochratoxin A, Red pepper.

INTRODUCTION

Spices and herbs are natural products that can be obtained from parts of certain plants including the roots, rhizomes, bulbs, bark, leaves, stems, flowers, fruits and seeds. They are valued for distinctive flavors, colors and aromas, and are among the most versatile and widely used ingredients in food preparation and processing throughout the world (McKee, 1995).

Pepper belongs to the nightshade family *Solanaceae*, and is a fruit of the genus *Capsicum*. It is harvested as a vegetable as well as a source of spice, and contains numerous essential vitamins and other nutrients. After salt, pepper is the world's most popular seasoning and the name is derived from 'old world' black pepper, as it resembled the latter in taste. Peppers can be categorised as sweet or hot (Rajeev, 2010), and are widely used for culinary purposes because of the number of varieties available. For example, red pepper is used as a spice, and as an ingredient in other food products (e.g. Lecsó).

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As with many other agricultural products, herbs and spices may be exposed to a wide range of microbial contaminants before, during and post-harvest (McKee, 1995; Koci-Tanackov *et al*., 2007). Although used in small quantities, herbs and spices are recognized as significant carriers of microbial contamination primarily xerophilic storage molds and some bacteria (Dimic *et al*., 2000; Romagnoli *et al*., 2007). Whilst fungi are the most common contaminants, most are probably commensal residents of the plant. Spices are collected in tropical areas using traditional methods, which mean products are exposed to contaminants from the soil and air, before being sufficiently dry to prevent microbial growth (Kneifel and Berger, 1994), as well as during harvesting, handling and packing.

Molds reduce the quality of food, and create a potential risk for human health with the production of toxic metabolites known as mycotoxins. Tropical climates (wide ranges of temperature, humidity and rainfall) favor mycotoxin contamination, but it is poor hygiene during post-harvest treatment that promotes the growth of molds leading to the production of mycotoxins (Martins *et al*., 2001). Aflatoxins and ochratoxin A are the most important mycotoxins with worldwide occurrence and pose a risk to human health because they are resistant to heat (e.g. cooking) and damage specific tissues and organs (Galvano *et al*., 2005; Jay *et al*., 2005). On a global scale, contamination of spices by mycotoxins has been reported as significant in Ethiopia (Fufa and Urga, 1996), Egypt (El-Kady *et al*., 1995; Selim *et al*., 1996; Aziz *et al*., 1998) Turkey (Gurbuz *et al*., 2000), Portugal (Martins *et al*., 2001), Italy (Romagnoli *et al*., 2007) and Morocco (Zinedine *et al*., 2006). Control of microbial contamination relies on the application of good hygiene practice in production and harvesting and post-harvest processing including storage.

During the last decade of the 20th century, food-borne infections and intoxication due to spices increased in several European countries (Buckenhüskes *et al*., 2004; Jackson *et al*., 1995). There are no microbiological standards for dried spices in European Community legislation. However, the Codex Code of Hygienic Practice specifies that dried spices should be free from pathogenic microorganisms at levels that may represent a hazard to health and requires that *Salmonella* spp. should be absent in treated, ready-to-eat spices (Codex Alimentarius Commission, 1995). The European Spice Association (ESA) and the European Commission (EC) Recommendation 2004/24/EC also specify that *Salmonella* spp. should be absent in 25 g of spice (ESA, 2007), *Escherichia coli* must be less than 10^2 cfu g^{-1}, and other bacteria requirements should be agreed between the buyer and the seller (Muggeridge *et al*., 2001).

Studies on the microbiology of these commodities have shown the presence of high microbial total counts (up to 8 log CFU g^{-1}in black pepper, paprika, chilli powder and cumin seeds (Baxter and Holzapfel, 1982; Bhat *et al*., 1987; McKee, 1995). Thus, herbs and spices may provide a conduit to introduce food spoilage organisms to a range of meals (Garcia *et al*., 2001). When added to high moisture foods, low levels of microbial contamination in herbs and spices may develop quickly causing the food to deteriorate.

Razavi Khorasan (a province located in northeast of Iran) is one of the most important production areas for hot red pepper in Iran (Table1). However, due to the humid and warm climatic conditions, microbial and mycotoxin contamination of pepper is common.

The aim of this work was to assess the microbial contamination of Razavi Khorasan hot red pepper including mycotoxin levels to determine the extent of the risk. This is the first report on Iranian red pepper, which is used mostly in processed foods and ready-to-eat meals consumed daily in large quantities as culinary ingredients by households in Iran.

Table 1. Physicochemical properties of Iranian red pepper spice.

Quality characteristics	Value (%)
Color	Red
Taste	Hot
Moisture	9-11
Total ash	6.5 -7.5
Acid–insoluble ash	0.3 - 1
Volatile Oil	0.8 – 1

MATERIALS AND METHODS

Sampling

A native spice of hot red pepper, grown in Razavi Khorasan province and produced in Sabzevar, was selected. Harvest started at the end of summer and continued until early autumn. Samples (36) sun-dried in open air were collected randomly during this period. The average size of samples collected ranged between 250 g and 1 kg. The samples were transported to the laboratory under ambient temperature, and immediately analyzed for microbial assessment. Portions of each sample were kept in polyethylene bags and stored at -20ºC for mycotoxin analysis.

Sample Preparation

Samples were ground and mixed to uniform consistency using a laboratory mill or mixer (Omni-Mixer, Sorvall, Newton, CT, USA).

Physicochemical Analysis

All chemicals were analytical reagent grade. All solutions were prepared with deionized water.

The samples were analyzed for moisture, total ash, and volatile oil- and acid-insoluble ash content. These analyses were performed according to standard and reference methods including ISO 939:1980, ISO 928:1997, ISO 6571, 2:2008 and ISO 930: 1997, respectively. All analyses were performed in triplicate.

Microbiological Analysis

For the detection and enumeration of microorganisms, standard media were prepared. All media and reagents were purchased from Merck (Darmstadt, Germany).

Samples (25 g) were homogenized with 225 ml of sterile saline containing 0.1% (w/v) peptone and 0.85% (w/v) NaCl using a stomacher apparatus (Seward Medical, London, UK). Serial dilutions were performed in sterile Ringer's solution. The molten media was mixed with 1 ml of each diluted sample before plating (anaerobic). Diluted samples (0.1 ml) were applied to the surface of the media using a surface spreading technique (aerobic). Duplicate plates were prepared in all cases.

The total aerobic mesophilic count (TAMC) was estimated by applying plate count agar and incubating at 30º C for 48 hours. Yeasts and molds were determined with yeast glucose chloraphenicol agar and incubating at 25º C, also for 48 hours. Sulphite-reducing Clostridia spp. was determined by pouring aliquots (10 ml) into molten sulphite polymyxin sulfadiazine agar (20 ml). The agar was overlaid with 5 ml of sterile paraffin, and samples were incubated at 35º C for 24 hours. Coliforms were estimated by pouring in chromocults coliform agar and incubating at 35ºC for 24 hours. *Escherichia coli (E. coli*) was detected by gas and indol production on lauryl sulfate broth, *E. coli* (EC) broth and peptone water incubated at 37-44ºC for 48 hours. *Salmonella* spp. were estimated by a pre-enrichment step in salmosysts broth at 35ºC for 24 hours followed by inoculation on xylose lysine desoxycholate agar, and incubation at 35ºC for a further 48 hours.

Qualitative Analysis of Aflatoxins and Ochratoxin A

Each dried sample (25 g) was de-fatted by extraction with 50 ml normal hexane for 10 hours using a soxhlet-type extractor. The de-fatted residue was re-extracted for a further 10 hours with 50 ml chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered and evaporated under vacuum to near dryness. The residue was diluted with chloroform (to 1 ml). The chloroform extracts and mycotoxin standards (5 µl of 1 $\mu g ml^{-1}$ concentration of B1, B2, G1 and G2 aflatoxins and OTA standards [Sigma-Aldrich, Germany]) were spotted onto TLC plates (20×20 cm MERCK aluminium sheets coated with 0/25-mm layer thickness of silica gel G). The chromatogram was obtained at room temperature (25ºC) in an unsaturated chamber containing a solvent system composed of toluene, ethyl acetate and formic acid (50:40:10). Visualization was performed under UV light at 365 nm (Scott et al. 1970, Thrane, 1986 and Van Egmond, 1981).

RESULTS AND DISCUSSION

The results of microbial analysis including TAM, coliform, sulphite-reducing clostridia, mold and yeast counts, *E. coli* and *Salmonella* spp. from 36 samples of Razavi Khorasan hot red pepper are described in Table 2. These results show that all collected samples were contaminated with both fungi and bacteria species.

Bacterial Contamination

Total Aerobic Mesophiles

All Razavi Khorasan hot red pepper samples were contaminated with total aerobic mesophiles (TAM). The average count was 4.55×10^5 cfu g^{-1} (10^3- 4×10^6 cfu g^{-1}, Table 2). TAM counts were greater than 10^6 cfu g^{-1} in approximately 8% of the samples whilst levels in the remainder were between 10^3 and 10^6 cfu g^{-1} (Table 3). Generally, in spices, TAM equal to or more than 10^6 cfu g^{-1} is not acceptable based on the international commission on microbiological specification for food (ICMSF, 2005). The high TAM count found in our samples may reflect poor handling, inappropriate drying conditions or just a general lack of hygiene (Gillespie *et al*., 2000; Richardson and Stevens, 2003). Based on Recommendation 2004/24/EC and European Spice Association (ESA) specifications, 33 out of 36 samples were satisfactory, i.e. were of an acceptable microbiological quality, but three (ca. 8%) were unsatisfactory due to high levels of TAM contamination (Table 4).

High incidences and numbers of TAM could be considered part of the normal flora or exposure to the environment pre- and post-harvesting. It has been reported that microbial counts vary according to the region, year of production, harvest month and storage conditions prior to drying. Thus, the observed TAM counts are likely to reflect the original bio-load during growth (Farkas *et al*., 2000). These findings conform to existing literature (King *et al*., 1981; Seenappa and Kempton, 1981; Banerjee and Sarkar, 2003), which reported high microbial counts on dried spices of export quality due to preparation methods and handling. Some reports suggest that black pepper contains TAMB ca. greater than *7 log CFU* g^{-1} (Banerjee and Sarkar, 2003; Baxter and Holzapfel, 1982; Christensen *et al*., 1967; Geeta and Kulkarni, 1987; Julseth and Deibel, 1974; Kneifel and Berger, 1994) compared with *5.65 log CFU* g^{-1} in Razavi Khorasan hot red pepper samples from this study.

Coliform

Coliforms were found at high levels in Razavi Khorasan hot red pepper samples

Table 2. Microbiological counts of Iranian red pepper samples.

Samples	TAM [a]	Coliform	*E. coli*	SRC [b]	Salmonella	Mold/Yeast
1	1×10^3	2.85×10^3	–	$< 10^2$	–	4.5×10^5
2	2.75×10^3	5.97×10^3	–	$< 10^2$	–	8.4×10^5
3	7×10^3	2.74×10^4	+	$< 10^2$	–	9×10^4
4	1.9×10^3	7.7×10^5	–	$< 10^2$	–	3.2×10^5
5	3.47×10^5	6.137×10^4	+	$< 10^2$	–	3.48×10^5
6	4×10^6	3.52×10^6	–	$< 10^2$	–	4×10^6
7	1.425×10^4	9.3×10^4	+	$< 10^2$	–	3.24×10^6
8	1.02×10^4	4×10^2	+	$< 10^2$	–	1.52×10^4
9	2.5×10^3	9×10^2	–	$< 10^2$	–	2.4×10^3
10	8.5×10^5	8.1×10^3	+	$< 10^2$	–	2.17×10^5
11	5.85×10^3	2×10^2	–	$< 10^2$	–	4.3×10^5
12	1.16×10^4	2×10^3	–	$< 10^2$	–	2.6×10^5
13	9.45×10^4	1.74×10^4	+	$< 10^2$	–	6.9×10^3
14	2.12×10^5	7×10^3	–	$< 10^2$	–	4×10^5
15	1.81×10^4	2.14×10^5	–	$< 10^2$	–	5.1×10^5
16	6.7×10^4	1.41×10^4	–	$< 10^2$	–	8.5×10^3
17	8.5×10^4	1.2×10^5	+	$< 10^2$	–	8.5×10^5
18	3.76×10^6	2.25×10^6	+	$< 10^2$	–	9.35×10^3
19	4.75×10^5	6.95×10^5	–	$< 10^2$	–	1.23×10^5
20	4×10^5	7.7×10^5	+	$< 10^2$	–	8.9×10^4
21	1.22×10^3	2.05×10^3	–	$< 10^2$	–	1.6×10^5
22	1.6×10^3	2.6×10^3	–	$< 10^2$	–	2.3×10^5
23	2.2×10^3	1.25×10^4	+	$< 10^2$	–	2.75×10^5
24	2.4×10^3	1.69×10^4	–	$< 10^2$	–	3.1×10^5
25	2.8×10^5	3.12×10^6	–	$< 10^2$	–	4.6×10^6
26	3.9×10^6	2.5×10^4	+	$< 10^2$	–	3.1×10^6
27	1.2×10^4	1.9×10^2	–	$< 10^2$	–	2.12×10^4
28	1.5×10^4	4.3×10^2	–	$< 10^2$	–	2.8×10^3
29	6.81×10^3	6.15×10^3	+	$< 10^2$	–	3.6×10^5
30	4.25×10^5	8.5×10^2	–	$< 10^2$	–	4.12×10^5
31	1.64×10^4	7.2×10^3	+	$< 10^2$	–	4.4×10^5
32	1.1×10^4	6.6×10^4	+	$< 10^2$	–	6.5×10^3
33	4.5×10^5	7.9×10^3	–	$< 10^2$	–	4.7×10^5
34	8.8×10^4	1.45×10^5	–	$< 10^2$	–	7.85×10^5
35	7.53×10^4	9×10^4	+	$< 10^2$	–	8.74×10^3
36	7×10^5	2.6×10^5	–	$< 10^2$	–	9×10^3

[a] total aerobic mesophiles, [b] sulphite-reducing clostridia

Table 3. Distribution of the contamination levels among the red pepper samples.

Microorganism	$< 10^2$	$10^2 - 10^3$	$10^3 - 10^4$	$10^4 - 10^5$	$10^5 - 10^6$	$> 10^6$
TAM [a]	0	0	11(30.55%)	13(36.11%)	9(25%)	3(8.34%)
Coliform	0	6(16.66%)	10(27.78%)	10(27.78%)	7(19.44%)	3(8.34%)
SRC [b]	36(100%)	0	0	0	0	0
Mold/Yeast	0	0	8(22.22%)	4(11.11%)	20(55.56%)	4(11.11%)

[a] total aerobic mesophiles, [b] sulphite-reducing clostridia

with a mean count of 3.43×10^5 (1.9×10^2 to 3.52×10^6 cfu g^{-1}, Table 2). In ca. 16% of samples coliform counts were less than 10^3 cfu g^{-1}, but 83% of samples were between 10^3 and10^6 cfu g^{-1}. Thus, according to Recommendation 2004/24/EC and the ESA specifications, 30 (out of 36) samples were unsatisfactory due to high levels of microbial contamination (Table 4). In spices, coliforms are ubiquitous, but suggest faecal

Table 4. Microbiological quality of Iranian red pepper spice.

Microorganism	No. of samples examined	No. of samples contaminated	Analysis results		
			Acceptable levels	No. of satisfactory samples	No. of unsatisfactory samples
TAM	36	36	$5\times10^5 - 10^6$	31(86.12%)	3(8.34%)
Coliform	36	36	10^3	6(16.67%)	30(83.34%)
E.coli	36	15	Not detected	21(58.34%)	15(41.67%)
SRC	36	0	$10^2 - < 10^3$	36(100%)	0
Mold/Yeast	36	36	$5\times10^3 - 10^4$	2(5.56%)	28(77.78%)
Salmonella	36	0	Not detected in 25 g	36(100%)	0

contamination pre- or post-harvesting (Kneifel *et al*., 1994).

E. coli

Almost half of the samples (ca. 42%) were positive for *E. coli*, and thus of unsatisfactory quality (Tables 2 and 4). The presence of indicators of faecal contamination, such as *E. coli*, is important, and other studies have reported *E. coli* in a wide range of spices (Schwab *et al*., 1982; De Boer *et al*., 1985; McKee, 1995; Garcia *et al*., 2001; Banerjee & Sarkar, 2003; Sagoo *et al*., 2009).

SRC (Sulphite-reducing Clostridia)

In this study, sulphite-reducing *Clostridia* spp. (SRC) levels were below detection limits, fewer than 100 cfu g^{-1}, suggesting that all 36 samples were of satisfactory quality (Tables 2-4) according to Recommendation 2004/24/EC and the ESA specifications. Low levels of *Clostridia* spp. contamination may be because of the low water content of spices that is not conducive to growth of this microorganism. However, spores from *Clostridia* spp. may survive cooking and multiply in foods held at room temperatures, increasing the risk of food poisoning if foods containing these spices are not properly stored and cooked prior to consumption.

***Salmonella* spp.**

No *Salmonella* spp. was detected in any of the samples (Table 2). Recommendation directive 2004/24/EC and ESA specifications clearly state that *Salmonella* spp. should be absent in all 25 g portions of spices meaning all the Razavi Khorasan hot red pepper samples were of a satisfactory quality (Table 4). It is most likely that the absence of *Salmonella* spp. count can be attributed to the nature of spice products**.** Our results are consistent with others from the literature, which suggest *Salmonella* spp. is uncommon in spices (Abou Donia, 2008; Garcia *et al*., 2001; Julseth and Deibel, 1974) although Banerjee and Sarkar (2003) found two positive samples in their study. Sospedra *et al.* (2010) analyzed the microbial quality of 53 spice samples, and found contamination with mesophilic aerobic counts (10%) and *Enterobacteriaceae* (20%) as well as pathogenic microorganisms like *Staphylococcus aureus*, *Yersinia intermedia*, *Shigella* spp., *Enterobacter* spp., *Acinetobacter calcoaceticus* and *Hafni alvei*. Contaminated spices have been responsible for outbreaks of salmonellosis making the presence of *Salmonella* spp. of particular concern in spices added to ready-to-eat foods (Vij *et al*., 2006; Lehmacher *et al*., 1995; Gustavsen & Breen, 1984).

Fungal Contamination, and Aflatoxins and Ochratoxin A Production

Fungi

Fungal populations obtained from the Razavi Khorasan hot red pepper samples are shown in Table 2. Generally, mold and yeast counts were high, ranging from 2.4×10^3 to 4.6×10^6 cfu g^{-1}. The average number of colonies per gram, in all 36 samples, was 6.5×10^5 cfu g^{-1}. In spices, mold and yeast counts greater than 10^4 cfu $g^{-1}$are unsatisfactory according to the international commission on microbiological specification for food. Thus, only two samples (ca. 6%) were satisfactory whilst six (ca. 17%) were contaminated but of sufficient microbiological quality to be consumed and the remaining 28 (ca. 78%) were unsatisfactory (Table 4).

Our findings agree with other studies that state mold and yeasts are present in spices (Baxter & Holzapfel, 1982; Freire and Offord, 2002; Kneifel and Berger, 1994). Mold and yeast are common in all types of spices, but mold contamination is more frequent (Kneifel and Berger, 1994) and are generally recognized as spoilage organisms in spices. Spices are known as a major source of mold contamination in meat products, and the presence of molds is important since their survival, or the presence of fungal toxins, following cooking may cause food poisoning or deterioration of valuable food products (Christensen *et al*., 1967).

Occurrence of Aflatoxins and Ochratoxin A

Samples of Razavi Khorasan hot red pepper contaminated with *Aspergillus* and *Penicillium* species were analyzed for aflatoxins and ochratoxin A. The method used does not provide definitive proof of the identity of mycotoxins, but offers a qualitative assessment as to their presence that can be used to indicate specific methods for subsequent quantitative analysis.

Analysis of 36 Razavi Khorasan hot red pepper samples for naturally-occurring mycotoxins showed 25 samples (ca. 70%) were contaminated with aflatoxins. Aflatoxin B_1 and B_2 were found in nine (of 36) samples (25%). Aflatoxin G_1 and G_2 were not detected. Six samples (17%) were contaminated with ochratoxin A (Figure1).

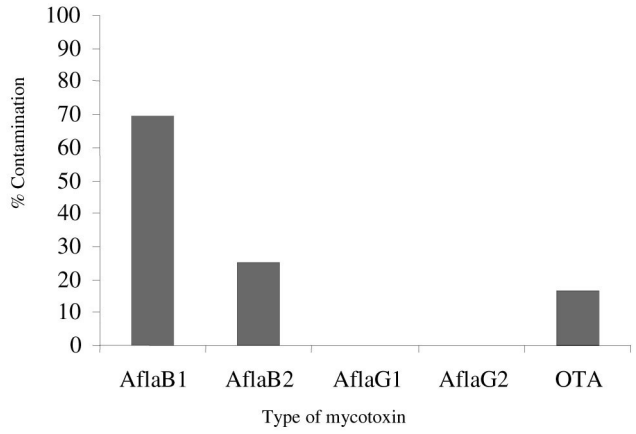

Figure 1. Percentages of aflatoxins and ochratoxin A present in 36 red pepper samples: (AflaB1) Aflatoxin B1; (AflaB2) Aflatoxin B2; (AflaG1) Aflatoxin G1; (AflaG2) Aflatoxin G2, (OTA) Ochratoxin A.

These results suggest that mycotoxins contamination is a genuine problem where spices are stored for prolonged periods, without adequate temperature and moisture controls, which renders spices susceptible to mold growth and mycotoxins production (Bugno *et al*., 2006). Rani and Singh (1990) found that 89% of their fennel, coriander and cumin samples were contaminated with aflatoxin B_1 at 3000 ppb, 1640 ppb and 1580 ppb, respectively. Similarly, Roy *et al*. (1988) and Roy and Chourasia (1990) determined that the seeds of *Piper nigrum* and *Mucuna prurians*, and the barks of *Acacia catechu*, *Coriandrum sativum* and *Elettaria cardamomun* were contaminated with aflatoxin B_1 at or below 20 µg kg^{-1}. Results similar to those described in this study have also been reported by El-Kady *et al*. (1995). Most of the molds identified have been reported to produce mycotoxins, and mycotoxins may be produced on plants in the field before harvest or later, post-harvest and during storage (Gedek, 1985).

CONCLUSIONS

Razavi Khorasan hot red pepper samples analyzed in this study contained a high number and wide range of microorganisms with only two (ca. 6%) fit-for-purpose/safe for human consumption. These results suggest sanitary conditions at different stages in production must be improved to reduce the potential hazard to human health. However, it is difficult to select a single microbial index for the determination of quality because herbs and spices are used as ingredients in a variety of products prepared in different ways. The need to provide control mechanisms and establish best practice and to improve the quality and safety of spices, means more studies are needed, particularly to determine effective methods of decontamination but also safe processing methods pre- and post-harvest, and better transportation and storage.

Dried spices are used in a variety of ways by food manufacturers and caterers as well as domestic kitchens, and microbiological concerns are governed by end use. Although spices are not major sources of food-borne disease, they are nevertheless a potential hazard, particularly if spices are added at the end of cooking or to foods prepared without (further) cooking (Little *et al*., 2003). Since all spices are susceptible to a variety of microbial contamination, protection of human health lies in the application of good manufacturing and distribution practices. The Codex-Code of Hygienic Practice for spice suppliers will, if applied appropriately, minimize microbial food safety hazards (Codex Alimentarius Commission, 1995) whilst studies continue.

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ارزیابی کیفیت میکروبیولوژیکی و آلودگی مایکوتوکسینی ادویه فلفل قرمز ایرانی

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چکیده

هدف از این مطالعه ارزیابی کیفیت میکروبی فلفل قرمز ایرانی بود. میزان حضور آفلاتوکسین‌ها و اکراتوکسین A نیز در این نمونه‌ها مورد بررسی قرار گرفت. بدین منظور ۳۶ نمونه از این نوع فلفل از سطح مزرعه جمع آوری شد و در زیر نور خورشید خشک گردید. برای آزمون‌های میکروبی و شناسایی مایکوتوکسین‌ها، روش‌های تدوین شده و استاندارد مورد استفاده قرار گرفت. شمارش کلی باکتری‌های مزوفیل هوازی در نمونه‌ها از $۱۰^{۲}$ تا $۱۰^{۲}*۴$ کلنی بر گرم متغیر بود. کلیفرم‌ها در تمامی نمونه‌ها در مقادیر بالایی بین $۱۰^{۲}*۱/۹$ کلنی تا $۱۰^{۲}*۳/۵۲$ کلنی بر گرم حضور داشتند که می‌تواند نشان دهنده کیفیت بهداشتی نامناسب نمونه‌ها باشد. ۴۱/۶۷ درصد از نمونه‌ها به دلیل حضور اشرشیا کلی در محدوده غیرقابل قبول قرار داشتند. کلستریدیوم احیا کننده سولفیت در تمامی نمونه‌ها پایین تر از حدود مشخص شده بود و سالمونلا نیز شناسایی نشد. رشد قارچ‌ها در تمامی نمونه‌ها مشاهده شد. تعداد کلنی کپک و مخمر در یک گرم نمونه عموماً بالا و در محدوده $۱۰^{۲}*۲/۴$ تا $۱۰^{۲}*۴/۶$ به دست آمد. بر اساس نتایج به دست آمده، نمونه های موررد بررسی حاوی سطوح بالایی از میکروارگانیسم‌ها بوده و بر طبق نظریه اتحادیه اروپا، تنها ۲ نمونه (۵/۵۶٪) از نظر تمامی فاکتورهای میکروبی دارای حدود قابل قبول بودند و ۹۴/۴۴ درصد از نمونه‌ها در محدوده غیر قابل قبول قرار داشتند. این نتایج نشان دهنده کیفیت پایین میکروبی نمونه‌ها می باشد که سوالاتی را از نقطه نظر شرایط بهداشتی آنها در مراحل برداشت، خشک کردن و جابجایی به دنبال خواهد داشت. عمده جنس‌های قارچی شناسایی شده آسپرژیلوس و پنی سیلیوم بودند. همچنین آلودگی به آفلاتوکسین‌ها و اکراتوکسین Aبه ترتیب در ۶۹/۴٪ و ۱۶/۷٪ از نمونه‌ها مشاهده شد که ممکن است مخاطراتی را برای سلامتی انسان‌ها به دنبال داشته باشد.