Rev Argent Microbiol. 2017;49(4):339–346

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

Microbiological quality of Argentinian paprika

María G. Melo González[a], **Stella M. Romero**[b,*], **Mila Arjona**[c], **Ada G. Larumbe**[b], **Graciela Vaamonde**[b]

[a] *Facultad de Ciencias de la Salud, Universidad Nacional de Catamarca, Av. Belgrano 300, (K4700AAP), Catamarca, Argentina*
[b] *Instituto de Micología y Botánica (INMIBO), Universidad de Buenos Aires-CONICET, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Pabellón II, 4to piso (C1428EGA), Buenos Aires, Argentina*
[c] *Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, Centro de Biología Molecular y Biotecnología (CEBIOTEC), Universidad Nacional de Catamarca, Av. Belgrano 300, (K4700AAP), Catamarca, Argentina*

Received 27 November 2016; accepted 21 February 2017
Available online 29 May 2017

KEYWORDS
Paprika;
Microbial contamination;
Toxigenic fungi;
Mycobiota

Abstract The aim of this study was to evaluate the microbiological quality of paprika produced in Catamarca, Argentina. Microbiological analyses were carried out for the enumeration of total aerobic mesophilic bacteria, coliforms, yeasts and molds, and the detection of *Salmonella* in samples obtained from different local producers during three consecutive years. The mycobiota was identified paying special attention to the mycotoxigenic molds. Standard plate counts of aerobic mesophilic bacteria ranged from 2.7×10^5 to 3.7×10^7 CFU/g. Coliform counts ranged from <10 to 8.1×10^4 CFU/g. *Salmonella* was not detected in any of the samples tested. Fungal counts (including yeasts and molds) ranged between 2×10^2 and 1.9×10^5 CFU/g. These results showed a high level of microbial contamination, exceeding in several samples the maximum limits set in international food regulations. The study of the mycobiota demonstrated that *Aspergillus* was the predominant genus and *Aspergillus niger* (potential producer of ochratoxin A) the most frequently isolated species, followed by *Aspergillus flavus* (potential producer of aflatoxins). Other species of potential toxigenic fungi such as *Aspergillus ochraceus*, *Aspergillus westerdijkiae*, *Penicillium chrysogenum*, *Penicillium crustosum*, *Penicillium commune*, *Penicillium expansum* and *Alternaria tenuissima* species group were encountered as part of the mycobiota of the paprika samples indicating a risk of mycotoxin contamination. *A. westerdijkiae* was isolated for the first time in Argentina.

* Corresponding author.
E-mail address: smromero@conicet.gov.ar (S.M. Romero).

http://dx.doi.org/10.1016/j.ram.2017.02.006

PALABRAS CLAVE
Pimentón;
Contaminación microbiana;
Hongos toxinógenos;
Micobiota

Calidad microbiológica del pimentón argentino

Resumen El pimentón es considerado una de las especias más proclives a contaminarse con diversos tipos de microorganismos, incluyendo patógenos como *Salmonella* y hongos capaces de producir micotoxinas. Existen muy pocos datos acerca de la contaminación microbiana del pimentón producido en nuestro país. El objetivo del presente trabajo fue evaluar la calidad microbiológica del pimentón *(Capsicum annum* L.) producido en la provincia de Catamarca, una de las principales zonas productoras del norte argentino. Se realizó el recuento de bacterias aerobias mesófilas, coliformes totales y mohos y levaduras, y la búsqueda de *Salmonella* en muestras obtenidas de diferentes establecimientos productores locales durante 3 años consecutivos. Se identificaron todas las cepas fúngicas (1.622 aislamientos) a nivel de género y se determinaron las especies pertenecientes a los géneros potencialmente toxinógenos. Los recuentos totales de bacterias aerobias mesófilas variaron entre $2,7 \times 10^5$ y $3,7 \times 10^7$ UFC/g. Los coliformes totales estuvieron en el rango de < 10 a $8,1 \times 10^4$ UFC/g. *Salmonella* no fue detectada en ninguna de las muestras analizadas. Los resultados obtenidos muestran un alto nivel de contaminación, que excede en varias de las muestras los límites máximos establecidos en las regulaciones alimentarias internacionales. El estudio de la micobiota demostró que *Aspergillus* fue el género predominante. Otros géneros encontrados fueron *Cladosporium*, *Rhizopus*, *Alternaria* y *Penicillium*. *Aspergillus niger* (potencial productor de ocratoxina A) fue la especie aislada con mayor frecuencia, seguida de *Aspergillus flavus* (potencial productor de aflatoxinas). También se encontraron otras especies toxinógenas, lo que indica un riesgo potencial de contaminación con micotoxinas. *Aspergillus westerdijkiae* fue aislado por primera vez en Argentina.

Introduction

Spices and herbs have been used for centuries for the aroma and flavor characteristics they convey to foods. The increasing popularity of highly spiced cuisines as well as a desire for flavorful foods which are low in sodium and fat have resulted in a continuing interest in the use of spices and herbs in food products[29]. Paprika (in Spanish referred to as ''pimentón'') is a powdered spice with a deep orange-red color and a characteristic non-pungent flavor resulting from the dried and ground fruits of certain varieties of pepper (*Capsicum annuum* L. belonging to the family Solanaceae). Microbiological studies carried out with species, including paprika, have indicated high microbial loads which could pose a problem for food manufacturers [4,7,9,25,29,31,33,39]. These commodities normally carry a great number of bacteria and molds, often of soil origin, and could be a major source of microbial contamination in foods. Current practices of harvesting, handling and production often cause additional contamination and microbial growth. Many spices are grown and harvested in poor sanitary conditions, which increase the risk of contamination even with pathogens such as *Salmonella*. A significant outbreak of human salmonellosis due to paprika and paprika-powdered potato chips was described and well documented in Germany in 1995[26]. Owing to production conditions and poor storage practices, products derived from *Capsicum* are also susceptible to fungal contamination. Spoilage caused by fungi decreases the quality of the products and also imply a risk for health due to potential contamination with different mycotoxins. Toxigenic molds such as *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. have been detected by several researchers in the mycobiota of *Capsicum* powder as well as natural contamination with aflatoxins and ochratoxin A[16,19,23,28,42,43,45].

In Argentina, paprika production is a regional industry of increasing importance in some northern provinces (Catamarca, Salta and Tucumán). This study aimed to determine the microbiological quality of paprika from this region, paying a special attention to pathogenic microorganisms such as *Salmonella* and mycotoxigenic fungi.

Materials and methods

Samples

Fifteen samples of paprika (*Capsicum annuum* L.) from Santa María Department, Catamarca Province, Argentina, belonging to 2010, 2011 and 2012 consecutive harvests were analyzed. These samples were obtained from different local producers. For each sample, 250 g of paprika were collected in sterile containers; all samples were kept at 5 °C until analysis.

Water activity

Water activity was measured with a water activity meter (Aqualab, Decagon Devices CX3 02734) with an accuracy of ±0.002. Measurements were performed in duplicate.

Aerobic plate count (APC)

One gram of paprika was homogenized in 9 ml of 0.1% peptone water in an Erlenmeyer flask. Subsequent decimal dilutions were prepared in sterile peptone water. One ml of each dilution was plated by the pour plate method with agar plate count, in duplicate. The plates were incubated at 35 °C for 48 h[5].

Total coliform count

One ml of subsequent decimal dilutions up to 1:1000 (previously prepared for the APC) was transferred to Petri dishes in duplicate. Ten ml of Violet Red Bile Agar (VRBA) was poured, swirled to mix and let to solidify. Then, it was overlaid with 10 ml of melted medium. The plates were incubated at 35 °C for 24 h. Purple-red colonies (0.5 mm or larger in diameter) surrounded by a zone of precipitated bile acids were counted. To confirm that the colonies were coliforms, 10 representative colonies were picked and each of them were transferred to a tube of Brilliant Green Lactose Bile Broth. The tubes were incubated at 35 °C and were examined at 24 and 48 h for gas production[5].

Salmonella spp.

Salmonella was investigated according to the reference method for paprika[5]. For the pre-enrichment, 25 g of paprika were added to 225 ml of Trypticase Soy Broth (TSB) and incubated at 35 °C for 24 h. The enrichment step was performed on selenite cystine broth and tetrathionate broth, and were incubated at 35 °C for 24 h. Isolations were examined on Bismuth Sulfite Agar (BSA) and Xylose Lysine Desoxycholate Agar (XLD), after incubation at 35 °C for 48 and 24 h, respectively. Suspected colonies of *Salmonella* pp. were tested on Triple Sugar Iron (TSI) and Lysine Iron (LIA) agar, incubated at 35 °C for 24 h. Colonies exhibiting typical reactions on TSI and LIA were purified and further characterized by traditional assays: urease, oxidase, phenylalanine descarboxylase, Voges-Proskauer, indole and citrate.

Mold and yeast counts

Mold and yeast counts were performed on dichloran 18% glycerol (DG18) agar, a medium that has lower water activity ($a_w = 0.955$) and favors xerophilic fungi development[36]. Plates were incubated at 25 °C for 5 days.

Mold identification

Preliminary characterization at genera level of the isolated strains was performed according to Pitt and Hocking[36]. The strains belonging to genera producers of mycotoxins were identified to species level.

Isolates of *Aspergillus* and *Penicillium* were plated on Malt Extract Agar (MEA) and were incubated for 7 days at 25 °C to obtain well-sporulated cultures. The spores of each strain were collected and placed in 1 ml of a sterile aqueous solution with 0.05% Tween 80 and 0.2% agar. *Aspergillus* strains were cultured in MEA and 25% glycerol nitrate agar (G25N) and incubated at 25 °C, and in Czapek Yeast Extract Agar (CYA) incubated at 25 °C and 37 °C. All plates were incubated in darkness for a standard time of 7 days for anamorphic species and 14 days for teleomorphic species. Taxonomic identification was done according to Klich[24] and Pitt and Hocking[36]. *Penicillium* strains were cultured in MEA and Czapek Agar (CZ) and incubated at 25 °C, and in CYA at 5, 25 and 37 °C for 7 days. Identification was done according to Pitt[35], Samson et al.[40] and Samson and Frisvad[41].

Alternaria strains were cultured in Potato Dextrose Agar (PDA) and incubated at 25 °C for 5–7 days. Strains were subcultured on tap water agar 18% (TWA) and incubated at 25 °C for 7–14 days under lights with a 12 h-photoperiod. Taxonomic identification was performed according to Simmons and Roberts[47] and Simmons[46].

Results and discussion

Table 1 shows the results of the aerobic plate count, total coliforms and water activity of the samples analyzed in the present study. Water activity of the samples showed levels between 0.299 and 0.534. Fernández-Trujillo and Escarabajal[11] reported and recommended a range between 0.5 and 0.7 during storage of paprika from Murcia, with an optimal level of $a_w = 0.5$ to prevent product caking.

The standard plate counts of aerobic mesophilic bacteria showed high levels of contamination in the samples, ranging from 2.7×10^5 to 3.7×10^7 CFU/g. Several other studies also reported high microbial loads in herbs and species according to Mc Kee's review[29]. Surveys conducted in different countries determined that aerobic mesophilic bacterial plate counts ranged from several hundreds to several millions per gram. High counts were observed for all types of spices, being paprika one of the most contaminated, often with values greater than 7 log CFU/g. The results of surveys conducted more recently revealed similarly high microbial loads in paprika[4,7]. Although microbiological criteria for spices have been recommended, few specific standards have been suggested in some countries[6,25]. Argentinian food regulations do not include microbiological specifications for paprika or other species. The International Commission on Microbiological Specifications for Foods (ICMSF)[21] set up a maximum limit of 10^6 CFU of total aerobic mesophilic bacteria/g of spice and 10^4 CFU/g as the bacterial counts that distinguish good from marginal quality. A total aerobic mesophilic count of $\leq 10^4$ CFU/g is of acceptable quality and 10^4–10^6 CFU/g is of marginal quality. Values above 10^6 are unacceptable. Applying these criteria, high rejection rates have been mentioned by several authors. Schwab et al.[44] informed that 44% of the samples of paprika analyzed would not meet the ICMSF standard. Banerjee and Sakar[7] reported that 51% of the samples of some spices marketed in India were in the unacceptable range ($\geq 10^6$ CFU/g). A similar proportion of unacceptable samples was detected in the present study. On the other hand, results reported by Garbowska et al.[14] report good hygienic conditions in the production process of spices and herbs available in the Polish market since 60% of the analyzed samples did not exceed 10^4 CFU/g whereas the level regarded as unacceptable ($\geq 10^6$ CFU/g) was not identified in any of the samples. However, most of the results published up to now confirm

Table 1 Total aerobic plate and total coliform counts (CFU/g), and a_w levels of paprika samples from Santa Maria Department from Catamarca, Argentina

Samples	Year	Aerobic plate count (CFU/g)	Total coliform count (CFU/g)	a_w
1	2010	2.7×10^7	<10	0.424
2	2010	5.7×10^6	<10	0.488
3	2010	7.9×10^6	4.0×10^2	0.325
4	2010	3.7×10^7	1.8×10^2	0.534
5	2011	8.8×10^5	7.3×10^3	0.350
6	2011	1.1×10^7	$>5.6 \times 10^4$	0.517
7	2011	2.7×10^5	2.9×10^4	0.371
8	2011	5.0×10^5	4.4×10^3	0.374
9	2011	1.2×10^6	1.8×10^2	0.346
10	2011	1.3×10^6	1.2×10^3	0.422
11	2012	7.4×10^5	5.0×10^2	0.299
12	2012	5.6×10^6	5.6×10^2	0.423
13	2012	1.1×10^6	8.1×10^4	0.413
14	2012	9.1×10^5	<10	0.413
15	2012	5.7×10^6	1.7×10^3	0.374

the poor hygienic quality of spices, including paprika, produced around the world [15,18,31,39,48]. In the second edition of the ICMSF book on sampling for microbiological analysis[22] the Commision accepted that, in retrospect, the above mentioned recommendations for spices were inappropriate because a considerable portion of these commodities in international commerce would not meet the suggested limits. Furthermore, failure to meet the limits might or might not be related to food quality or safety. The new recommendation was that spices be treated as raw agriculture commodities and, accordingly, the ultimate use of such products will be determinant. However, the original ICMSF specifications are still considered a guide to evaluate the microbiological quality of spices and herbs in international trade.

Gallardo Guerrero et al.[13] studied the evolution of the microflora during dehydration of red pepper fruits during a period of 9 days at temperatures ranging between 30 and 40 °C to obtain a high quality paprika protected by PDO (Product with Designation of Origin: ''Pimentón de La Vera''). Initial counts of total mesophilic aerobic bacteria ranging between 8 and 11 log CFU/g tended to decrease in the middle stages of processing and increased again at the end. These authors attribute such high microbial levels to soil pollution, the environment and feces of birds and other animals contaminating the red pepper fruits either during cultivation, their time in the dryer or handling operations. Similar sources of contamination, characteristic of handcrafted produced products, could be present in the region considered in the present work.

Total coliforms are used generally as indicators of hygienic quality. Coliform counts in the samples ranged from <10 to 8.1×10^4 (Table 1). A few samples exceeded the maximum count set by the ICMSF for this group of microorganisms (10^4 CFU/g). These results agree with those of other authors[7,44].

Salmonella spp. is a pathogen of greatest concern in spices due to its ability to persist in low a_w environments and low dose for infection. In the present study *Salmonella* spp. was not detected in any of the samples tested. Other surveys also revealed that this pathogen was not present in spices[7–9]. However, Vij et al.[49] reviewed spice recalls that took place in the United States from 1970 to 2004 involving 12 spice types contaminated with bacterial pathogens and reported that in all but one instance the recalled spices contained *Salmonella spp.*. Paprika was the spice most often involved in the recalls. Furthermore, different spices were identified as the contaminated food vehicle in several outbreaks of salmonellosis[33]. One of the most widespread outbreaks associated with contaminated paprika used to season potato chips ocurred in Germany in 1993[26]. Some, if not all, of the paprika was imported from South America. There were an estimated 1000 cases of illness, mostly affecting children younger than 14 years of age. The estimated infectious dose in that outbreak was reported to be 4–45 salmonellae. The high fat content associated with the paprika-powdered chips may have protected *Salmonella* spp. from gastric acid in the stomach, resulting in the low infectious dose. It is important to note that many spices are frequently used in ready-to-eat foods (e.g. crackers and chips) or in cooked foods that are seasoned before consumption increasing the risk of foodborne disease. Although it could be apparent that *Salmonella* spp. contamination is rare or sporadic in spices, including paprika, this pathogen is of great concern and should be included in any sampling plan to monitor the microbiological quality of these products. Other pathogens such as *Bacillus cereus* and *Clostridium perfringens* seem to be rarely detected.

Table 2 shows the total molds and yeast counts and the percentage of molds detected in the samples. In most of the samples (10/15) molds account for more than 80% of the fungal load. However, yeasts were present in high numbers in some samples and they were absent in some others. Santos et al.[42] reported that predominant contamination of *Capsicum* products produced in Spain was caused by yeasts. Baxter and Holzapfel[8] analyzed selected spices and herbs in South Africa and also found that paprika contained a high number of viable yeasts. In the present study, fungal

Table 2 Mold and yeast count (CFU/g) and percentage of molds detected in paprika samples from Santa Maria Department from Catamarca, Argentina

Samples	Year	Molds and yeast (CFU/g)	Molds (CFU/g)	Molds (%)
1	2010	2.4×10^4	2.3×10^4	95.8
2	2010	2.6×10^3	2.4×10^3	92
3	2010	1.9×10^5	1.2×10^4	6
4	2010	1.1×10^4	3.3×10^3	30
5	2011	1.5×10^4	1.5×10^4	100
6	2011	4.0×10^3	3.4×10^3	85
7	2011	1.2×10^3	1.1×10^3	92
8	2011	1.7×10^5	8.8×10^3	5
9	2011	2.3×10^4	2.1×10^4	91
10	2011	2.0×10^2	2.0×10^2	100
11	2012	4.2×10^4	4.2×10^4	100
12	2012	1.2×10^4	2.1×10^3	17.5
13	2012	6.6×10^4	4.2×10^4	64
14	2012	1.6×10^3	1.3×10^3	81
15	2012	4.3×10^2	4.3×10^2	100

counts (including molds and yeasts) ranged between 2×10^2 and 1.9×10^5 CFU/g. These results are consistent with other studies[29]. Santos et al.[42] reported 2.3×10^4 mean total CFU/g of paprika in MEA and 3.8×10^2 CFU/g of paprika in DG18. The low a_w of this media, also used in the present work for fungal counts, mainly selects xerophilic species, being suitable for dried foods. The ICMSF[21] set up a maximum limit of 10^4 CFU of molds and yeasts/g of spices. In the present study six samples had acceptable counts, other six showed slightly higher levels while the other three reached the 10^5 level. A survey carried out in Australia in order to develop a database for unacceptable levels of fungal contamination of foods provided some information on species. Specifications for less than 1000 yeasts and molds/g were informed by most of the Australian food manufacturers. Some of them included a marginally acceptable category which allowed up to 2000 fungi/g provided there was no organoleptic deterioration of the raw material. A few companies set the unacceptable level at 10^4 or 10^5 CFU/g. The variation in acceptable levels by the different organizations is a reflection of the different end uses of the spices[3].

The study of molds in spices and herbs is of significance because the quality of the products has decreased as a consequence of fungal spoilage and there is also a health risk due to the potential production of mycotoxins. In the present work, the mycobiota of paprika was identified paying special attention to the mycotoxigenic molds. A total of 1622 isolates of molds were recovered from samples by the dilution plate method. All colonies were subcultured for taxonomic identification. Seven genus were identified and the percentage of each genus over the total number of isolates was calculated for each sample (Table 3). *Aspergillus* was prevalent since it was present in all but one sample and accounted for more than 50% of the mycobiota observed in most of the samples. Other genera widely distributed were *Penicillium* and *Alternaria*. *Rhizopus* was abundant in some of the samples, making it difficult to isolate the rest of the fungi. *Cladosporium, Paecilomyces* and *Fusarium* were less frequently isolated.

Strains belonging to mycotoxigenic genera *Aspergillus, Penicillium* and *Alternaria* were identified at species level. Table 4 shows the distribution of these fungal species among the samples analyzed. Due to the new single name nomenclature for fungi[30], Hubka et al.[20] provided a necessary nomenclatural revision and transferred all *Eurotium* species to *Aspergillus*. These changes were incorporated into the present study. *Aspergillus montevidensis, Aspergillus chevalieri, Aspergillus glaucus* and *Aspergillus ruber*, formerly considered to be *Eurotium* species, are xerophilic fungi very common in dry foods and were found in the mycobiota of *Capsicum* powder by other authors[16,42]. Furthermore, *A. montevidensis* is the current name of the well-known *Eurotium amstelodami*. The name change was due to the fact that *E. amstelodami* was an illegitimate name[20,34].

Several toxigenic species of *Aspergillus* were detected, belonging to sections *Flavi* (*Aspergillus flavus* and *Aspergillus parasiticus*), *Nigri* (*Aspergillus niger)* and *Circumdati* (*Aspergillus ochraceus* and *Aspergillus westerdijkiae*). *A. flavus*, a potential producer of type B aflatoxins and cyclopiazonic acid, was quite frequent, being isolated from five samples, whereas *A. parasiticus*, generally a strong producer of type B and G aflatoxins, was present in only one sample. *A. niger*, reported as a potential producer of ochratoxin A (OTA), was the most frequently isolated toxigenic mold, being present in all but one of the samples. The predominance of *Aspergillus* section *Nigri* in the dried spice was not unexpected because members of this group can survive the drying process due to the relative resistance of black spores to sunlight and UV radiation[38]. Another OTA producer, *Aspergillus carbonarius*, very commonly found in grape products, was not detected.

Other species capable of producing OTA were *A. ochraceus*, isolated from three samples and *A. westerdijkiae*, present in one sample and isolated in Argentina for the first time.

Table 3 Percentage of fungal genera detected in each paprika sample analyzed from Santa Maria Department from Catamarca, Argentina

Samples	*Aspergillus*	*Penicillium*	*Alternaria*	*Rhizopus*	*Cladosporium*	*Paecilomyces*	*Fusarium*	*Mycelia sterilia*
1	98.3							1.7
2	75.0		8.3	16.7				
3	62.1	12.1	15.5		5.2		3.4	1.7
4	76.7	0.6	8.0	9.2	4.3			1.2
5	79.7	6.3	2.8	9.8	0.7	0.7		
6	52.9			47.1				
7	66.7	12.5	12.5	6.3	2.1			
8	24.4	4.4	44.4		6.7		20.0	
9	6.6	9.4		0.9	0.9			82.1
10			35.0	60.0				5.0
11	97.0	1.5	1.3				0.2	
12	31.1	9.7	55.3					3.9
13	35.5	2.0	4.4					58.1
14	16.2	20.6	1.5	5.9				55.9
15	47.7	15.9	9.1	18.2				9.1

Table 4 Distribution of species belonging to mycotoxigenic genera (*Alternaria*, *Aspergillus* and *Penicillium*)

Species	Samples														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Alternaria tenuissima[a]										*					*
Aspergillus chevalieri											*				
Aspergillus flavus	*			*	*	*	*								
Aspergillus glaucus	*				*										
Aspergillus montevidensis			*												*
Aspergillus niger	*	*	*	*	*	*	*	*	*		*	*	*	*	*
Aspergillus ochraceus			*	*						*					
Aspergillus parasiticus												*			
Aspergillus restrictus															*
Aspergillus ruber			*												
Aspergillus terreus							*								
Aspergillus westerdijkiae[b]															*
Penicillium atramentosum									*						
Penicillium chrysogenum					*				*			*			*
Penicillium commune											*			*	
Penicillium crustosum			*												
Penicillium expansum			*												
Penicillium italicum			*												

[a] *Alternaria tenuissima* species group.
[b] Cited for the first time in Argentina.
* Presence of the species in the sample.

Although ochratoxin A was first described from *Aspergillus ochraceus*, molecular studies indicate that *A. westerdijkiae* is the major ochratoxin A-producing species in *Aspergillus* Section Circumdati[12]. *A. westerdijkiae* is morphologically similar to *A. ochraceus*, though it is unable to grow at 37 °C. Gil Serna et al.[17] reported that *A. westerdijkiae* achieved the highest values of both growth and OTA production in a paprika-based medium in comparison with different matrix-based media, probably due to the sugar composition of *Capsicum annuum* fruits which might positively affect OTA production by this species.

The only *Alternaria* species identified, *Alternaria tenuissima* species group, is a potential producer of tenuazonic acid, alternariol, alternariol monomethyl ether, altenuene and altertoxins. However, it was present in only two samples. Some of the *Penicillium* species listed in Table 4 are able to produce toxic secondary metabolites such as cyclopiazonic acid (*Penicillium commune*), tremorgens (*Penicillium crustosum*) and patulin and citrinin (*Penicillium expansum*), but they were sporadically isolated.

Results of the present study are supported by numerous reports which consistently indicate that *Aspergillus* is the most commonly occurring genus in *Capsicum* powder

as well as in other spices[1,9,16,27,28,42,43,45]. The same fungal genera and species isolated in the present work were isolated by Gherwaby et al.[16] from chili in Saudi Arabia, being *A. niger* and *A. flavus* the most prevalent toxigenic molds. Santos et al.[42] reported that *Aspergillus* and *Eurotium* were the predominant fungi in samples of paprika and chili produced in Spain. The most abundant species was *A. niger*. These authors also isolated *A. flavus* with relatively high frequency (60% of the paprika samples). Other *Aspergillus* species able to produce OTA (*A. carbonarius*, *A. ochraceus* and *A. westerdijkiae)* were also detected in the paprika samples but less frequently. Martín et al.[28] analyzed the fungal contamination of smoked paprika and identified *Aspergillus*, *Cladosporium*, *Penicillium* and *Fusarium* as predominant genera. Among the toxigenic species isolated they mentioned *A. niger* (the most frequent), *Fusarium verticillioides*, *Penicillium expansum* and *Penicillium citrinum*, but they did not detect any aflatoxin-producing fungi.

Table 4 shows the co-occurrence of *A. niger* and *A. flavus* in several of the analyzed samples. This finding is in agreement with other studies which report a high co-occurrence of *Aspergillus* species able to produce aflatoxins and OTA[16,43]. Although the presence of toxigenic molds in foods does not necessary imply the presence of the toxins they can produce, it may be considered as an indicator of potential contamination with mycotoxins. Numerous surveys have confirmed the natural occurrence of aflatoxins and OTA in *Capsicum* products, including paprika, from different countries[2,10,16,19,23,32,33,37,42], indicating a need to establish maximum levels for regulation.

Conclusions

From the results of the present study, it can be concluded that paprika produced in the northern region of Argentina presents a high microbial load as was expected according to the conditions of production and storage of this commodity. Sundrying of the fruits in contact with the soil is a critical step as well as the storage in environmental conditions conductive to microbial proliferation. Similar levels of microbial contamination of *Capsicum* products have been reported worldwide, indicating that a high proportion of the samples analyzed by several workers exceeded the limits established by food regulations. In order to improve the microbiological quality of paprika, good agricultural practices and good manufacturing practices should be applied throughout the supply chain. The presence of molds capable of producing mycotoxins in this product should be considered a potential hazard for public health.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We are grateful to Nilda Arapa for her technical assistance. S.M. Romero, A.G. Larumbre and G. Vaamonde are members of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina), InMiBo publication N° 215.

References

1. Adegoke GO, Allamu AE, Akingbala JO, Akanni AO. Influence of sundrying on the chemical composition, aflatoxin content and fungal counts of two pepper varieties – *Capsicum annuum* and *Capsicum frutescens*. Plant Foods Hum Nutr. 1996;49:113–7.
2. Ahn J, Kim D, Jang HS, Kim Y, Shim WB, Chung DH. Occurrence of ochratoxin A in Korean red paprika and factors to be considered in prevention strategy. Mycotoxin Res. 2010;26:279–86.
3. Andrews S. Specifications for fungi in Australian foods. In: Samson RA, Hocking AD, Pitt JI, King AD, editors. Modern methods in food mycology. Publishers BV: Elsevier Science Amsterdam; 1992. p. 97–110.
4. Aguilera MO, Stagnitta PV, Micalizzi B, de Guzmán AMS. Prevalence and characterization of *Clostridium perfringens* from spices in Argentina. Anaerobe. 2005;11:327–34.
5. American Public Health Association (APHA). Compendium of methods for the Microbiological Examination of Foods, fourth ed Washington, DC: American Public Health Association; 2001.
6. Anonymous, 2009. http://www.otcasea.gob.do/wp-content/uploads/2009/06/propuesta-regl-sanitario-alimentos-rd.pdf 08/10/2016.
7. Banerjee M, Sarkar PK. Microbiological quality of some retail spices in India. Food Res Int. 2003;36:469–74.
8. Baxter R, Holzapfel WH. A microbial investigation of selected spices, herbs, and additives in South Africa. J Food Sci. 1982;47:570–4.
9. Donia MA. Microbiological quality and aflatoxinogenesis of Egyptian spices and medicinal plants. Global Vet. 2008;2:175–81.
10. Fazekas B, Tar A, Kovacs M. Aflatoxin and ochratoxin A content of spices in Hungary. Food Addit Contam. 2005;22:856–63.
11. Fernández-Trujillo JP, Escarabajal D. El proceso tradicional de elaboración del pimentón de Murcia y sus posibles innovaciones. Grasas y Aceites. 2006;57:433–42.
12. Frisvad JC, Frank JM, Houbraken JAMP, Kuijpers AF, Samson RA. New ochratoxin A producing species of *Aspergillus* section Circumdati. Stud Mycol. 2004;50:23–43.
13. Gallardo-Guerrero L, Pérez-Gálvez A, Aranda E, Mínguez-Mosquera MI, Hornero-Méndez D. Physicochemical and microbiological characterization of the dehydration processing of red pepper fruits for paprika production. LWT-Food Sci Technol. 2010;43:1359–67.
14. Garbowska M, Berthold-Pluta A, Stasiak-Różańska L. Microbiological quality of selected spices and herbs including the presence of *Cronobacter* spp. Food Microbial. 2015;49:1–5.
15. García S, Iracheta F, Galván F, Heredia N. Microbiological survey of retail herbs and spices from Mexican markets. J Food Prot. 2001;64:99–103.
16. Gherbawy YA, Shebany YM, Hussein MA, Maghraby TA. Molecular detection of mycobiota and aflatoxin contamination of chili. Arch Biol Sci. 2015;67:223–34.
17. Gil-Serna J, Patiño B, Cortes L, Gonzalez-Jaen MT, Vazquez C. *Aspergillus steynii* and *Aspergillus westerdijkiae* as potential

risk of OTA contamination in food products in warm climates. Food Microbiol. 2015;46:168–75.
18. Hara-Kudo Y, Ohtsuka K, Onoue Y, Otomo Y, Furukawa I, Yamaji A, Segawa Y, Takatori K. *Salmonella* prevalence and total microbial and spore populations in spices imported to Japan. J Food Prot. 2006;69:2519–23.
19. Hernández Hierro JM, Garcia-Villanova RJ, Rodríguez Torrero P, Toruno Fonseca IM. Aflatoxins and ochratoxin A in red paprika for retail sale in Spain: occurrence and evaluation of a simultaneous analytical method. J Agric Food Chem. 2008;56:751–6.
20. Hubka V, Kolarik M, Kubátová A, Peterson SW. Taxonomic revision of the genus *Eurotium* and transfer of species to *Aspergillus*. Mycologia. 2013;105:912–37.
21. ICMSF (International Commission on Microbiological Specifications for Foods). Microorganisms in foods 2, sampling for microbiological analysis: principles and specific applications. first ed. Oxford: Blackwell Scientific; 1974.
22. ICSMF (International Commission on Microbiological Specifications for Foods). Microorganisms in foods 2, sampling for microbiological analysis: principles and specific applications. second ed. Oxford: Blackwell Scientific; 1986.
23. Iqbal SZ, Paterson RRM, Bhatti IA, Asi MR. Survey of aflatoxins in chilies from Pakistan produced in rural, semi-rural and urban environments. Food Addit Contam. 2010;3:268–74.
24. Klich MA. Identification of common *Aspergillus* species. first ed. Utrecht: Centraalbureau Voor Schimmelcultures; 2002.
25. Koohy-Kamaly-Dehkordy P, Nikoopour H, Siavoshi F, Koushki M, Abadi A. Microbiological quality of retail spices in Tehran, Iran. J Food Prot. 2013;76:843–8.
26. Lehmacher A, Bockemühl J, Aleksic S. Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. Epidemiol Infect. 1995;115:501–11.
27. Mandeel QA. Fungal contamination of some imported spices. Mycopathologia. 2005;159:291–8.
28. Martín A, Aranda E, Benito MJ, Pérez-Nevado F, Cordoba MG. Identification of fungal contamination and determination of mycotoxigenic molds by micellar electrokinetic capillary chromatography in smoked paprika. J Food Prot. 2005;68:815–22.
29. McKee LH. Microbial contamination of spices and herbs: a review. Food Sci Technol-LEB. 1995;28:1–11.
30. McNeill J, Barrie FR, Buck WR, DemoulinV, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold KM, Prado J, Prud'homme Van Reine WF, Smith GF, Wiersema JH. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Reg Veg. 2012;154:208.
31. Moreira PL, Lourenção TB, Pinto JPAN, Rall VLM. Microbiological quality of spices marketed in the city of Botucatu, Sao Paulo, Brazil. J Food Prot. 2009;72:421–4.
32. Mwangi WW, Nguta CM, Muriuki BG. Aflatoxin contamination in selected spice preparations in the Nyahururu retail market, Kenya. J Scientific Res Reports. 2014;3:917–23.
33. Pinkas JM, Keller SE. Spices. In: Gurtler JB, Doyle MP, Kornacki JL, editors. The microbiological safety of low water activity foods and spices. New York: Springer; 2014. p. 99–114.
34. Pitt JI. Nomenclatorial and taxonomic problems in the genus Eurotium. In: Samson RA, Pitt JI, editors. Advances in *Penicillium* and *Aspergillus* systematics. New York: Plenum Press; 1985. p. 383–96.
35. Pitt JL. A laboratory guide to common *Penicillium* species. North Ryde: CSIRO; 1988.
36. Pitt JI, Hocking AD. Fungi and food spoilage. Springer: New York; 2009.
37. Reddy SV, Kiran-Mayi D, Uma Reddy M, Thirumala-Devi K, Reddy DVR. Aflatoxins B1 in different grades of chilies (*Capsicum annum* L.) in India as determined by indirect competitive-ELISA. Food Addit Contam. 2001;18:553–8.
38. Romero SM, Comerio RM, Larumbe G, Ritieni A, Vaamonde G, Fernández Pinto V. Toxigenic fungi isolated from dried vine fruits in Argentina. Int J Food Microbiol. 2005;104:43–9.
39. Salari R, Najafi H, Boroushaki MT, Mortazavi SA, Fathi Najafi M. Assessment of the microbiological quality and mycotoxin contamination of Iranian red pepper spice. J Agric Sci Tech. 2012;14:1511–21.
40. Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. Introduction to food and airborne fungi. Utrecht: Centraalbureau Voor Schimmelcultures; 2002.
41. Samson RA, Frisvad JC. *Penicillium* subgenus *Penicillium*: new taxonomic schemes and mycotoxins and other extrolites. Stud Mycol 49, Centraalbureau Voor Schimmelcultures, Utrecht. 2004.
42. Santos L, Marín S, Mateo EM, Gil-Serna J, Valle-Algarra FM, Patiño B, Ramos AJ. Mycobiota and co-occurrence of mycotoxins in *Capsicum* powder. Int J Food Microbiol. 2011;151:270–6.
43. Sardiñas N, Gil-Serna J, Santos L, Ramos AJ, González-Jaén MT, Patiño B, Vázquez C. Detection of potentially mycotoxigenic *Aspergillus* species in *Capsicum* powder by a highly sensitive PCR-based detection method. Food Control. 2011;22:1363–6.
44. Schwab AH, Harpestad AD, Swartzentruber A, Lanier JM, Wentz BA, Duran AP, Barnard RJ, Read RB. Microbiological quality of some spices and herbs in retail markets. Appl Environ Microbiol. 1982;44:627–30.
45. Shundo L, de Almeida AP, Alaburda J, Lamardo LC, Navas SA, Ruvieri V, Sabino M. Aflatoxins and ochratoxin A in Brazilian paprika. Food Control. 2009;20:1099–102.
46. Simmons EG. *Alternaria*: an identification manual. Utrecht: CBS Fungal Biodiversity Centre; 2007.
47. Simmons EG, Roberts RG. *Alternaria* themes and variations (73). Mycotaxon. 1993;48:109–40.
48. Sospedra I, Soriano JM, Mañes J. Assessment of the microbiological safety of dried spices and herbs commercialized in Spain. Plant Foods Hum Nutr. 2010;65:364–8.
49. Vij V, Ailes E, Wolyniak C, Angulo FJ, Klontz KC. Recalls of spices due to bacterial contamination monitored by the US Food and Drug Administration: the predominance of *Salmonella*. J Food Prot. 2006;69:233–7.