doi: 10.5219/95

CONTAMINATION OF RAISIN BY FILAMENTOUS FUNGI – POTENTIAL PRODUCERS OF OCHRATOXIN A

Lusine Hakobyan, Karine Grigoryan, Ara Kirakosyan

ABSTRACT

The forty-one samples of Armenian made and eleven samples of imported raisins collected in several markets in Yerevan were studied. The sample collections were carried out during of years 2004 to 2008. Thirty two species of filamentous fungi from *Aspergillus*, *Penicillium, Alternaria Trichoderma* and *Syncephalastrum* genera were isolated and identified. Among species isolated from studied samples species belonging to *Aspergillus* genera have a very high frequency of occurrence, 65.2% of all investigated filamentous fungi. Species from *Nigri* section show the highest occurrence: 66.7% of all isolated fungi belonging to *Aspergillus* genera. Species *A. carbonarius* and *A. niger* were dominated among isolated fungi from section *Nigri*. Both Armenian and imported samples of raisin had a high contamination level by these fungi which are potential producers of ochratoxin A. In Armenian samples were detected two more ochratoxigenic species belonging to *Aspergillus* section *Nigri*: *A. sclerotioniger* and *A. lacticoffeatus.* But their frequency of occurred was low. Thirty seven strains of *A. flavus* were isolated, 92% of them were isolated from Armenian samples. Influence of pH and aw on contamination level of raisin by fungi was studied. It was revealed that highest contamination level by filamentous fungi occurred in raisins with relatively high aw value. Contamination level of raisin doesn't depend on pH.

Keywords: raisin, ochratoxin A, water activity, contamination, filamentous fungi

INTRODUCTION

Many researchers reported that dried fruits are often contaminated with species from Aspergillus Nigri section **(Alghalibi et al. 2004, Almeida, Alaburda, Ruvieri et al. 2007, Iamanaka, Taniwaki et al. 2005, Macdonald et al. 1999, Magnoli et al. 2004, Varga, Kocsube, Koncz et al. 2006)**. **Barkai-Golan and Paster (2008)** have shown that species of Aspergillus, Penicillium and Alternaria are major species presented in dried fruits. In wines, raisins and other grape-based products ochratoxin A is predominant. This mycotoxin has been associated with A. carbonarius and, to a lesser extent with A. tubingensis or A. niger. Studies of 60 samples of dried fruits (raisins, dates and figs) carried out by **Saeed, Alghalibi and Abdul-Rahman (2004)** in Yemen Republic have shown high level of fungal contamination. 23 species and one variety belonging to 15 genera including A. niger, A. flavus, A. fumigatus, A. ochraceus, Penicillium chrysogenum and Rhizopus stolonifer were dominated among species isolated from dried fruits. The analyses of samples of dried vine fruit in Argentina **(Magnoli et al., 2004)** revealed six species of Aspergillus section Nigri. The predominant species were A. niger var. niger, A. niger var. awamory and A. carbonarius. The frequency of occurrence of these species was lower in white dried vine fruit than in black dried vine fruit.

Samson, Houbraken, Kuijpers et al. (2004) showed that some species belonging to Aspergillus Nigri section are ochratoxin A producers. Fifteen species from Nigri section were isolated and identified in different food products. Four of them were producers of ochratoxin A. Ochratoxin A producing species from section Nigri occurring on grapes, raisins and in wine include A. carbonarius and to a lesser extent *A. niger*. Four species recovered from coffee, *A. carbonarius, A. niger, A. lacticoffeatus and A. sclerotioniger* produce ochratoxin A. Other species of section Nigri including *A. japonicus* and *A. tubingensis* also occurred. *A. niger* occurred very often in dried fruits (black sultanas, white sultanas, dates, dried plums, dried figs and apricots), especially in dried vine fruits as reported in **(Iamanaka et al., 2005)**. This specie is ochratoxigenic. The contamination level by A. niger in black sultanas, plums, figs, dates and white sultanas were 22.0, 8.0, 4.0, 1.5 and 0.5%, respectively. Thirty three (26.3%) samples of 117 analyzed contained more than 5 $\mu g.kg^{-1}$ of ochratoxin A.

Valero, Sanchis, Ramos et al. (2007) isolated 11 fungi: *Alternaria alternata, Cladosporium herbarum, Eurotium amstelodami, Penicillium janthinellum, P. decumbens, Trichoderma harzianum, Candida sp., Aspergillus carbonarius* OTA-negative, A. carbonarius OTA-positive, *A. niger var. niger*. and *A. japonicus var. aculeatus* from grapes and sun-dried grapes at different growth conditions (aw - 0.82-0.97 and TO- 20-40 OC). They showed that *Aspergillus section Nigri* is dominant.

According to **Rita et al. (2006)** there are 19 accepted taxonomies of the black aspergilla. *Moreover*, identification of species of *Aspergillus section Nigri* is often problematic because of their morphological similarity to each other. New *Aspergillus strains* of the section Nigri, which did not produce detectable amounts of ochratoxin A (OTA) but have a similar morphology to A. carbonarius, were isolated from wine grapes and dried vine fruit. The strains described belong to a new species, named A. ibericus.

As shown by **Crespo, Sánchez, Ramón et al. (2007)** black Aspergillus species (section Nigri) are main source of OTA contamination in grapes and also in dried grapes worldwide. Most of the isolates that produced ochratoxin A belong to A. carbonarius species. **Romero, Comerio, Larumbe et al. (2005)** have isolated ochratoxin A producer fungi from dried vine fruits even after surface disinfection. Species from *Aspergillus section Nigri* ("black aspergilli") were dominant. *Aspergillus* (50.2%), Eurotium (21.4%) and *Penicillium* (13.5%) genera have relatively low frequency. OTA was found in only 3 of 293

isolated strains of *A. niger*. A. carbonarius have less frequency but 96% of 48 strains were ochratoxigenic. **Naresh and David (2007)** reported that in northern Europe the major species of fungi producing ochratoxin A are *P. verrucosum*, *A. ochraceus* and species from *Aspergillus section Nigri*, especially *A. carbonarius*.

O TA was detected in grape, grape juice and dried vine fruit in many countries: Morocco **(Zinedine et al., 2007), Australia (Su-Lin, Ailsa and Eileen, 2005)**, France, **(Macdonald et al., 1999, Bejaoui, Mathieu, Taillandier et al., 2006)**, Hungary **(Varga et al., 2006)**, Brazile **(Almeida et al., 2007)**, Argentina **(Romero et al., 2005, Rocha Rosa et al., 2002)**, Greece **(Tjamos, Antoniou, Kazantzidou, et al., 2004)**, Portugal **(Rita, Mendonça and Venâncio, 2005, Rita, Lourenc, Ali´Pio and Venancio 2006)**, Turkey **(Turk, Rengin, AÞkun et al., 2004)**, Spain **(Esteban, Abarca, Bragulat et al., 2006)**, Egypt **(Zohri and Abdel-Gawad, 2007)** and others. In some cases the level of OTA in products was exceeded maximum allowed quantity.

MATERIAL AND METHODS

Sampling

11 samples of imported and 41 samples of Armenian black and white raisins realizing in markets of Yerevan were studied. The sampling has been carried out between 2004 and 2008 by dot method **(SANCO/1208/2005-rev. 1)**.

Mycological analyses of raisin

Samples were sterilized with 3% solution of sodium hypochlorite for 15 minutes. After surface disinfection samples were washed with fresh distillate water. The analyses were carried out with direct plating and dilution plating methods. For plating method direction food particles were placed directly on solidified agar media. For dilution 1:10, 10g of sample was dissolved in 90 ml sterilized water and mixed for 15 minutes **(Pitt and Hocking, 1997)**. For isolation of filamentous fungi CYA (Chapek-Yeast Agar medium, HiMedia Ltd.), GYA (Glucose-Yeast Agar medium, HiMedia Ltd.), and MEA (Malt-Extract Agar medium, HiMedia Ltd.) were used. The plates were incubated at 28° C for 7 days **(NF ISO 7954 - 88)**. After incubation the colony forming unit (CFU) was accounted according to **NF ISO 7698-91**, and frequency of occurrence was detected **(El-Kady, Abdel-Hafez and El-Maraghy, 1982)**.

The growing fungi were identified morphologically based on macro- and microscopic characteristics using following manual: **Raper and Fennell (1977)**, **Pitt (1979)**, **Samson, Hoekstra, Frisvad and Filtenborg (1995)**, **Samson, Noonim, Meijer, Houbraken, Frisva and Varga (2007)**.

Determination of aw, pH and sulfur dioxide

Determination of aw was spent with AquaLab (Decagon Devices, Pullman, WA, USA). Definition of pH was spent with pH-meter (Oakton, USA). Sulfur dioxide in samples of raisin was determined by aspiration method **(Wood, Foster, Damant et al., 2004)**.

RESULTS AND DISCUSSION

Fungal flora of raisin

A comparative analysis of 41 samples of Armenian raisin and 11 samples of imported raisin from Iran were carried out. 32 species of filamentous fungi belonging to two classes: *Zygomycetes* and *Hyphomycetes*, and 6 genera: *Mucor, Aspergillus, Penicillium, Alternaria, Trichoderma* and *Syncephalastrum,* were isolated and identified (Table 1).

Table 1 The classification of isolated fungi from studied samples

Class	Order	Family	Genera	Quality of species
Zygomycetes	*Mucorales*	*Mucoraceae*	*Mucor*	2
		Piptocefalidaceae	*Syncephalastrum*	1
Hyphomycetes	*Hyphomycetales*	*Moniliaceae*	*Aspergillus*	15
			Penicillium	12
			Trichoderma	1
		Dematiaceae	*Alternaria*	1

The most dominant fungal species were *A. carbonarius* (58,8%) and species from *Mucor* genera (52,9%): *M. racemosus* and *M. mucedo*. Species *A. sclerotiicarbonarius* (29.4%), *A. tubingensis* (29.4%), *A. foetidus* (29.4%), *A. flavus* (23.5%), *P. variabile* (29.4%) and *P. lanosum* (23.5%) were with average frequency of occurrence (Table 2 and 3). Studied samples of raisin have a high contamination level by fungi from *Mucor* genera. The presence of these fungi is a result of secondary contamination of raisin. These fungi were detected only on surface of samples. Surface disinfection of samples with 3% solution of sodium hypochlorite inhibited the growth of species of *M. mucedo* and *M. racemosus*.

Table 2 Fungi isolated from Armenian and imported raisin during of 2004 to 2008 years.

Species of fungi	Frequency of occurrence (%)
Penicillium	
P. ciatophora	11.8
P. corymbiferum	5.9
P. lanosum	23.5
P. variabile	29.4
P. clavigerum	3.57
P. cyclopium	3.57
P. puberulum	3.57
P. velutinum	14.3
P. diversum	7
P. rubrum	3.57
P. steckii	3.57
P. brevicompactum	3.57
Mucor	
M. mucedo	27.8
M. racemosus	32.1
Trichoderma *viride*	3.57
Syncephalastrum *racemosus*	3
Alternaria *alternata*	3

The other species of section *Aspergillus Nigri: A. japonicus* (8%), *A. aculeatus* (17.7%), *A. uvarum* (5.9%), *A. lacticoffeatus* (6%) have a lower frequency of occurrence. Species belonging to *Aspergillus* genera

(65.2%) were most prevalent among all isolated species. 15 species from *Aspergillus* genera were isolated, 10 of them (66.7%) belonged to section *Nigri*.

The comparative analyses of local and imported raisin have shown that contamination level of Armenian samples was much higher than Iranian samples. No filamentous fungi were identified in some imported samples. The quantity of species isolated from Armenian samples and belonging to *Aspergillus* genera exceeds the quantity of species isolated from imported raisin samples. Only 6 species of *Aspergillus* genera were isolated from imported raisin and 14 from local samples (Table 3).

Table 3 The results of comparative analyze of species of *Aspergillus* genera isolated from local and imported raisin

Species of *Aspergillus* genera	Frequency of occurrence (%)	Armenian samples	Imported samples
A. niger	47	+	+
A. sclerotioniger	17.7	+	-
A. carbonarius	58.8	+	+
A. sclerotiicarbonarius	29.4	+	-
A. tubingensis	29.4	+	+
A. foetidus	29.4	+	-
A. lacticoffeatus	6	+	-
A. uvarum	5.9	-	+
A. aculeatus	17.7	+	-
A. japonicus	8	-	+
A. fumigatus	11.8	+	-
A. ochraceus	5.9	+	-
A. flavus	23.8	+	+
A. nomius	11.8	+	-
A. orizea	5.9	+	-

Species *A. niger var. niger*, *A. carbonarius*, *A. sclerotiicarbonarius*, *A. foetidus* and *A. tubingensis* occurred nearly in all samples. But these fungi were detected at a higher frequency in locally produced raisin rather than in imported (Table 4).

Table 4 The frequency of occurrence of species belonging to section *Nigri* in Armenian and imported samples

Species from section *Aspergillus Nigri*	The frequency of occurrence in Armenian raisin (%)	The frequency of occurrence in imported raisin (%)
A. niger	61.5	59.5
A. scelrotioniger	21	-
A. carbonarius	59	35
A. sclerotiicarbonarius	29	-
A. tubingensis	43	42
A. foetidus	50	-
A. uvarum	-	7
A. japonicus	-	17
A. aculeatus	7.6	-
A. lacticoffeatus	7.6	-

Species of *Aspergillus* section *Nigri* are difficult to identify because of their morphological similarity to each other. . Several morphological characteristics, particularly conidia size allows to separate species of *A. carbonarius* (7–9 µm) and *A. niger* (3–5 µm) from others.

According to **Samson et al. (2007)** not only specie *A. carbonarius* is potential producer of ochratoxin A but also species of *A. niger, A. lacticoffeatus* and *A. sclerotioniger* among filamentous fungi from *Aspergillus Nigri* section. These species were found in studied samples. The contamination of raisin by fungi was quite high especially by pathogenic fungi *A. carbonarius* and *A. niger.* The frequency of occurrence of *A. carbonarius* was higher in Armenian raisins - 59%, rather than in imported raisins - 35%. 39 strains of *A. carbonarius* were isolated, 26 of which from Armenian and 10 from imported samples.

A. foetidus, A. sclerotioniger and *A. sclerotiicarbonarius* species were identified only in locally produced samples. Among ochratoxigenic species only *A. niger* and *A. carbonarius* were detected in imported samples. Toxigenic fungi occurred more frequently in locally produced raisin (Figure 1).

The results showed that the contamination level of raisin by filamentous fungi sharply increases every year and new species from *Aspergillus* genera are appeared. In our samples of 2007-2008 years frequency of occurrence of *A. carbonarius*, *A. foetidus* and *A. tubingensis* species is comparatively higher than in samples collected between 2004 and 2006. The occurrence of ochratoxigenic fungi *A. carbonarius* has sharply increased (from 70 to 80 %) recently in both locally produced and imported samples (Figure 2).

Species belonging to *Aspergillus flavi* section: *A. flavus* and *A. nomius* have average frequency of occurrence. These species are potential producers of mycotoxins from aflotoxins group. Specie *A. flavus* has been found in both Armenian and imported raisin (23.5% frequency). *A. nomius* was detected only in local samples at a low frequency (17%). Contamination level of raisin by *A. flavus* has also been raised during of recent years. In 2007 to 2008, for example, high contamination level of raisin by *A. flavus* was observed especially in local samples (Figure 3). 37 strains of *A. flavus* were isolated, 82% of which from Armenian raisin.

Influence of pH, aw and sulfur dioxide content on contamination level

For growth of filamentous fungi and mycotoxin production some growth factors such as pH, water activity (aw) and others are considered. Therefore, influence of pH and aw on contamination level of raisin by fungi was studied (Table 5).

The correlation between pH and contamination level is not simple. As such contamination level of raisin doesn't depend on pH. Values of pH of analyzed samples were 4.42- 4.8 which is favorable for growth and sporulation of fungi (including toxigenic).

A positive correlation between water activity and contamination level of Armenian raisin by filamentous fungi is revealed. These results are presented in table 5. The highest contamination levels are detected in samples of Armenian raisin with relatively high value of aw.

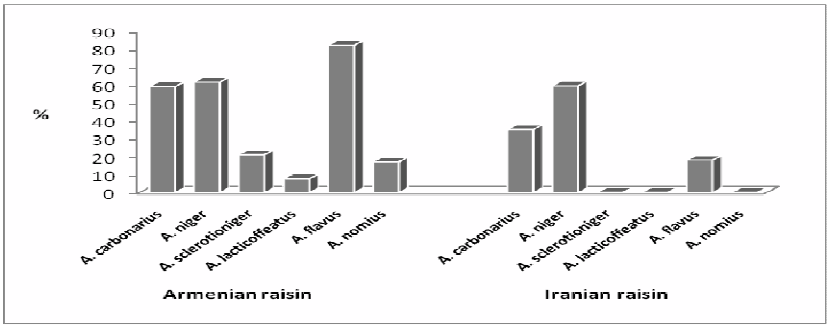

Figure 1 Frequency of occurrence of toxigenic fungi detected in Armenian and Iranian raisin samples

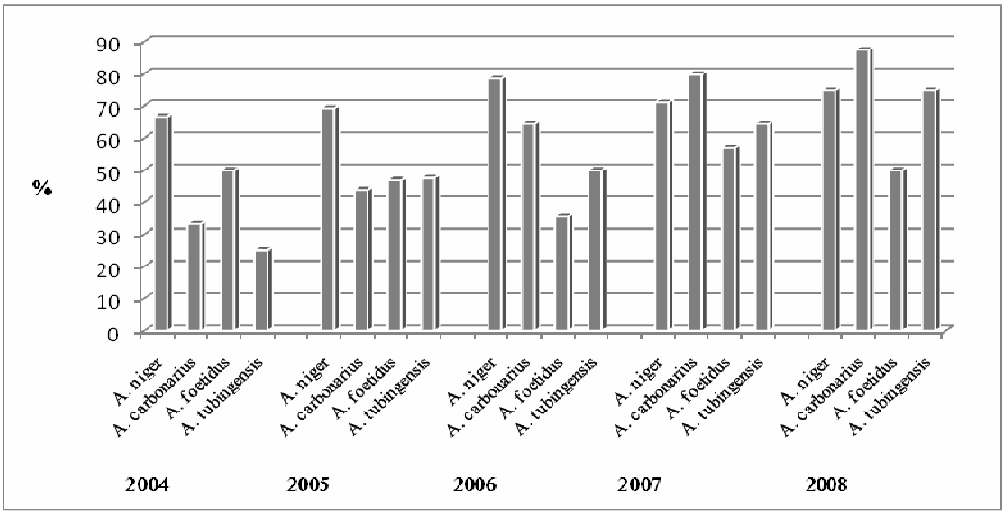

Figure 2 The species from *Aspergillus Nigri* section isolated from raisin samples between 2004 and 2008 years

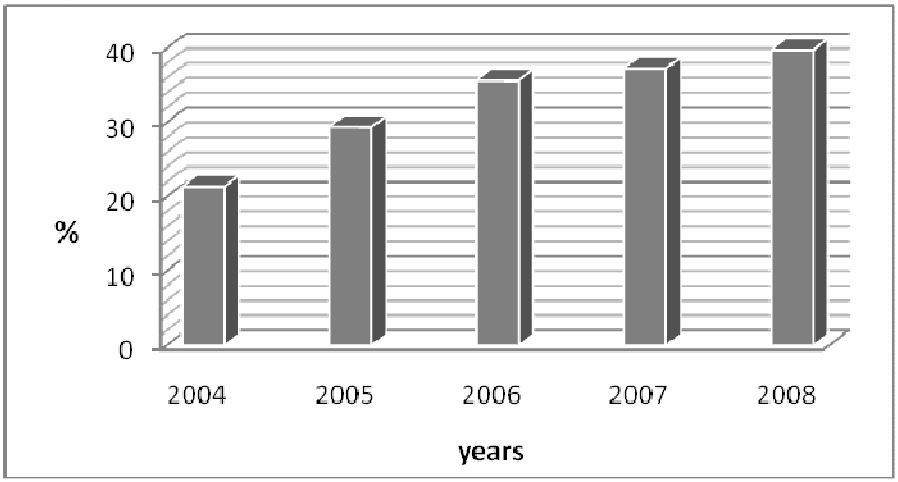

Figure 3 frequency of occurrence of *A. flavus* from years 2004 to 2008

Colony forming units (cfu.g^{-1}) of Iranian samples was possible because Iranian raisins have a high content of sulfur dioxide. "Free" SO_2 in Iranian gold raisin is about 45to 80 mg/kg, which can inhibit fungal growth. Total SO_2 was about 900-1200 mg/kg. It is noteworthy, however the quantity of sulfur dioxide in samples of Iranian gold raisin did not exceed the maximum allowed level such as 2000 mg.kg^{-1} **(Christensen, 2000)**. Sulfur dioxide was not detected in Armenian black raisins. Armenian black raisins were not treated with sulfur dioxide as commonly used to prevent natural color development. Subsequently, the frequency of occurrence of filamentous fungi was high in these samples.

It can be implied that the contamination of raisins by filamentous fungi can be prevented by use of control environmental conditions.

Table 5 The Influence of pH, aw and SO_2 on contamination level of raisin by filamentous fungi

Samples	pH	aw	Level of contamination by fungi (cfu.g^{-1})	SO_2 mg.kg^{-1}	
				Free SO_2	Total SO_2
Iranian	4.60	0.437	20	45	950
Iranian	4.42	0.432	10	62	900
Iranian	4.58	0.560	40	65	1100
Iranian	4.50	0.437	-	80	1200
Iranian	4.71	0.780	30	60	1000
Iranian	4.51	0.560	10	75	1200
Iranian	4.75	0.781	30	60	1000
Iranian	4.60	0.620	30	65	1100
Armenian	4.80	0.479	60	-	-
Armenian	4.75	0.485	70	-	-
Armenian	4.70	0.850	150	-	-
Armenian	4.50	0.901	170	-	-
Armenian	4.70	0.850	140	-	-
Armenian	4.67	0.459	40	-	-

'-' not detected

CONCLUSION

As a result of this and many other reports it is obvious that raisin is a favorable substrate for fungi, particularly for potential producers of mycotoxins such as ochratoxin A (*A. carbonarius, A. niger*). Six species of potential producers of mycotoxins, *A. carbonarius, A. niger, A. sclerotioniger, A. lacticoffeatus, A. flavus and A. nomius*, were isolated. *A. sclerotioniger, A. lacticoffeatus and A. nomius* occurred only in Armenian samples of raisin (Figure 1). In connection with climate change in recent years in Armenia the contamination level of raisin by potential ochratoxigenic fungi such as *A. carbonarius* has been increased. The frequency of occurrence of *A. niger* is turned to be very high. In 2004 it was about 66.7%, compare to 75% for recent five years (Figure 2). However, the occurrence of *A. carbonarius* has risen to 54.2%. Potential producer of aflatoxin A species such as *A. flavus* and *A. nomius* have occurred more often in raisin. And contamination of raisin by these species of toxigenic fungus has also risen within recent years, which is an actual and serious problem in Armenia.

REFERENCES

ALMEDIA, P. A., ALABURDA, J., RUVIERI, V., SABINO, M. 2007.Ochratoxin A in raisins samples marketed very low - 0 to 40, despite of high water activity. This is in São Paulo, Brazil. In Revista. – ISSN, vol. 38, 2007, p. 14-20.

BARKAI-GOLAN, R., PASTER, N. 2008. Mouldy fruits and vegetables as a source of mycotoxins: part 1. In *World Mycotoxin Journal*, vol. 1, 2008, no. 2, p. 147-159.

BEJAOUI, H., MATHIEU, F., TAILLANDIER, P., LEBR IHI,A. 2006. Black aspergilli and ochratoxin A production in French vineyards. In *International journal of food microbiology*, vol. 111, 2006, no. 1, p. 46-52.

CRESPO, A., SA'NCHEZ, M., RAMO'N, D., MARTINEZ-CULEBRAS, P. V. 2007. Molecular characterization of Aspergillus isolates responsible for OTA contamination in grapes and wine. In XII International IUPAC Symposium on Mycotoxins and Phycotoxins. Istanbul. Turkey. 2008, 5-9 August.

CHRISTENSEN, P. 2000. In Raisin production manual. Agriculture & Natural Resources. University of California, 2008, p. 214-225.

EL-KADY, I. A., ABDEL-HAFEZ, J. N., EL-MARAGHY, S. S. 1982. Contibution to the fungal flora of cereal grains in Egipt. In *Journal Mycopathologia*, 1982, no. 77, p. 103-109.

ESTEBAN, A., ABARCA, M. L., BRAGULAT, M. R., CABANES, F. J. 2006. Effect of pH on ochratoxin A production by Aspergillus niger aggregate species. In *Food Additives and Contaminants*, vol. 23. 2006, no. 6, p. 616-622(7).

IAMANAKA, B. T., TANIWAKI, M. H., MENEZES, H. C.,VICENTE, E., FUGARO, M. H. 2005. Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. In *Food Additive and Contaminant*. vol. 22, 2005, no 12, p.1258-1263.

MACDONALD, S., WILSON, P., BARNES, K., DAMANT, A., MASSEY, R., MORTBY, E., SHEPHERD, M. J. 1999.Ochratoxin A in dried vine fruit: method development and survey. In *Food Additives and Contaminants*. vol. 16, 1999, no 6, p. 253-260(8).

MAGNOLY, C., ASTOREC, A., PONSONE, L., COMBINA, M., PALACIO, G., ROSA, C. A. R., DALCERO, A. M. 2004. Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. In *Letters in Applied Microbiology*, vol. 39, 2004, no 4, p. 326-331(6).

NARESH, M. and DAVID, A., 2007. Conditions of formation of ochratoxin A in drying, transport and in different commodities. In *Food Additives & Contaminants*: Part A, vol. 22, 2007, Issue 1,p. 10 – 16.

PITT, J. I., HOCKING, A. D. 1997. Fungi and Food Spoilage. Blackie Academic and Professional, London.1997, p. 350.

RAPER K. B. and FENNELL, D. I. 1977. The genus Aspergillus. Krieger Publishing Company. Huntingon, NewYork, USA.

RITA, S., MENDONCA, C. and VENANCIO, A. 2005. Fungi and ochratoxin A detected in healthy grapes for wine production. In *Letters in Applied Microbiology*, vol. 42, 2005, no 1, p. 42 – 47.

RITA, S., LOURENC, A., ALI'PIO, P., VENANCIO, A., 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on Aspergillus and Penicillium species. In *Mycological Research*. vol. 110, 2006, p. 971-978.

RITA, S., CABANES, F. J., PERRONE, G., CASTELLA', G., VENANCIO, A., MULE', G., KOZAKIEWICZ, Z., 2006.Aspergillus ibericus: a new species of

section Nigri isolated from grapes. In *Mycology*, vol. 98, 2006, no2, p. 295-306.

ROMERO, S. M., COMERIO, R. M., LARUMBE, G., RITIENI, A., VAAMONDE, G., FERNA'NDEZ, P. V., 2005. Toxigenic fungi isolated from dried vine fruits in Argentina. In *International journal of food microbiology*; vol. 104, 2005, no.1, p. 43-9.

ROSA, C. A. R., PALACIOS, V., COMBINA, M., FRAGA, M. E., REKSON, A. O., MAGNOLY, C. E., DALCERO, A. M., 2002. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. In *Food Additives & Contaminants*: Part A, vol. 19, 2002, no. 4, p. 408 – 414.

SAEED, M. S., ALGHALIBI, M. S. and ABDUL-RAHMAN, M. S., 2004. Mycoflora and mycotoxin contamination of some dried fruits in Yemen Republic. Biology Department, Faculty of Science, Sana'a University, Sana'a, Yemen. In *Univ. Bull. Environ. Res*, vol. 7, 2004, no. 2, p. 235-243.

SAMSON, R. A., HOEKSTRA, E. S., FRISVAD, J., FILTENBORG, O. 1995. In Introduction to Food-borne fungi. 4thed. Cenraalbureau Voor Schimmelcultures, Baarn, Nether-Lands. 1995, p.450.

SAMSON, R. A., HOUBRACEN, J. A., KUIJPERS, F. A., FRANK, J. M., FRISVAD, J. C. 2004. New ochratoxin A or sclerotium producing species in *Aspergillus section Nigri*. In *Studies in Mycology*. vol. 50, 2004, p. 45–61.

SAMSON, R. A., NOONIM, P., MEIJER, M., HOUBRAKEN, J., FRISVAD, J. C., VARGA, J. 2007. Diagnostic tools to identify black aspergilli. In *Mycology*. vol. 59, 2007, no. 13, p.129-145.

SU-LIN, L. L., AILSA, D. H., and EILEEN, S. S. 2006. Survival and growth of Aspergillus carbonarius on wine grapes before harvest. In *International Journal of Food Microbiology*, vol. 111, 2006, no 1, p. S83-S87.

TJAMOS, S. E., ANTONIOU, P. P., KAZANTAIDOU, A., ANTONOPOULOS, D. F., PAPAGEORGIOU, I., TJAMOS, E. C. 2004. Aspergillus niger and Aspergillus carbonarius in Corinth Raisin and Wine-Producing Vineyards in Greece: Population Composition, Ochratoxin A Production and Chemical Control. In *Journal of Phytopathology*. vol. 152, 2004, no. 4, p. 250 – 255.

TURK, J. B., RENGIN, E., AÞKUN, T., SARIG, L. N., EVRIM, Z. T., HAFIZE, E. 2004. Colonial and Morphological Characteristics of Some Aspergillus Fr.:Fr. Species Isolated from Vineyards in Manisa and Üzmir Provinces (Turkey). Turk. In Journal Botany, vol. 28, 2004, p. 287-298.

VALERO, A., SANCHIS, V., RAMOS, A. J., MARIN, S. 2007. Studies on the interaction between grape-associated filamentous fungi on a synthetic medium. In Int**.** Journal of *Food Microbiology*. vol. 113, 2007, no. 3, p. 271-276.

VARGA, J., KOCSUBE, S., KONCZ, Z., TEREN, J. 2006. Mycobiota and ochratoxin a in raisins purchased in Hungary. In *Acta alimentaria* ISSN 0139-3006 CODEN ACALDI . vol. 35, 2006, no. 3, p. 289-294.

ZINDENDINE, A., SORIANO, J. M., JUAN, C., MOJEMMI, B., MOLT, J. C., BOUKLOUZE, A., CHERRAH, Y., IIDRISSI, L., AOUAD, R., MAES, J. 2007. Incidence of ochratoxin A in rice and dried fruits from Rabat and Sal area, Morocco. In *Food Additives & Contaminants*: part A, vol. 24, 2007, no. 3, p. 285 – 291.

ZOHRI, A. A., ABDEL-GAWAD, M. K. 2007. Survey of mycoflora and mycotoxins of some dried fruits in Egypt. In Journal of Basic Microbiology. vol. 33, 2007, no. 4, p. 279-288.

WOOD, R., FOSTER, L., DAMANT, A., KEY, P. 2004 . Analytical methods for food additives.

Woodhead Pudl. In *Food Science &Technology*. CRC, 2004, p 208.

Controle de la qualite des produits alimentaires controle microbiologique. 1993. NF ISO 7954-88. Direktives generales pour le denombrement des levures et moisissures.

Controle de la qualite des produits alimentaires controle microbiologique. 1993. NF ISO 7698-91. Direktives generales pour le denombrement des levures et moisissures.

Guidannce Document for Competent Authorities for the Control of compliance with EU Legislation on Afaltoxins Version 16/10/2006, SANCO/1208/2005-rev.1.P.1-44.

Contactact address:

Lusine Hakobyan, Department of Biology, Faculty of Biology, Yerevan State University, Armenia, E-mail: lusinehl@yahoo.com

Karine Grigoryan, Department of Biology, Faculty of Biology, Yerevan State University, Armenia, E-mail: foodlab@inbox.ru

Ara Kirakosyan, Department of Cardiac Surgery, University of Michigan, Ann Arbor, USA. E-mail: akirakos@umich.edu