Raisins and Currants as Conventional Nutraceuticals in Italian Market: Natural Occurrence of Ochratoxin A

Francesca Fanelli, Giuseppe Cozzi, Assunta Raiola, Irene Dini, Giuseppina Mulè, Antonio F. Logrieco, and Alberto Ritieni

Abstract: The healthy consumers make a strong pressure to natural products that can prevent the chronic diseases and improve the general health status, and therefore an important aspect that have to be considered is the safe level of the nutraceuticals. This study reports the occurrence of Ochratoxin A (OTA) and associated fungal contamination in 35 samples of dried vine fruits imported in the European community potentially used for the development of new nutraceutical supplements. High pressure liquid chromatography analysis identified 18 samples as contaminated by OTA with an average level of 2.6 μg/kg. OTA was measured in 4 samples of currants (mean value of 6.6 μg/kg) and 13 samples of raisins (mean value of 1.4 μg/kg). In one sample of currants and one of raisins from Turkey OTA exceeded the limits set by European Commission of 10 μg/kg, being contaminated with 12.61 and 15.99 μg/kg, respectively. All the positive samples were confirmed by Orbitrap Q Exactive through their molecular weight and the corresponding fragmentation. The worldwide consumption of dried vine fruits contributed to OTA exposure in several group of consumers. In particular, considering the potential nutraceutical approach, this consumption may be represent a severe risk for healthy consumers that consider these products like healthy and salutistic for their contents in antioxidants, flavonoids, and polyphenols. Data reported in this study confirmed the need to regularly monitor mycotoxin levels in these food products and optimize the process of fruits drying in order to reduce the development of toxigenic molds.

Keywords: *Aspergillus* spp., dried vine fruits, food safety, nutraceutical food, ochratoxin A, supplement

Introduction

In the last few years, consumers have shown a significant interest to include a high level of bioactive compounds in their standard diet, preferably derivated from natural sources such as plants and fruits (Bhat and others 2010), encouraging the development of new nutraceutical foods and supplements by researchers and pharmaceutical companies. The term nutraceutical was coined by DeFelice (1995), referring to any substance that may be considered a food or part of a food providing medical or health benefits. More recently, the Dietary Supplement Health and Education Act defines nutraceuticals as a dietary supplement that may contain an herb or other botanical, or a concentrate, metabolite, constituent, extract, or combination of any ingredient from the other categories (Frankos and others 2010). In this frame, numerous studies have strongly suggested that the inclusion of grapes and grape products as supplements incorporated in daily intake of foods may generate significant health benefits, mainly due to the presence of phenolic compounds, that are potent antioxidants recognized as preventative agents against several diseases and protecting the body tissues against oxidative stress (Shalaby and Horwitz 2015). Global raisin production for 2014/2015 is forecast at a record 1.2 million metric tons, up 5% from the previous year (USDA 2014). Consumption, which lagged production of the previous 4 years, is also at a record 1.2 million tons. Dried vine fruits are produced by dehydrating grape berries which is of particular interest because bioactive molecules must be ingested in a strict dose-dependent manner to achieve beneficial therapeutic.

On the other side, raisins and currants are often affected by toxigenic fungal colonization, in particular *Aspergillus* section *Nigri* or black aspergilli, with higher occurrence of *Aspergillus niger* aggregate (Perrone and others 2007; Palumbo and others 2011), which are known to produce Ochratoxin A (OTA). This is a nephrotic mycotoxin classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B; IARC 1993). OTA was evaluated by the Scientific Committee on Food (SCF) in 1998 when it was concluded that it has carcinogenic, nephrotoxic, teratogenic, immunotoxic, and possibly neurotoxic properties (SCF 1998).

The presence of OTA has been widely reported in dried vine fruits (MacDonald and others 1999; Ostry and others 2002; Möller and Nyberg 2003; Stefanaki and others 2003) in many countries (Lombaert and others 2004; Covarelli and others 2012) and with different levels, usually much higher than wine, as reported in Europe by Miraglia and Brera (2002). Dried vine fruits from Athens and Thebes market were analyzed; 23% of samples were found to be contaminated at high levels and 18 exceeded the EU limit (Kollia and others 2013). Samples purchased from Tunisian and Spanish markets were analyzed for the presence of 16 mycotoxins and revealed the contamination with 2 to 6 mycotoxins in 51% of the analyzed samples (Azaiez and others 2015).

These evidences confirmed the concern related to the consumption of these products, which contributed to a significant extent to OTA exposure, especially in vulnerable groups of

JFDS-2017-0424 Submitted 3/9/2017, Accepted 7/27/2017. *Authors Fanelli, Cozzi, Mulè, and Logrieco are with Inst. of Sciences of Food Production, CNR, via Amendola 122/0, 70126 Bari, Italy. Author Raiola is with Dept. of Agricultural Sciences, Univ. of Naples "Federico II," Via Università 100, Portici, 80055 Naples, Italy. Authors Dini and Ritieni are with Dept. of Pharmacy, Univ. of Naples Federico II, Via Domenico Montesano 49, Napoli 80141, Italy. Direct inquiries to author Raiola (E-mail: assuntaraiola@hotmail.com).*

Journal of Food Science A Publication of the Institute of Food Technologists

doi: 10.1111/1750-3841.13854

consumers such as children (Raiola and others 2015). For this reason, European Commission (EC) established the limit of 10.0 μg/kg for OTA in dried vine fruit (EC 2006).

The health hazard of nutraceutical formulations potentially obtained from dried vine fruits products largely depends on the presence of unusually high concentrations of toxic compounds. More purposely, if these products are used for infusion preparations or extracted and dehydrated from pharmaceutical companies in order to concentrate the antioxidant compounds, also an higher level of undesirable compounds such as mycotoxins would be available for consumers (Kalny and others 2007).

In addition, a significant increase of health risk in contaminated supplements could derive from the different physical state compared with the food source. In fact, the matrix in which a contaminant is present plays an important role in its bioaccessibility and bioavailability.

For these multiple reasons, a particular attention should be addressed to the control quality of the foodstuffs used as a source of nutraceutical supplements. However, an important limit for the safety of nutraceutical preparations is represented by the absence of a formal legislation regulating these products (Gulati and Ottaway 2006; Martínez-Domínguez and others 2014).

The first goal of this study was to evaluate the incidence of fungal and yeast contamination in raisins and currants of different origins intended to use as raw materials for the development of nutraceutical formulations in Italian market. Successively, we investigated the OTA occurrence in the same products by an innovative and optimized method developed in our laboratory, based on the confirm of positive samples by UHPLC-HRMS Orbitrap Q Exactive.

Materials and Methods

Samples

A total of 35 dried vine fruit samples from worldwide origin and the packaged samples were obtained from an importing company from Turkey (Fundacio Privada International Tree Nut, Antalya) or purchased from different markets in Bari (Italy). Samples were transported to laboratory and stored in air-tight containers at room temperature until mycological and chemical analysis.

Mycological analysis

Mycological analysis of dried vine fruits was performed by direct plating onto Dichloran Rose Bengal Chloramphenicol (DRBC, Oxoid) agar (King and others 1979; Pitt and Hocking 1997). Representative monoconidial isolates, based on diversity and incidence within each sample, were finally stored in cryotubes From each sample, 50 dried berries were aseptically plated (5 for plate) in DRBC agar petri dishes (90 mm).

Plates were incubated in darkness for 7 days at 25 °C and examined daily. After incubation, the number of berries showing fungal infection were recorded and the incidence of fungal genus for each sample was determined. Fungal colonies were subcultured both on malt extract agar (MEA) and Czapek–Dox Agar containing 100 mg/L of streptomycin and 50 mg/L of neomycin sulphate as antibacterial agents.

The strains were identified to genus/section level by culture and morphological observation under stereoscope (Pitt 1979; Samson and others 2004). Representative monoconidial isolates from each sample were finally stored in cryotubes under 20% glycerol at −80 °C and under liquid nitrogen for successive molecular characterization.

Chemical and materials

OTA stock solution (1 mg/mL) was prepared by dissolving the solid standard purchased from Sigma Aldrich (Milan, Italy) in methanol (99:1, v/v). Standard solutions for HPLC calibration or spiking purposes were prepared by diluting stock solution. Acetonitrile, methanol, water (HPLC grade), and glacial acetic acid (CH_3COOH) were purchased from Mallinckrodt Baker (Milan, Italy).

Potassium chloride (KCl), monosodium phosphate (NaH_2PO_4), sodium sulphate ($NaSO_4$), sodium chloride (NaCl), and sodium bicarbonate ($NaHCO_3$) were obtained from Sigma Aldrich.

Acetonitrile, methanol, and water for UHPLC mobile phase and organic solvents were LC/MS grade from Merck (Darmstadt, Germany), whereas formic acid and ammonium formate were obtained from Fluka (Milan, Italy).

Sample preparation

The samples were extracted and purified according to Vicam (1997) procedure for OTA in dried vine. As regards the retail unpacked dried vine fruits, 1 kg was purchased, from which 5 incremental samples weighing 100 g each were taken; consequently, the laboratory samples were 500 g that were homogenized in a Waring blender. Analytical method was similar to that described by Karami-Osboo and others (2015) with slight modifications.

Fifty grams of homogenized sample was used for extraction with 100 mL methanol containing 1% sodium bicarbonate solution (70:30) in Polytron (Lucerne, Switzerland). The mixture was homogenized for 1 min and filtered with filter paper. An aliquot of 10 mL was diluted with 40 mL phosphate-buffered saline (0.2 g KH_2PO_4, 1.1 g anhydrous Na_2HPO_4, 8.0 g NaCl, 0.20 g KCl, pH 7.0) and filtered. An aliquot of 10 mL was passed through the Ochratest TM (Ochratest, VICAM, Watertown, Mass., U.S.A.) immunoaffinity column (2 drops/s), then the column was washed with 20 mL of water and eluted with 4 mL of methanol. The methanolic extract was dried with a centrifugal evaporator, resuspended in 1 mL of methanol, filtered with a 0.22 μm filter (Phenomenex, Torrance, Calif., U.S.A.) and injected in the LC apparatus. Each sample was analyzed in triplicate.

Recovery experiments and linearity range

Recovery tests were carried out dried vine in triplicate. OTA-free samples were spiked with OTA standard (Sigma) and maintained at room temperature in a dark room overnight. Four contamination levels used were: 1, 10, 50, and 100 μg/kg. Linearity was estimated in the working range of 0, 1 to 50 ng/mL for OTA, with 4 calibration levels, each injected in duplicate.

HPLC analysis

OTA analysis was performed using an LC (Shimadzu-Japan) equipped with an autosampler SIL-20A, 2 pumps LC-20AD and a fluorimetric detection RF- 20AXL (OTA: λ_{ex} = 360 nm; λ_{em} = 460 nm). An Onyx Monolithic column with 3 μm particle size C_{18} (100 × 3.0 mm; Phenomenex) was employed. Mobile phase was used in isocratic conditions: 65% A (1% CH_3COOH in H_2O), 35% B (1% CH_3COOH in CH_3CN) at the flow rate of 1 mL/min (Raiola and others 2012).

OTA confirmation by Orbitrap Q Exactive analysis

Positive samples for the presence of OTA found by HPLC were confirmed by the high-resolution mass spectrometry

Table 1–Recovery ± RSD (%), LOD and LOQ (ng/g).

OTA level (μg/kg)	Recovery ± RSD (%)	LOD (ng/g)	LOQ (ng/g)
1	85.2 ± 2.3	0.05	0.15
10	88.1 ± 5.1	0.05	0.15
50	84.6 ± 3.5	0.05	0.15
100	89.0 ± 5.7	0.05	0.15

(HRMS-Orbitrap) analysis, by using an optimized and innovative method carried out in our laboratory.

Qualitative profile of OTA has been obtained using ultra high pressure liquid chromatography (UHPLC, Thermo Fisher Scientific, Waltham, Mass., U.S.A.) equipped with a degassing system, a Dionex Ultimate 3000 a Quaternary UHPLC pump working at 1250 bar, an auto sampler device and a thermostated (T = 50 °C) Thermo fisher AccucoreAq C18 (100 × 2.1 mm) column 2.6 μm particle size. Injection volume was of 5 μL. Eluent phase was formed as follow: phase A (H_2O in 0.1% formic acid and 5 mM ammonium formate), phase B (methanol in 0.1% formic acid and 5 mM ammonium formate). OTA has been eluted using a 0.4 mL/min flow rate with a gradient programmed as follows: 0 min—60 % of phase B, 9 min—100% of phase B, 12 min—100% of phase B, 12.1 min—60% of phase B, 15 min—60% of phase B.

For the mass spectrometry analysis a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific) has been used. An ESI source (HESI II, Thermo Fischer Scientific) operating inpositive (ESI+) ion mode for OTA analysis. Ion source parameters in ESI+ mode were: spray voltage 3.50 kV, sheath gas (N_2 > 95%) 30, auxiliary gas (N_2 > 95%) 10, capillary temperature 320 °C, S-lens RF level 50, auxiliary gas heater temperature 300 °C.

Value for automatic gain control (AGC) target was set at 1 × 10^6, with a resolution of 70000 full width at half maximum (FWHM), and a scan rate in the range between 90 and 1000 m/z. The experiment was performed in full MS scan.

The accuracy and calibration of the Q ExactiveOrbitrap LC-MS/MS was checked daily using a reference standard mixture obtained from Thermo Fisher Scientific. Data analysis and processing has been performed using the Xcalibur software, v. 3.0.63 (Xcalibur, Thermo Fisher Scientific).

OTA was identified by its retention time and exact mass at 5 decimal places from the total ion chromatogram (TIC) (Figure 1).

Results

Recovery and LOD

Table 1 showed values for recovery, the detection limit (LOD), and limit of quantification (LOQ) of OTA obtained by HPLC analysis. Values of recovery were 85.2 ± 2.3%, 88.1 ± 5.1%, 84.6 ± 3.5%, and 89.01 ± 5.7% for the levels of contamination of 1, 10, 50, and 100 μg/kg, respectively. LOD obtained was 0.05 ng/g for OTA whereas LOQ was 0.15 ng/g. Although interday precision was calculated by the relative standard deviation (RSD_R) calculated from concentration results of spiked samples generated under reproducibility conditions by one determination per concentration on 6 d, RSD_R for the procedures validated at LQ and 10 LQ were lower than 12% and 8%, respectively. These results demonstrated that the developed methods yielded acceptable recoveries from the analyzed samples.

Fungal contamination in dried vine fruits

Table 2 reported the incidence of contamination by *Aspergillus sect. Nigri* spp. and other species in dried vine fruits samples. The global incidence of contamination by *Aspergillus sect. Nigri* resulted very high. In 19 over 35 samples 100% of the fruits resulted contaminated by fungi belonging to this section. With respect to the type of dried fruits, although in 10/17 raisins sample the contamination was 100%, the average incidence of *Aspergillus* section *Nigri* was slightly higher on currants (74%) than on raisins (72%). With respect to the origin, samples from Turkey were the most contaminated by *Aspergillus sect. Nigri* (range of incidence between 86% and 100%), with an average occurrence of 94%. Eighteen samples showed 100% of contamination, whereas in only 1 sample out of 21 no *Aspergillus* occurred. *Penicillium* spp. were isolated in 4 samples, from Iran, Chile, South Africa, and the U.S., whereas *Erotium* spp. were found in 2 samples from Chile, with an incidence of 58% and 44% (Table 2).

In the only sample from Turkey where no *Aspergillus* was detected, we found a high incidence of *Monascus* spp. (46%; Table 2). In the samples from Chile, *Aspergillus* incidence was in the range

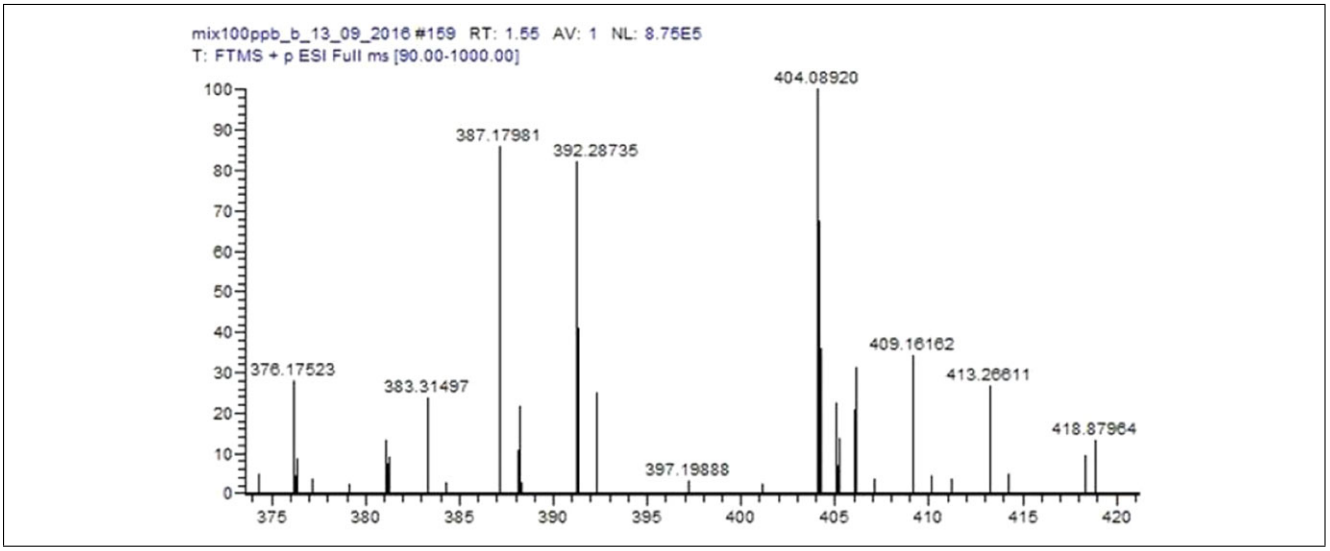

Figure 1–Orbitrap Q Exactive spectrum of OTA.

Table 2–Incidence of fungal and yeast contamination in dried vine fruits.

Sample	*Aspergillus sect. Nigri* (%)	*Aspergillus sect. Flavi* (%)	*Penicillium* spp. (%)	*Eurotium* spp. (%)	*Monascus* spp. (%)	Yeasts (%)
DF1	100	–	–	–	–	–
DF2	25	12.5	–	–	–	–
DF19	100	–	–	–	–	–
DF25	100	–	–	–	–	–
DF26	13	–	–	–	–	–
DF41	54	–	6	–	–	–
DF52	100	–	–	–	–	–
DF53	44	–	–	–	–	12
DF78	90	–	–	–	–	–
DF79	86	–	–	–	–	–
DF80	–	–	–	–	–	–
DF81	8	–	–	–	–	–
DF82	100	–	–	–	–	–
DF83	–	–	–	–	46	–
DF91	100	–	–	–	–	–
DF95	42	–	2	58	–	–
DF96	12	–	–	44	–	–
DF97	100	–	–	–	–	–
DF105	100	–	–	–	–	–
DF106	100	–	–	–	–	–
DF107	100	–	–	–	–	–
DF108	100	–	–	–	–	–
DF109	100	–	–	–	–	–
DF110	100	–	–	–	–	–
DF111	100	–	–	–	–	–
DF112	100	–	–	–	–	–
DF113	100	–	–	–	–	–
DF114	100	–	–	–	–	–
DF115	100	–	–	–	–	–
DF120	–	–	2	–	–	–
DF121	72	–	–	–	–	–
DF122	42	–	2	–	–	–
DF123	–	–	–	–	–	–
DF124	100	–	–	–	–	–
DF125	82	–	–	–	–	–

between 12% and 72%, whereas 2 of 7 samples resulted not contaminated by any fungal species. In 2 samples of currants from Iran, levels of *Aspergillus* incidence equal to 8% and 54% were observed, whereas in one sample *Penicillium* spp. were isolated.

In 2 samples of green raisins from China, *Aspergillus sect. Nigri* incidence was between 82% and 100%, whereas in 2 raisins samples from the U.S., the levels were 42% and 44%. The sample of golden raisins from South Africa was not contaminated by *Aspergillus* but by *Penicillium* spp., however with a low incidence of 2% (Table 2).

OTA contamination in dried fruits

Table 3 shows the incidence of OTA contamination in dried vine fruits samples from different geographical origin. The presence of OTA in positive fruits found by HPLC was confirmed by Orbitrap Q Exactive analysis.

With respect to the type of dried fruits, the relative occurrence of contaminated samples in raisins (14/22) was higher than in currants (4/13). Nevertheless, in the latter, the average level of contamination was higher than in raisins (4.1 μg/kg versus 1.35 μg/kg). Levels of OTA ranged from 0.05 μg/kg to 15.99 μg/kg in currants and from 0.95 μg/kg to 12.61 μg/kg in raisins.

With respect to geographical origin, the higher average contamination was measured in samples from Turkey (3.1 μg/kg). In 65% of samples from this state OTA levels were between 0.05 and 15.99 μg/kg. In 2 samples levels of OTA over the European Union (EU) legal limit of 10 μg/kg were measured (15.99 and 12.61 μg/kg in currants and raisins respectively).

OTA occurred in only 2 above 7 samples from Chile, with the values of 1.09 and 1.18 μg/kg for golden raisins and raisins, respectively. In both 2 samples of currants from Iran, as in the samples from China, although a high fungal contamination was observed, OTA was not detected.

In 2 samples of raisins from the U.S., OTA was measured at levels equal to 1.07 and 1.14 μg/kg. The sample of golden raisins from South Africa was contaminated by OTA in the amount of 1.91 μg/kg.

Discussion

This study examined an important problematic in the context of food safety that is the quality control of the raisins and currants, potentially adopted for the development of nutraceutical supplements, because it is widely reported their healthy effect, attributable to the high amount of polyphenols, including flavonoids, anthocyanins, proanthocyanins, and stilbenes.

In particular, we evaluated the incidence of fungal contamination and OTA amount originating from several countries of the world by an innovative and optimized method of detection.

We found low levels of OTA contamination in sample of dried vine fruits imported from countries outside Europe, despite the high incidence of potential toxigenic fungi occurring in the samples. These data are in agreement with previous studies performed in different countries and regions, which described dried vine fruits as susceptible of fungal growth and subsequent mycotoxin

Table 3–OTA contamination in dried vine fruits.

Sample	Dried vine fruits	Origin	OTA (μg/kg)
DF1	Currants	Turkey	n.d.
DF2	Currants	Chile	n.d.
DF19	Currants	Turkey	n.d.
DF25	Currants	Turkey	n.d.
DF26	Raisins	Chile	n.d.
DF41	Currants	Iran	n.d.
DF52	Currants	Turkey	n.d.
DF53	Raisins	USA	1.14
DF78	Currants	Turkey	n.d.
DF79	Currants	Turkey	n.d.
DF80	Raisins	Chile	n.d.
DF81	Currants	Iran	n.d.
DF82	Currants	Turkey	0.23
DF83	Currants	Turkey	0.13
DF91	Green raisins	China	n.d.
DF95	Raisins	Chile	n.d.
DF96	Raisins	Chile	n.d.
DF97	Currants	Turkey	15.99
DF105	Currants	Turkey	0.05
DF106	Raisins	Turkey	12.61
DF107	Raisins	Turkey	1.18
DF108	Raisins	Turkey	n.d.
DF109	Raisins	Turkey	1.3
DF110	Raisins	Turkey	2.17
DF111	Raisins	Turkey	n.d.
DF112	Raisins	Turkey	0.95
DF113	Raisins	Turkey	1.41
DF114	Raisins	Turkey	1.54
DF115	Raisins	Turkey	1.05
DF120	Golden raisins	South Africa	1.9
DF121	Raisins	Chile	1.18
DF122	Raisins	USA	1.07
DF123	Golden raisins	Chile	1.09
DF124	Sultana	Turkey	1.12
DF125	Green raisins	China	n.d.

n.d., not detected

production and accumulation. In 2002, Miraglia and Brera summarized data on dried fruits contamination provided by European countries, indicating these commodities to be highly exposed to OTA contamination. The incidence reported for dried vine fruits was 73% in Europe (over 800 samples analyzed) with OTA levels around 3 μg/kg.

Stefanaki and others (2003) investigated the presence of OTA in 81 samples of Greek domestic currants and sultanas. The latter resulted less contaminated than currants (average level of 2.1 and 2.8 μg/kg, respectively), and globally 7.5% of both types of dried vine fruit exceeded OTA limit of 10 μg/kg. In Italy, Imperato and others (2011) defined dried vine fruits as the main food matrix contaminated by OTA within the survey carried on 345 samples of imported foodstuffs. However, the contamination levels were below the maximum tolerable limit set by EU regulation.

Zinedine and others (2007) analyzed 20 samples of dried raisins purchased from retail shops in Morocco. OTA amounts ranged from 0.05 to 4.95 μg/kg in dried raisins in the samples.

Investigations of dried vine fruits samples from Turkey showed the presence of samples which overtook the EU limits: Aksoy and others (2007) reported OTA mean value of 1.36 μg/kg, whereas Bircan (2009) measured detectable levels in the range from 0.5 to 58 μg/kg in 53% of the samples.

It is important to note that, despite the high occurrence of *Aspergillus sect. Nigri*, potentially able to produce high amount of OTA, the average level of OTA measured in the positive samples was relatively low (2.56 μg/kg). However, 2 samples, 1 raisins and 1 currants, both from Turkey, exceeded EU legal limit with 12.61 and 15.99 μg/kg, respectively.

Biodiversity of fungal species isolated from our samples is consistent with contamination usually reported on dried vine fruits (Abarca and others 2003; Magnoli and others 2004; Romero and others 2005; Tournas and others 2015). Besides *Aspergillus* and *Penicillium* species, we also detected *Eurotium*, *Monascus*, and other yeasts. Interestingly, in one sample from Turkey (Table 2) only *Monascus* strains occurred. Organisms of the genus *Monascus*, known for the production of red pigments used as coloring agents in food and textiles (Fabre and others 1993), are able to grow over several substrates and food by-products, including dried fruits (Water Relations of Foods 1974; Golob and others 2002).

Results presented in this paper revealed a significant variance of the incidence of *Aspergillus* and OTA development. On the contrary, OTA occurrence is not correlated with the incidence of fungal contamination. This discrepancy may be due to the specific environmental conditions developed by processing grape berries. During ripening of the fruits (Covarelli and others 2012) temperature increases and the sugar is concentrated as the moisture content decreases. By this way drying fruits become an optimal and selective medium for xerotolerant molds such as *A. niger sect. Nigri* species. However, these conditions are not necessarily optimal for mycotoxin production as several studies confirmed (Leong and others 2006; Magan 2007). Recently, Passamani and others (2014) demonstrated that the best range for OTA production by *A. niger* and *A. carbonarius* was at 15 °C, aw 0.99, whereas the growth was optimal at 20 to 30 °C and 24 to 37 °C, respectively. Zhang and others (2014) analyzed a total of 56 dried vine fruits from Chinese markets. All samples were analyzed for OTA. Only one sample exceeded the EU. Heshmati and others (2015) evaluated the occurrence of OTA in 66 samples of dried grapes in Iran. OTA was detected in 57.5% of currants, 62.5% of sultanas, and 60% of raisins samples. None of the samples contained OTA level exceeding the maximum limit prescribed in the EU regulations, which is 10 μg/kg.

In general, to prevent or reduce the occurrence of species of molds able to produce OTA in dried fruits, good agricultural practices, good manufacturing practices, and hazard risk analysis should be applied. As an example, the use of drying chambers instead of sun drying could significantly reduce the fungal development and the corresponding OTA production.

However, an interesting aspect to consider in the frame of OTA occurrence in nutraceutical products is the potential protective action exercised by some antioxidants, such as α-tocopherol, cyanidin-3-o- β glucopyranoside ascorbic acid, and polyphenols against OTA effects on health.

These properties are probably due to the ability that the antioxidants have to act as superoxide anion scavengers, protecting cell membrane from the mycotoxin-induced damage (Atroshi and others, 2002). *In vitro* and *in vivo* studies (Baldi and others 2004; Costa and others 2007) showed that these substances could counteract some of the OTA toxic effects.

According to our study OTA occurrence is not at level of concern, except for 2 samples from Turkey. Despite these reassuring results, it should be considered that the low OTA levels found in analyzed samples, could represent a very significant exposure when these products are extracted to obtain nutraceutical supplements, where the level of phytochemicals, but also of contaminants are significantly concentrated, with the consequent risk to exceed the legislative limit fixed for OTA equal to 10 μg/kg.

Conclusion

This study confirms the need to control mycotoxin levels with a regular monitoring activity, especially in area where the production of dried vine fruits is widely practiced.

In addition, because healthy consumers tend to join and to follow a better life style and to insert in the diet the food integrators or nutraceuticals to improve health status, more rigorous controls over the safety and quality of these products could be achieved through good manufacturing practice, regulatory control, and research efforts (Kho and Woo 2000). Although storage is generally identified as the most critical stage for OTA contamination, control strategies applied during the preharvest time result effective to reduce fungal infection (Amézqueta and others 2009).

Regulators should also employ actions to minimize these risks, by introducing specific limits for mycotoxins and in particular OTA for this particular category of products.

Our study revealed a significant variance of the incidence of *Aspergillus* and OTA development, whereas OTA occurrence is not correlated with the incidence of fungal contamination. This effect may be due to the specific environmental conditions developed by processing grape berries. The confirmation of OTA identification was carried out by an innovative method that is represented by Orbitrap Q Exactive, based on the molecular weight and the corresponding fragmentation. In one sample of currants and one of raisins from Turkey OTA exceeded the limits set by European Commission of 10 μg/kg. Therefore, further investigations about the exposure to other mycotoxins from both conventional and innovative nutraceutical sources are need in order to obtain a complete risk assessment associated to their consumption. In addition, the impact of the different matrixes represented by dried vine fruits and nutraceutical supplements on bioaccessibility and bioavailability of OTA should be evaluated.

Acknowledgments

We thank Antonio Dimundo for his valuable technical assistance. This work was financially supported by EC KBBE-2007-222690-2 MYCORED.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

Authors' Contributions

F. Fanelli and G. Cozzi carried out analyses of fungal contamination and ochratoxin A in dried vine fruits and wrote the manuscript; A. Raiola, I. Dini, and G. Mulè analyzed data and wrote the manuscript; A.F. Logrieco and A. Ritieni designed the study, interpreted the results, and revised the manuscript.

References

Abarca ML, Accensi F, Bragulat MR, Castella G, Cabanes FJ. 2003. *Aspergillus carbonarius* as the main source of ochratoxin A contamination in dried wine fruits from the Spanish market. J Food Prot 66:504–6.

Amézqueta S, González-Peñas E, Murillo-Arbizu M, López de Cerain A. 2009. Ochratoxin A decontamination: a review. Food Control 20:326–33.

Aksoy U, Eltem R, Meyvaci KB, Altindisli A, Karabat S. 2007. Five year survey of ochratoxin A in processed sultanas from Turkey. Food Addit Contam 24:292–6.

Atroshi F, Rizzo A, Westermarck T, Ali-Vehmas T. 2002. Antioxidant nutrients and mycotoxins. Toxicology 180:151–67.

Azaiez I, Font G, Mañes J, Fernández-Franzón M. 2015. Survey of mycotoxins in dates and dried fruits from Tunisian and Spanish markets. Food control 51:340–6.

Baldi A, Losio MN, Cheli F, Rebucci R, Sangalli L, Fusi E, Bertasi B, Pavoni E, Carli S, Politis I. 2004. Evaluation of the protective effects of alpha-tocopherol and retinol against ochratoxin A cytotoxicity. Brit J Nutr 91:507–12.

Bhat R, Kiran K, Arun AB, Karim AA. 2010. Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. Food Anal. Methods 3:181–7.

Bircan C. 2009. Incidence of ochratoxin A in dried fruits and co-occurrence with aflatoxins in dried figs. Food Chem Toxicol 47:1996–2001.

Costa S, Utan A, Cervellati R, Speroni E, Guerra MC. 2007. Catechins: Natural free-radical scavengers against ochratoxin A-induced cell damage in a pig kidney cell line (LLC-PK1). Food Chem Toxicol 45:1910–7.

Covarelli L, Beccari G, Marini A, Tosi L. 2012. A review on the occurrence and control of ochratoxigenic fungal species and ochratoxin A in dehydrated grapes, non-fortified dessert wines and dried vine in the Mediterranean area. Food Control 26:347–56.

DeFelice SL. 1995. The nutraceutical revolution: its impact on food industry R&D. Trends Food Sci Technol 61:59–61.

Duckworth R, editor. 1974. Water relations of foods. In Proceedings of an International Symposium held in Glasgow.

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

Fabre CE, Goma G, Blanc PJ. 1993. Production and food applications of the red pigments of *Monascus ruber*. JFS 58:1099–110.

Frankos VH, Street DA, O'Neill RK. 2010. FDA regulation of dietary supplements and requirements regarding adverse event reporting. Clin Pharmacol Ther Oxford 87:239–44.

Golob P, Farrell G, Orchard JE. 2002. Crop post-harvest: science and technology: principles and practice. Vol. 1. Oxford: Wiley-Blackwell.

Gulati OP, Ottaway PB. 2006. Legislation relating to nutraceuticals in the European Union with a particular focus on botanical-sourced products. Toxicol 221(1):75–87.

Heshmati A, Nejad ASM. 2015. Ochratoxin A in dried grapes in Hamadan province, Iran. Food Additiv Contam, part B 8:255–9.

Imperato R, Campone L, Piccinelli AL, Veneziano A, Rastrelli L. 2011. Survey of aflatoxins and ochratoxin A contamination in food products imported in Italy. Food Control 22:1905–10.

International Agency for Research on Cancer. 1993. IARC monographs on the evaluation of carcinogenic risks to humans. Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxin. Lyon: Int Agency Res Cancer 56:489–521.

Kalny P, Fijalek Z, Daszczuk A, Ostapczuk P. 2007. Determination of selected microelements in polish herbs and their infusions. Sci Tot Environ 381:99–104.

Karami-Osboo R, Miri R, Javidnia K, Kobarfard F, AliAbadi MHS, Maham M. 2015. A validated dispersive liquid-liquid microextraction method for extraction of ochratoxin A from raisin samples. J Food Sci and Technol 52:2440–5.

King AD, Hocking AD, Pitt JI. 1979. Dichloran-rose Bengal medium for enumeration and isolation of moulds from foods. AEM 37:959–64.

Koh HL, Woo SO. 2000. Chinese proprietary medicine in Singapore: regulatory control of toxic heavy metals and undeclared drugs. Drug Saf 23(5):351–61.

Kollia E, Kanapitsas A, Markaki P. 2013. Occurrence of aflatoxin B_1 and ochratoxin A in dried vine fruits from Greek market. Surveillance 7:11–16.

Leong SL, Hocking AD, Scott ES. 2006. Effect of temperature and water activity on growth and ochratoxin A production by Australian *Aspergillus carbonarius* and *A. niger* isolates on simulated grape juice medium. Int J Food Microbiol 110:209–16.

Lombaert GA, Pellaers P, Neumann G, Kitchen D, Huzel V, Trelka R, Kotello S, Scott PM. 2004. Ochratoxin A in dried vine fruits on the Canadian retail market. Food Addit Contam 21:578–85.

MacDonald S, Wilson P, Barnes K, Damant A, Massey R, Mortby EA. 1999. Ochratoxin A in dried vine fruit: method development and survey. Food Addit Contam 16:253–60.

Magan N. 2007. Fungi in extreme environments. In Wicklow DT, Soderstrom B, editors. The Mycota, vol. 4. Environmental and ecological relationships. Berlin: Springer Verlag. P 99–114.

Magnoli C, Astoreca A, Ponsone L, Combina M, Palacio G, Rosa CAR, Dalcero A. 2004. Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. Letters in Applied Microbiology 39:326–31.

Martínez-Domínguez G, Plaza-Bolaños P, Romero-González R, Garrido-Frenich A. 2014. Analytical approaches for the determination of pesticide residues in nutraceutical products and related matrices by chromatographic techniques coupled to mass spectrometry. Talanta 118:277–91.

Miraglia M, Brera C. 2002. Assessment of dietary intake of ochratoxin A by the population of EU member states. In Reports on tasks for scientific cooperation. Report of experts participating in SCOOP Task 3.2.7. Rome, Italy: Directorate-General Health and Consumer Protection.

Möller TE, Nyberg M. 2003. Ochratoxin A in raisins and currants: basic extraction procedure used in two small marketing surveys of the occurrence and control of the heterogeneity of the toxins in samples. Food Add Contam 20:1072–6.

Ostry V, Ruprich J, Skarkova J. 2002. Raisins, ochratoxin A and human health. Mycotoxin Res. 18 Suppl 2:178–82.

Palumbo JD, O'Keeffe TL, Vasquez SJ, Mahoney NE. 2011. Isolation and identification of ochratoxin A-producing Aspergillus section Nigri strains from California raisins. Lett. Appl. Microbiol 52:330–6.

Passamani FR, Hernandes T, Lopes NA, Bastos SC, Santiago WD, Cardoso MD, Batista LR. 2014. Effect of temperature, water activity, and pH on growth and production of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazilian grapes. J Food Prot 77: 1947–5.

Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Meijer M, Noonim P, Mahakarnchanakul W, Samson RA. 2007. Biodiversity of *Aspergillus* species in some important agricultural products. Stud Mycol 59:53–66.

Pitt JI. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Academic Press Inc.

Pitt JI, Hocking AD. 1997. Fungi and food spoilage. London: Blackie Academic and Professional.

Raiola A, Meca G, Mañes J, Ritieni A. 2012. Bioaccessibility of deoxynivalenol and its natural co-occurrence with ochratoxin A and aflatoxin B_1 in Italian commercial pasta. Food Chem Toxicol 50:280–7.

Raiola A, Tenore GC, Manyes L, Meca G, Ritieni A. 2015. Risk analysis of main mycotoxins occurring in food for children: an overview. Food Chem Toxicol 84:169–80.

Romero SM, Comerio RM, Larumbe G, Ritieni A, Vaamonde G, Fernández Pinto V. 2005. Toxigenic fungi isolated from dried vine fruits in Argentina. Int J Food Microbiol 104:43–49.

Samson RA, Hoekstra ES, Frisvad JC. editors. 2004. Introduction to food and airborne fungi. 7th ed., Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures. P 389.

SCF 1998. Opinion on ochratoxin A. Expressed on 17 September 1998, the Scientific Committee on Food, the European Commission. Available from: http://europa.eu.int/comm/food/fs/sc/scf/out14_ en.html. Accessed 2017 March 20.

Shalaby S, Horwitz BA. 2015. Plant phenolic compounds and oxidative stress: integrated signals in fungal–plant interactions. Curr Genet 61:347–57.
Stefanaki I, Foufa E, Tsatsou-Dritsa A, Dias P. 2003. Ochratoxin A in Greek domestic wines and dried vine fruits. Food Addit Contam 20:74–83.
Tournas VH, Niazi NS, Kohn JS. 2015. Fungal presence in selected tree nuts and dried Fruits. Microbiol Insights 8:1–6.
United States Dept. of Agriculture Foreign Agricultural Service (USDA). 2014. Raisins: World Markets and Trade, Foreign Ag Service (FAS).
Vicam. 1997. OchraTest instruction manual. Watertown, Mass: Water Corporation.
Zhang X, Li J, Zong N, Zhou Z, Ma L. 2014. Ochratoxin A in dried vine fruits from Chinese markets. Food Addit Contam part B 7:157–61.
Zinedine A, Soriano JM, Juan C, Mojemmi B, Moltó JC, Bouklouze A, Cherrah Y, Idrissi L, El Aouad R, Mañes J. 2007. Incidence of ochratoxin A in rice and dried fruits from Rabat and Salé area, Morocco. Food Addit Contam 24:285–91.