Assessment of mycotoxins co-occurrence in Italian dried figs and in dried figs-based products

R. Di Sanzo1| S. Carabetta1| L. Campone1,2| S. Bonavita1| D. Iaria1| S. Fuda1|L. Rastrelli2| Mt. Russo1

1Food Chemistry, Safety and SensoromicLaboratory (FoCuSS Lab), University of ReggioCalabria, Reggio Calabria, Italy
2Department of Pharmacy, University ofSalerno, Salerno, Italy
Correspondence
Rosa Di Sanzo, Food Chemistry, Safety andSensoromic Laboratory (FoCuSS Lab),University of Reggio Calabria, Via Salita Melissari, 89124 Reggio Calabria,

Abstract
The possible contamination by aflatoxins (AFs), ochratoxin A (OTA), fumonisin B1(FB1), fusaric acid (FA), and beauvericin (BEA), was investigated in 55 samples of dried figs and dried figs-based products purchased from the South Italy (Calabria) market. A total of 41 samples showed contamination by at least one of the mycotoxins investigated. Aflatoxin B1was found in six samples(0.19—8.41μg/kg) total aflatoxins were found in 13 samples (0.5–17.12μg/kg), OTA was foundin 21 samples (<LOQ—158.58μg/kg), FB1was found in eight samples (153.81–5,412.96μg/kg),BEA was found in 12 samples (<LOQ—5,708.49μg/kg), and FA was found in 28 samples(<LOQ—74,520.20μg/kg). The analyzed samples were contaminated with one (41.8%), two(7.3%), three (5.5%), four (10.9%), and five (5.5%) mycotoxins. To the best of our knowledge, the incidence of contamination by FA and BEA has been shown for the first time in dried figs.

Practical applications
The present work was focused on mycotoxin mixtures contamination levels of dried figs and dried figs-based products. It is known that the co-occurrence of mycotoxins leads to additive orsynergistic effects. For some analyzed samples the AFB1, aflatoxins, and ochratoxin A (OTA)levels exceed the European Union countries legal limit. In addition, the simultaneous presence of FB1, FA, and BEA can be a potential threat to the health of consumers. Although different studies examined AFs, OTA, and Fusarium toxins as single mycotoxins in dried figs, to our knowledge, the co-occurrence of AFs, OTA, FB1, FA, and BEA in dried figs and in dried figs-based products samples has not been reported previously. Therefore, to prevent mycotoxins contamination, the employment of good practices in all the processing steps is necessary. Furthermore, the quality control of the ingredients used in figs preparations (spices, other dried fruits, cocoa, etc.), usually not adequately, is essential. Finally, it is of importance to establish more stringent rules to ensure food safety, even if no legal limit was still set for Fusarium toxins in dried fruit.

1|INTRODUCTION

Exposure to mycotoxins, natural toxic metabolites produced by some species of mold genera (Aspergillus, Penicillium, and Fusarium) which invade crops in the field and may grow on foods during storage, is awidely recognized health risk, which has been receiving an increasing attention. Most of these fungi are able to produce several mycotoxins simultaneously and to contaminate a wide variety of foodstuffs. Therefore, the risk of human co-exposure to multiple mycotoxins is real, raising agrowing concern about their potential impact on human health. Since 2008, the Committee on Food Additives of Joint FAO/WHO (JECFA) evaluated mycotoxins to be potential human carcinogens and urged that dietary exposure should be reduced to the lowest practicable levels so as to reduce the potential risk as far as possible (JECFA, 2008).At the present time, the legislation concerns the single toxins while no normative prevision takes into account the complex dynamics associated with the interactions between concurrent myco-toxins in foodstuffs.T he most important mycotoxins that have been detected in awide range of commodities, including cereals, spices, wine, coffee, andalso animal feeding stuffs (Di Stefano et al., 2015; Di Stefano, Pitonzo, Cicero, & D'Oca, 2014; Imperato, Campone, Piccinelli, Veneziano, &Rastrelli, 2011; O'Brien & Dietrich, 2005; Shephard, 2009) are aflatoxins (AFs), Ochractoxin A, and Fusarium toxins. AFs, a group of approximately 20 related secondary fungal metabolites, even if only AFs B1,B2,G1, and G2 are normally found in foods, are produced by different strains of Aspergillus flavusand Aspergillus parasiticus. A. flavusis a ubiquitous fungus which favoring the aerial parts of plants and produces mainly AFB1and AFB2.A. parasiticus, more adapted to a soil environment, produces both B-and G- AFs. Studies about dietary exposure assessment for total AFs(AF B1+B2+G1+G2) confirmed the risk for human health and the reduction of dietary AF exposure is an important public health goal.AFB1classified from IARC carcinogenic to human (Group 1) is one ofthe most potent known hepatocarcinogens (IARC, 2012). The current maximum legal limits for AFs—Commission Regulation (EC) No.1881/2006—are 2μg/kg for aflatoxin B1and 4μg/kg for total AFs indried fruit for direct human consumption and 5μg/kg for aflatoxin B1and 10μg/kg for total AFs in dried fruit subjected to sorting or other physical treatment before consumption or use as an ingredient in foodstuffs (European Commission [EC], 2006).Ochratoxins, contaminate grains,

legumes, coffee, dried fruits, beer, wine, and meat (Bayman et al., 2002; Iamanaka, Taniwaki,Menezes, Vicente, & Fungaro, 2005), are potent nephrotoxins, carcinogens, teratogens, and immunotoxins in rats and potentially in humans(IARC, 1993) classified from IARC as a possible human carcinogen(Group 2B).For European Union countries, the Commission Regulation(EC) No. 123/2005 sets legal limits in ochratoxin A (OTA) in 5μg/kg indifferent foods, spices, coffee, dried fruits, nuts, and figs. Studies carried out in Europe reported, in different dried fruits, the presence of ochratoxigenic fungi such as Aspergillus ochraceus, Aspergillus niger, and Aspergillus carbonarius as well as the presence of OTA (Iamanakaet al., 2005; Trucksess & Scott, 2008), and the widespread presenceof OTA in Turkish and Californian figs (Bayman et al., 2002; Doster,1996; European Commission (EC), 2005).Fusarium species have been recorded frequently as occurring in dried figs at a high incidence, and they are also considered the main agents of the endosepsis, a fungal disease so called for the internal rot that can affect fig fruits (Moretti et al., 2010). The main species involved in the disease was identified as *Fusarium proliferatum* and *Fusarium moniliforme* (Moretti et al., 2010).The fumonisins are produced by several species of the Fusarium genus, F. moniliforme and F. proliferatum are the most important(Akiyama, Uraroongroj, Miyahara, Goda, & Toyoda, 1997; Logrieco, Doko, Moretti, Frisullo, & Visconti, 1998; Ritieni et al., 1997; Shephard, 1998; Soriano & Dragacci, 2004; Williams, Meredith, & Riley,2004). An increasing number of structural analogues have been isoated from fungal cultures (Shephard, 1998; Williams et al., 2004) but the most important analogues found in naturally contaminated are fumonisin B1(FB1) and fumonisin B2(FB2) (Akiyama et al., 1997; Shephard, 1998; Williams et al., 2004).Among strategies applied in exposure assessments of mycotoxins of great interest are the food monitoring studies based on simple study design on adequate number of samples analyzed with multi methods. In order to analyze mycotoxins in foods, several samples preparation strategies have been developed including on-line solid-phase extraction (on-line SPE) (Campone et al., 2015), matrix solid-phase dispersion (Rubert, Soler, & Mañes, 2011), dispersive liquid–liquid micro-extraction (Campone et al., 2015; Campone, Piccinelli, Celano, &Rastrelli, 2011), accelerated solvent extraction (Campone, Piccinelli, Aliberti, & Rastrelli, 2009) and immunoaffinity columns (IACs) (Lattanzio, Ciasca, Powers, & Visconti, 2014).EFSA, as a result of the analysis of existing scientific studies, still in sufficient, has highlighted how the co-occurrence of mycotoxins in food products increases the probability of interactions, due to additiveor synergistic effects (Assunção, Silva, & Alvito, 2016; de Nijs et al.,2016), which may increase the toxicity and risk to human health. A preliminary study of our research team (unpublished data) concerning the monitoring of mycotoxin mixtures contamination levels, carried out in the years 2007–2009 on dried figs and dried figs-based products of southern Italy, in particular the region of Calabria, showed the presence of a worrying level of contamination. It highlighted the copresence of both aflatoxin B1(AFB1), aflatoxin B2(AFB2), aflatoxinG1(AFG1), aflatoxin G2(AFG2), OTA, FB1and further mycotoxins :fusaric acid (FA), and beauvericin (BEA). F. moniliforme produces not only fumonisins but also other secondary mycotoxins: FA and BEA (Bacon, Porter, Norred, &Leslie, 1996). Furthemore, different strains of F. subglutinans and F. Proliferatum produce both FA and BEA (Gruber-Dorninger, Novak, Nagl, & Berthil-ler, 2017).FA, 5-butilpicolinic acid, may increase the overall toxicity of other mycotoxins (Bacon et al., 1996). Thus, the major importance of FA to animal toxicity may be synergistic interactions with other naturally co-occurring mycotoxins as well as BEA (Bacon, Porter, &Norred, 1995).BEA, a cyclic hexadepsipeptide containing an alternatingsequence of threeN-methylL-phenylalanyl and three D-R-hydroxyisovaleryl residues, has been detected first time as a natural contaminant in a polish maize (Logrieco, Bottalico, Mulé, Moretti, &Perrone, 2003; Ritieni et al., 1997; Shephard, Sewram, Nieuwoudt, Marasas, & Ritieni, 1999) and also has been found in the maize grown in Italy (Ritieni et al., 1997). Such activity by BEA could increase the toxicity of other Fusarium mycotoxins that cooccur with BEA in contaminated cereals (Logrieco et al., 2003). We undertook this work with the aim to agree with EFSA concerns about consumers' health. The EFSA has recently launched an innovative initiative to urge scientists to develop validated analytical protocols for the evaluation of the combined toxicity, especially in the complex mixtures issue, to evaluate the impact of food contamination and to carry out the necessary risk assessment.

Mycotoxins contamination is the major problem for commercial dried figs and their derived products. The Mediterranean area, in particular South of Italy and the region of Calabria are the most important producers and consumers of these products. Different studies examined AFs, OTA, and Fusarium toxins as single mycotoxins in dried figs (Doster, 1996; Iamanaka et al., 2005; Iamanaka,de Menezes, Vicente, Leite, & Taniwaki, 2007; Karbancioglu-Güler &Heperkan, 2009; Karbancioğlu-Güler & Heperkan, 2008; Steiner, Rieker, & Battaglia, 1988; Trucksess & Scott, 2008) but, to the best of our knowledge, a comprehensive study regarding the co-occurrence of multiple mycotoxins in dried figs, in line with EFSA guidelines, has not yet been reported. Due to the significant health risks associated with mycotoxins in foods, to the importance of the amount of a single food ingredient consumed which influences the potential dietary exposure of humans to mycotoxins, to the EFSA declarations on risk assessment of multiple mycotoxins in food and related call launched in 2016 focused on this topic and to the cited preliminary study, the present work was focused on mycotoxin mixtures contamination levels of dried figs and dried figs-based products.

2|METHODS.

2.1|Samples

During 2016, a total of 55 samples of figs and dried figs-based products were purchased at the market in South of Italy (Calabria): 28 dried figs, 8 baked dried figs, and 19 dried fig-based products: 3 dried figs molasses, 8 baked dried figs stuffed with almonds, 6 dried figs flavored or covered with chocolate, and 2"salame di fichi". Each sample was representative of all production lot. Dried fig molasses and "salame di fichi" were two traditional products directly obtained by a local maker. Dried figs molasses preparation involves the slow cooking of shredded small-sized dried figs in water and subsequent separation from the residual solids exhausted of a syrup with a honey-like texture. The syrup looks like a highly vis-cous liquid, brown in color, sweet taste with a slightly bitter after taste are used in confectionery or also for seasoning cheeses. The "salame di fichi" is obtained from small-sized dried figs, baked and ground together with other ingredients: almonds, spices, and cocoa. In order to evaluate the mycotoxins contamination due to the ingredients, the same almonds and the cocoa powder used to prepare the "salame di fichi" samples were also analyzed separately. Sampling was conducted as described in Commission Regulation(EC) N. 401/2006. For each sample, an aliquot of 100 g was cut in a small pieces then the sample was homogenized by thoroughly blending in a Moulinex (double-force compact fp542, Groupe SEB, France)blender, immediately frozen and kept at−20 C until the time of analysis.

2.2|Chemicals and reagents

Mycotoxin standards (OTA, AFLA MIX, FB1, BEA, and FA) were purchased from Sigma-Aldrich (Milan, Italy). Ultrapure water, methanol, and acetonitrile of high-performance liquid chromatography grade were purchased from Sigma-Aldrich. Tween-20, sodium bicarbonate, potassium dihydrogen phosphate, anhydrous disodium hydrogenphosphatem, acetic acid, formic acid, NaH2PO42H2O, H3PO4, NaCl, KCl, o-phtaldialdehyde, Na2B4O710H2O, 2-CH3CH2SH, and CH3Clwere purchased from Sigma-Aldrich. AflaTest and OchraTest IACs were purchased from Vicam (Watertown, MA), a strong anion exchange Discovery DSC-SAX cartridge were purchased from Supelco (Milan, Italy).

2.3|AF analysis

The AFs extraction was performed modifying the method previously described by Stroka, Van Otterdijk, and Anklam (2000). Briefly, the sample was totally homogenized and 5 g were taken up in 20 ml of methanol: water (8:2) and 1 g of NaCl. The mixture was homogenized for 3 min and filtered in qualitative filter paper. An aliquot of 5 ml was diluted with 20 ml of Tween-20 (10%) and passed in a microfiber fil-ter. Then, 4 ml were introduced in an AflaTest (Vicam) IAC (1–2drops/s), followed by washing with distilled water. The AFs were eluted with 1 ml of methanol using a 1–2 drops/s flow. The total vol-ume was collected in a dark flask. At room temperature, 1 ml of dis-tilled water was added to the extract and analyzed by high liquid pressure chromatography (HPLC).The chromatographic separation of AFs was carried out using an HPLC Nexera X2 (Shimadzu, Japan) with fluorescence detection equipment set at 360 nm excitation and 440 nm emission and a Phenomenex Luna 3μC18 (150×3 mm) (Phenomenex, Torrance, CA)reversed-phase column. The used mobile phases in isocratic elution were methanol:water (45:55 [vol/vol]) with a flow rate of 1.0 ml/min

.2.4|OTA analysis

The OTA extraction was performed modifying Vicam method for OTA in currants and roasted coffee. The sample was totally homogenized in a blender and 2.5 g were taken for ocratoxin extraction using 50 mlof 1% sodium bicarbonate. The mixture was homogenized for 1 min and filtered in a microfiber filter. An aliquot of 20 ml was added with20 ml of phosphate buffered saline (0.20 g potassium dihydrogenphosphate, 1.10 g anhydrous disodium hydrogen phosphate, 8.0 g NaCl, 0.20 g KCl/0.01% Tween 20, pH 7.0) and filtered in a glass microfiber filter. An aliquot of 10 ml was introduced in an Ochratest (Vicam) IAC (1–2 drops/s), followed by washing with 10 ml phosphate buffer/ 0.01% Tween 20 and 10 ml purified water. The OTA were eluted with 1.5 ml of methanol using a 1–2 drops/s flow. The total volume was collected in a dark flask. A total of 1.5 ml of distilled water was added to the solution and analyzed by HPLC. The chromatographic separation of OTA was performed by HPLC Nexera X2 with fluorescence detection equipment set at 333 nm excitation and 477 nm emission and a Phenomenex Luna 3μC18(150×3 mm) (Phenomenex) reversed-phase column. The used mobile phases in isocratic elution were acetonitile:water:acetic acid (99:99:2[vol/vol]) with a flow rate of 0.8 ml/min

2.5|FB1analysisThe extraction of FB1was performed according to the method previously described by Logrieco et al. (1998): 2.5 g of the sample were homogenized in 25 ml of 1:1 methanol/1% KH2PO4pH 3 for 3 min. The homogenized product was centrifuged to 4,000 rpm for 5 min and extracted three times with 35 ml of CHCl3. The total volume was evaporated to dryness. The extracts were reconstituted into 1 ml of mobile phase prior to injection. Once again, no clean-up was per-formed on the samples.

The chromatographic separation of FB1was performed also by HPLC Nexera X2 with fluorescence detection equipment set at335 nm excitation and 440 nm emission and a Phenomenex Luna 3μC18 (150×3 mm) (Phenomenex) reversed-phase

column. The used mobile phases in isocratic elution were acetonitile:0.1 M NaH2-PO42H2O (75:25 [vol/vol]) adjusted to pH 3.35 with H3PO4with a flow rate of 1 ml/min.

2.6|BEA analysis

The extraction of BEA was performed modifying the procedure of Ritieni et al. (1997). A total of 2.5 g of the sample were homogenized in 25 ml methanol for 3 min and filtered in qualitative filter paper. The total volume was evaporated to dryness. The extracts were reconstituted into 1 ml of methanol prior to injection. Once again, no clean-up was performed on the samples. The chromatographic separation of BEA was carried out using an HPLC Nexera X2 equipped with ultraviolet (UV) detection set at205 nm and a Phenomenex Luna 3μC18 (150×3 mm)(Phenomenex) reversed-phase column. The toxins were separated by binary gradient elution according to the method previously described by Sewram Sewram, Nieuwoudt, Marasas, Shephard, and Ritieni (1999).

2.7|FA analysis FA extraction was carried out modifying the procedure of Baconet al. (1996): 2.5 g of the sample were homogenized in 25 ml of 1:1methanol/1% KH2PO4pH 3 for 3 min. The homogenized product was centrifuged to 4,000 rpm for 5 min and extracted three times with35 ml of CHCl3. The total volume was evaporated to dryness. The extracts were reconstituted into 1 ml of mobile phase prior to injection. Once again, no clean-up was performed on the samples. The chromatographic separation of FA was carried out using an HPLC Nexera X2 equipped with UV detection set at 271 nm. Phenomenex Luna 3μC18 (150×3 mm) (Phenomenex) reversed-phase column was used as stationary phase and methnol:water:1%KH2PO4(pH 3.4) (70,20:10) at flow rate of 1 ml/min was used as mobile phases in isocratic mode.

2.8|Method validation

The developed method was validated for dried figs in order to pro-duce accurate and reproducible results. For each analyte, the limit of detection LOD and limit of quantification LOQ were calculated by the signal-to-noise ratio, which should be more than 3 and 10, respectively. Three levels of contamination were prepared in matrices: AFB1(1, 2, and 5μg/kg), OTA (2, 10, and 20μg/kg), FB1(20, 50, and100μg/kg), FA, and BEA (50, 100, and 500μg/kg). The three levels were injected six times each in the HPLC. The validation data (recovery, RDS, LOD, and LOQ) for AFB1, OTA, FB1, FA, and BEA in dried figs are summarized in Table 1. The mycotoxins concentrations in the sample extracted were determined by the interpolation of resulting peak areas from the calibration graph.

3|RESULTS AND DISCUSSION

AFs, OTA, FB1, FA, and BEA occurrence in 55 samples of dried figs and dried figs-based products were investigated. Almonds and cocoa powder used as ingredients were also analyzed. The distribution of mycotoxins is given in Table 2 and 3.

3.1| Incidence of AFs and OTA

Total aflatoxins (AFTs) were detected in 13 (23.6%) samples(0.50–17.12μg/kg), of these five samples were above the EU legal limit. AFB1was detected in 10 (18.2%) samples (0.19–8.41μg/kg), of these one sample was above the EU legal limit. Both analyzed samples of almonds and cocoa powder were contained by AFTs. OTA was detected in 21 (38.2%) samples (<LOQ—158.58μg/kg) and of these eight were above the EU legal limit. Both samples of almonds and cocoa powder were contaminated by OTA. Figs have been known to be susceptible to AF and OTA contamination (Heperkan, Güler, & Oktay, 2012; Heshmati, Zohrevand, Khaneghah, Mozaffari Nejad, & Sant'Ana, 2017; Iamanaka et al., 2005;Karbancioğlu-Güler & Heperkan, 2008; Kaya & Tosun, 2013; Şenyuvaet al., 2008).Previous studies report that approximately 59 and 45% of the fig samples were contaminated with AFB1and OTA, respectively (Heshmati et al., 2017). In another survey, 14.3 and 52.4% of dried fig samples from Iran were contaminated with AFTs and OTA (Kaya & Tosun, 2013). High levels of AFB1(98%) and OTA (64%) were found in naturally contaminated individual figs (Şenyuva et al., 2008). Iamanaka et al. (2005) reported the highest incidence of OTA in fig samples(95%).In our study, the incidence of contaminated samples by AF and OTA is less than the literature mentioned.

3.2|Incidence of Fusarium toxin

FB1was detected in eight (14.5%) samples(153.81–5,412.96μg/kg).Both samples of cocoa powder were contaminated by FB1. Commission Regulation (EC) No 1126/2007 provide for a maximum of1,000μg/kg of fumonisins for mais and corn-based foods, no legal limits are required for other foods so in this study was assumed1,000μg/kg the reference limits for FB1in dried figs and dried figs-based products. FB1above reference limit was detected in four sam-ples analyzed and in both samples of cocoa powder. FA was detected in 28 (50.9%) samples (<LOQ—74,520μg/kg).BEA was detected in 12 (21.8%) samples (<LOQ—16,354.35μg/kg). Both analyzed samples of almonds and cocoa powder were contaminated by FA and BEA. Previous studies report that 74.7% of the fig samples were contaminated with FB1 (Heperkan et al., 2012). In another survey, 71.8and 79.5% of dried fig samples from Turkey collected between 2003and 2004 were contaminated

with FB1(Karbancioglu-Güler &Heperkan, 2009). Kaya and Tosun (2013) found that 63.1% of the dried fig samples collected in Turkey contained fumonisin. Finally, Moretti et al. (2010) showed that in Apulia there is a high risk of fig contamination by Fusarium species. They analyzed the toxigenicity of the identified Fusarium species and they found that some Fusarium strain were able to produce FA, BEA, and FB1. This study shows that the Fusarium species could contribute to Fusarium-toxin contamination of dried figs but the real Fusarium-toxin contamination of the collected figs was not studied. To the best of our knowledge, the incidence of contamination by FA and BEA has been shown for the first time in dried figs.

3.3|Mycotoxins co-occurrence

The research shows the widespread contamination in dried figs. A total of 41 (74.5%) samples analyzed showed contamination by at least one of the mycotoxins investigate. The analyzed samples were contaminated with one (41.8%), two (9.1%), three (5.9%), four (11.8%),and five (5.9%) mycotoxins. A total of 25.4% of the samples were not contaminated. The highest contaminated sample was the “salame di fichi”, simultaneously contaminated by AFs (1.95–17.12μg/kg), OTA (4.01–139.61μg/kg), FB1(249.97–1,112.35μg/kg), FA (56,221.6–62,988.5μg/kg), and BEA (476.24–1,307.16μg/kg).The “salame di fichi”, obtained by a local maker, is a traditional products obtained by grinding dried figs together with almonds, spices and cocoa. In order to evaluate the mycotoxins contamination due to the ingredients, the local maker provided us the same almonds and the cocoa powder used to prepare the “salame di fichi”. The cocoa power was simultaneously contaminated by AFs (2.64–4.04μg/kg), OTA (1.05–1.46μg/kg), FB1(1,245.01–1,467.15μg/kg), FA (27,418.07–41,779.20μg/kg),and BEA (12,012.46–16,354.35μg/kg). The almonds were simultaneously contaminated by AFs (0.52–2.55μg/kg), OTA (1.25–2.09μg/kg), FA (717.40–741.70μg/kg), and BEA (3,535.53–4,219.64μg/kg).The high contamination of cocoa powder and almonds, used as ingredients, might explain the high concentration of mycotoxins in both samples of salame di fichi. Also, salame di fichi samples showed concentrations well above the legal limit for all mycotoxins (OTA, AFT, and AFB1).The samples of fig molasses analyzed showed high level of contaminations, especially in Fusarium toxins. Maybe, this is related with the use, in the preparation, of poor quality or damaged figs. Thre esamples (one sample of dried figs, two samples of dried figs salami)showed the co-occurrence of OTA, AFs (total and B1) and all Fusarium-toxins (FB1, FA, and BEA). Six samples (one sample of dried figs, two samples of baked dried figs, two samples of dried figs salami)showed the co-occurrence of OTA, AFs (total and B1) and fusarium toxins (FA and BEA).The co-occurrence of AFs, OTA, FB1, FA, and BEA in dried figs and in dried figs-based products samples has not been reported previously. Kaya and Tosun (2013) researched AF, OTA, and fumonisin contamination in dried figs and fig molasses; however, the number of samples that contained all three mycotoxins was not mentioned. AFs and OTA were found together in 18.2% of the dried figs collected in Iran (Heshmati et al., 2017). The co-occurrence of AFs and OTA was reported by Şenyuva et al. (2008) in 3.8% in 2003 and 4.9% in 2004of the dried figs samples analyzed in Turkey. Heperkan et al. (2012)report the co-occurrence of AFs, cyclopiazonic acid, FB1, and OTA in dried figs. They found that 7.3% of the samples were contaminated with two, 5.5% by three, 10.9% by four, and 5.5% by five mycotoxins. Although the maximum level of BEA and FA tolerated in foods has not been set, it is known their toxic action and, also the synergetic action with other mycotoxins. Klarić, Rumora, Ljubanović, and Pepeljnjak (2008)) have allowed to determine individual and combined effects of FB1,BEA,andOTAon porcine kidney epithelial PK15 cell. They report that combined treatment with FB1, BEA, and OTA resulted mostly in additive effects on lactate dehydrogenase assay activity, and additive and synergistic effects on caspase-3 activity and apoptotic index. FB1,BEA, and OTA are able to induce apoptosis and necrosis in porcine kidney PK15 cells and thus evoke dominant additive or synergistic effect depending on the concentration used and time of exposure. Due to the potent toxic effects of FB1,BEA,andOTA,simultaneousexposure to those mycotoxins might be an important trigger for development of chronic renal diseases in humans, especially after long-time exposure.4|

CONCLUSION

Considering the coincident production of AFs, ochratoxin with other mycotoxins, it is very likely, that humans are always exposed to mixtures rather than to individual compounds (de Nijs et al., 2016).Therefore, mixture toxicity data are needed for an improved and more realistic risk assessment (Assunção et al., 2016).Figs seem to be an optimal substrate for fungi probably due to their high sugar content, making them more susceptible than other dry fruits. Sun drying on the trees might provide conditions that are highly conducive to mycotoxins accumulation. The infection by fungi on the exterior fruit surface as well as carried into the interior of the fruits by the insects and the temperature conditions generally prevailing during fruit sun drying, would seemingly be ideal for growth of the fungi. The Fusarium species are widely distributed mainly in soil and grown in soils of both the temperate and the tropical areas and inorganic substrates. Consequently, just to avoid the contaminations, the collection of figs fell to the ground must be avoided. The occurrence of mycotoxins has been determined in dried figs and dried figs-based products. The contamination level of AFs, OTA,FB1, FA, and BEA for some analyzed matrices was quite high and74.5% samples analyzed showed contamination by at least one of investigate mycotoxins. In this study, the incidence of contaminated samples by AF, OTA, and FB1is very low compared

with the findings in other papers. Mycotoxins were absent in 25.4% of the analyzed samples. Only one sample contained AFB1levels higher than 2µg/kg, five samples contained AFs levels higher than 4µg/kg and eight samples contained OTA levels higher than 5µg/kg, the maximum limit recommended by the European Union. The co-occurrence of AFs, OTA, FB1, FA, and BEA were noted in three (5.9%) of the samples. The "salame di fichi", obtained by grinding dried figs with almonds, spices and cocoa, was the highest contaminated sample and simultaneously contaminated by all the investigated micotoxins. The results of this study have a broad significance and are no limited to Calabria, because figs are widely cultivated around the world. Therefore, the co-occurrence of mycotoxins in dried figs and dried figs-based products is a worrying phenomenon. As 61.8% of the analyzed samples showed contamination by at least one of the investigate Fusarium toxins, it would be necessary to set the legal limit for these mycotoxins in dried fruit. Furthermore, it would be necessary, in setting the cautionary limits, to take in to account the possible copresence of other mycotoxins.

ACKNOWLEDGMENT
This work was supported by Research Infrastructure Saf@med-Food-Safety platform (PONa3_00016).

CONFLICT OF INTEREST The authors declare no potential conflict of interests.

AUTHOR CONTRIBUTIONS RMt, FS, and DSR contributed in collecting sample and classification. DSR, CS, and CL contributed to chromatographic analysis running the laboratory work. RMt, DSR, BS, and ID contributed to analysis of the data. RMt, DSR, CS, and CL contributed in drafting the paper. RMt and LR designed the study, supervised the laboratory work and con-tributed to critical reading of the manuscript. All the authors have readthe manuscript and approved the submission.

REFERENCES

Akiyama, H., Uraroongroj, M., Miyahara, M., Goda, Y., & Toyoda, M. (1997). Quantitation of fumonisins in corn by HPLC with o-phthalaldehyde postcolumn derivatization and their identification by LC/MS. Mycopathologia, 140(3), 157–161. https://doi.org/10.1023/A: 1006818226376

Assunção, R., Silva, M. J., & Alvito, P. (2016). Challenges in risk assessment of multiple mycotoxins in food. World Mycotoxin Journal, 9(5), 791–811. https://doi.org/10.3920/WMJ2016.2039

Bacon, C. W., Porter, J. K., & Norred, W. P. (1995). Toxic interaction of fumonisin B1 and fusaric acid measured by injection into fertile chicken egg. Mycopathologia, 129(1), 29–35. https://doi.org/10.1007/
BF01139334

Bacon, C. W., Porter, J. K., Norred, W. P., & Leslie, J. F. (1996). Production of fusaric acid by Fusarium species. Applied and Environmental Microbiology, 62(11), 4039–4043.

Bayman, P.,Baker, J. L., Doster,M.A., Themis, J.,Mahoney, N. E.,Bayman, P.,Mahoney, N. E. (2002). Ochratoxin Production by the Aspergillus ochraceus Group and Aspergillus alliaceus. Applied and Environmental Microbiology, 68(5), 2326–2329. https://doi.org/10.1128/AEM.68.5.2326

Campone, L., Piccinelli, A. L., Aliberti, L., & Rastrelli, L. (2009). Application of pressurized liquid extraction in the analysis of aflatoxins B1, B2, G1 and G2 in nuts. Journal of Separation Science, 32(21), 3837–3844.
https://doi.org/10.1002/jssc.200900329

Campone, L., Piccinelli, A. L., Celano, R., & Rastrelli, L. (2011). Application of dispersive liquid-liquid microextraction for the determination of aflatoxins B 1, B 2, G 1 and G 2 in cereal products. Journal of Chromatography A, 1218(42), 7648–7654. https://doi.org/10.1016/j.chroma.2011.05.028

Campone, L., Piccinelli, A. L., Celano, R., Russo, M., Valdés, A., Ibáñez, C., & Rastrelli, L. (2015). A fully automated method for simultaneous determination of aflatoxins and ochratoxin A in dried fruits by pressurized liquid extraction and online solid-phase extraction cleanup coupled to ultra-high-pressure liquid chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry, 407(10), 2899–2911. https:// doi.org/10.1007/s00216-015-8518-4

De Nijs, M., Mengelers, M. J. B., Boon, P. E., Heyndrickx, E., Hoogenboom, L. A. P., Lopez, P., & Mol, H. G. J. (2016). Strategies for estimating human exposure to mycotoxins via food. World Mycotoxin Journal, 9(5), 831–845. https://doi.org/10.3920/WMJ2016.2045

Di Stefano, V., Avellone, G., Pitonzo, R., Capocchiano, V. G., Mazza, A., Cicero, N., & Dugo, G. (2015). Natural co-occurrence of ochratoxin A, ochratoxin B and aflatoxins in Sicilian red wines. Food Additives & Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment, 32(8), 1343–1351. https://doi.org/10.1080/19440049.2015.1055521

Di Stefano, V., Pitonzo, R., Cicero, N., & D'Oca, M. C. (2014). Mycotoxin contamination of animal feeding stuff: Detoxification by gamma-irradiation and reduction of aflatoxins and ochratoxin A concentrations.
Food Additives & Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment, 31(12), 2034–2039. https://doi. org/10.1080/19440049.2014.968882

Doster, M. a. (1996). Species and mycotoxins in figs from California orchards. Plant Disease, 80, 484. https://doi.org/10.1094/PD-80-0484

European Commission (EC). (2005). Commission regulation (EC) no 123/2005 of 26 January 2005 amending regulation no 466/2001 as regards ochratoxin A. Official Journal of the European Union, L 25/3, 3–5 Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?
European Commission (EC). (2006). Commission regulation (EC) no 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities,
L 364/5, 5–24.
Gruber-Dorninger, C., Novak, B., Nagl, V., & Berthiller, F. (2017). Emerging mycotoxins: Beyond traditionally determined food contaminants. Journal of Agricultural and Food Chemistry, 65(33), 7052–7070. https://doi.
org/10.1021/acs.jafc.6b03413
Heperkan, D., Güler, F. K., & Oktay, H. I. (2012). Mycoflora and natural occurrence of aflatoxin, cyclopiazonic acid, fumonisin and ochratoxin A in dried figs. Food Additives & Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment, 29(2), 277–286. https://doi.
org/10.1080/19440049.2011.597037
Heshmati, A., Zohrevand, T., Khaneghah, A. M., Mozaffari Nejad, A. S., & Sant'Ana, A. S. (2017). Co-occurrence of aflatoxins and ochratoxin A in dried fruits in Iran: Dietary exposure risk assessment. Food and
Chemical Toxicology, 106, 202–208. https://doi.org/10.1016/j.fct. 2017.05.046
Iamanaka, B. T., de Menezes, H. C., Vicente, E., Leite, R. S. F., & Taniwaki, M. H. (2007). Aflatoxigenic fungi and aflatoxins occurrence in sultanas and dried figs commercialized in Brazil. Food Control, 18(5), 454–457. https://doi.org/10.1016/j.foodcont.2005.12.002
Iamanaka, B. T., Taniwaki, M. H., Menezes, H. C., Vicente, E., & Fungaro, M. H. P. (2005). Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. Food Additives and Contaminants, 22(12),
1258–1263. https://doi.org/10.1080/02652030500260447
IARC (1993). Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. In IARC monographs on the evaluation of carcinogenic risk of chemicals to humans (Vol. 56) Geneva, WHO Press. https://doi.org/10.1002/food.19940380335
IARC (2012). Aflatoxins. In IARC monographs on the evaluation of carcinogenic risks on humans Chemical Agent and Related Occupations (Vol. 100F, pp. 225–248). Geneva, WHO Press.
Imperato, R., Campone, L., Piccinelli, A. L., Veneziano, A., & Rastrelli, L. (2011). Survey of aflatoxins and ochratoxin A contamination in food products imported in Italy. Food Control, 22(12), 1905–1910. https://
doi.org/10.1016/j.foodcont.2011.05.002
JECFA. (2008). Evaluation of certain food additives and contaminants. World Health Organization. Tech. Rep. series. Geneva, WHO Press Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24779311
Karbancioğlu-Güler, F., & Heperkan, D. (2008). Natural occurrence of ochratoxin A in dried figs. Analytica Chimica Acta, 617(1–2), 32–36. https://doi.org/10.1016/j.aca.2008.01.009
Karbancioglu-Güler, F., & Heperkan, D. (2009). Natural occurrence of fumonisin B1 in dried figs as an unexpected hazard. Food and Chemical Toxicology, 47(2), 289–292. https://doi.org/10.1016/j.fct.2008.11.003
Kaya, S. B., & Tosun, H. (2013). Occurence of total aflatoxin, ochratoxin A and fumonisin in some organic foods. Journal of Pure and Applied Microbiology, 7(4), 2925–2932.
Klarić, M. Š., Rumora, L., Ljubanović, D., & Pepeljnjak, S. (2008). Cytotoxicity and apoptosis induced by fumonisin B1, beauvericin and ochratoxin A in porcine kidney PK15 cells: Effects of individual and combined
treatment. Archives of Toxicology, 82(4), 247–255. https://doi.org/10. 1007/s00204-007-0245-y
Lattanzio, V. M. T., Ciasca, B., Powers, S., & Visconti, A. (2014). Improved method for the simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in cereals and derived products by liquid
chromatography-tandem mass spectrometry after multi-toxin immunoaffinity clean up. Journal of Chromatography A, 1354, 139–143. https://doi.org/10.1016/j.chroma.2014.05.069
Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., & Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. European Journal of Plant Pathology, 109(7),
645–667. https://doi.org/10.1023/A:1026033021542
Logrieco, A., Doko, B., Moretti, A., Frisullo, S., & Visconti, A. (1998). Occurrence of fumonisin B1 and B2 in Fusarium proliferatum infected asparagus plants. Journal of Agricultural and Food Chemistry, 46(1988),
5201–6204. https://doi.org/10.1021/Jf9804903
Moretti, A., Ferracane, L., Somma, S., Ricci, V., Mulè, G., Susca, A., Logrieco, A. F. (2010). Identification, mycotoxin risk and pathogenicity of Fusarium species associated with fig endosepsis in apulia, Italy.
Food Additives & Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment, 27(5), 718–728. https://doi.org/10. 1080/19440040903573040
O'Brien, E., & Dietrich, D. R. (2005). Ochratoxin A: The continuing enigma. Critical Reviews in Toxicology, 35(1), 33–60. https://doi.org/10. 1080/10408440590905948
Ritieni, A., Moretti, A., Logrieco, A., Bottalico, A., Randazzo, G., Monti, S. M., Fogliano, V. (1997). occurrence of fusaproliferin, fumonisin B-1, and beauvericin in maize from Italy. Journal of Agricultural and Food Chemistry, 45(10), 4011–4016. https://doi.org/10.1021/jf9702151
Rubert, J., Soler, C., & Mañes, J. (2011). Evaluation of matrix solid-phase dispersion (MSPD) extraction for multi-mycotoxin determination in different flours using LC-MS/MS. Talanta, 85(1), 206–215. https://doi.

org/10.1016/j.talanta.2011.03.046
Şenyuva, H. Z., Gilbert, J., Samson, R. a., Özcan, S., Öztürko_glu, Ş., & Önal, D. (2008). Occurrence of fungi and their mycotoxins in individual Turkish dried figs. World Mycotoxin Journal, 1, 79–86. https://doi.
org/10.3920/WMJ2008.x009
Sewram, V., Nieuwoudt, T. W., Marasas, W. F., Shephard, G. S., & Ritieni, A. (1999). Determination of the Fusarium mycotoxins, fusaproliferin and beauvericin by high-performance liquid chromatography-electrospray ionization mass spectrometry. Journal of Chromatography A, 858(2), 175–185. https://
doi.org/https://doi.org/10.1016/S0021-9673(99)00814-6
Shephard, G. S. (1998). Chromatographic determination of the fumonisin mycotoxins. Journal of Chromatography A, 815(1), 31–39. https://doi. org/10.1016/S0021-9673(98)00187-3
Shephard, G. S. (2009). Aflatoxin analysis at the beginning of the twenty-first century. Analytical and Bioanalytical Chemistry, 395(5), 1215–1224. https://doi.org/10.1007/s00216-009-2857-y
Shephard, G. S., Sewram, V., Nieuwoudt, T. W., Marasas, W. F. O., & Ritieni, A. (1999). Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the Fusarium section Liseola.
Journal of Agricultural and Food Chemistry, 47(12), 5111–5115. https://doi.org/10.1021/jf9903713
Soriano, J. M., & Dragacci, S. (2004). Intake, decontamination and legislation of fumonisins in foods. Food Research International, 37(4),367–374. https://doi.org/10.1016/j.foodres.2004.01.008
Steiner, W. E., Rieker, R. H., & Battaglia, R. (1988). Aflatoxin contamination in dried figs: Distribution and association with fluorescence. Journal of Agricultural and Food Chemistry, 36(1), 88–91. https://doi.org/10.
1021/jf00079a022
Stroka, J., Van Otterdijk, R., & Anklam, E. (2000). Immunoaffinity column clean-up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices. Journal of Chromatography
A, 904(2), 251–256. https://doi.org/10.1016/S0021-9673 (00)00930-4
Trucksess, M. W., & Scott, P. M. (2008). Mycotoxins in botanicals and dried fruits: A review. Food Additives & Contaminants. Part A Chemistry, Analysis, Control, Exposure & Risk Assessment, 25(2), 181–192. https://
doi.org/10.1080/02652030701567459
Williams, L. D., Meredith, F. I., & Riley, R. T. (2004). Fumonisin-ortho-phthalaldehyde derivative is stabilized at low temperature.
Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 806(2), 311–314. https://doi.org/10.1016/j. jchromb.2004.03.052

TABLE 1 Validation results in dried figs

Compound	Calibration curve			Calibration range (µg/kg)	Validation (µg/kg)	Recovery (%)	RDS	LOD	LOQ
	a	b	r^2						
AFB_1	5,040,761	−11,862	0.9999	1–50	1, 2, 5	84	11.7	0.06	0.15
OTA	19,658,475	−9,231	0.9985	1–200	2, 10, 20	74	12.8	0.07	0.22
FB_1	838,807	6,584	0.9999	1–6.000	20, 50, 100	76	17.3	0.02	0.06
FA	18,510	−12,852	0.9999	500–20,000	50, 100, 500	86	9.5	0.83	2.52
BEA	3,929,927,045	170,178	0.9996	500–80,000	50, 100, 500	82	10.2	0.91	3.04

Abbreviations: AFB_1 = aflatoxin; BEA = beauvericin; FA = fusaric acid; FB_1 = fumonisin B_1; OTA = ochratoxin A.

TABLE 2 Number of contaminated samples with AFB_1, AFTs, and OTA and range of contamination

Samples	Number	AFB_1			Total AFs			OTA		
		Contaminated samples	>2 µg/kg[a]	Range of contamination (µg/kg)	Contaminated samples	>4 µg/kg[a]	Range of contamination (µg/kg)	Contaminated samples	>5 µg/kg[b]	Range of contamination (µg/kg)
Dried figs	28	2	-	1.14 ± 0.07–1.33 ± 0.07	4	2	1.85 ± 0.13–9.27 ± 0.46	7	1	<LOQ–41.17 ± 0.10
Baked dried figs	8	2	-	0.34 ± 0.02–1.14 ± 0.16	2	1	0.71 ± 0.02–7.46 ± 0.37	5	2	<LOQ–158.58 ± 0.16
Dried figs-based products	19	6	1	0.19 ± 0.04–8.41 ± 0.42	7	2	0.50 ± 0.03–17.12 ± 0.86	9	5	1.15 ± 0.06–139.61 ± 0.4
TOTAL	55	10	1	0.19 ± 0.04–8.41 ± 0.42	13	5	0.50 ± 0.03–17.12 ± 0.86	21	8	<LOQ–158.58 ± 0.36
Ingredients										
Cocoa	2	2	-	0.98 ± 0.02–1.08 ± 0.05	2	1	2.64 ± 0.14–4.04 ± 0.20	2	-	1.05 ± 0.02–1.46 ± 0.07
Almonds	2	2	-	0.48 ± 0.01–0.95 ± 0.03	2	-	0.52 ± 0.03–2.55 ± 0.08	2	-	1.25 ± 0.07–2.09 ± 0.10

Abbreviations: AF = aflatoxin; AFTs = total aflatoxins; OTA = ochratoxin A.
[a] Commission Regulation (EC) No. 1881/2006.
[b] Commission Regulation (EC) No. 123/2005.

TABLE 3 Number of contaminated samples with Fusarium toxins: FB_1, BEA, and FA and range of contamination

Samples	Number	FB_1			BEA		FA	
		Contaminated samples	>1,000 µg/kg[a]	Range of contamination (µg/kg)	Contaminated samples	Range of contamination (µg/kg)	Contaminated samples	Range of contamination (µg/kg)
Dried figs	28	5	2	153.81 ± 7.69–5,412.96 ± 37.30	3	<LOQ–3,979.97 ± 29.00	13	1,048.00 ± 1.05–11,283.00 ± 26.15
Baked dried figs	8	-	-	-	3	<LOQ–1,269.58 ± 7.11	4	<LOQ–20,287.72 ± 31.39
Dried figs-based products	19	3	2	249.97 ± 2.50–1,368.94 ± 25.62	6	<LOQ–5,708.49 ± 35.42	11	<LOQ–74,520.20 ± 42.08
TOTAL	55	8	4	153.81 ± 7.69–5,412.96 ± 37.30	12	<LOQ–5,708.49 ± 35.42	28	<LOQ–74,520.20 ± 42.08
Ingredients								
Cocoa	2	2	2	1,245.01 ± 9.03–1,467.15 ± 13.36	2	12,012.46 ± 10.90–16, 354.35 ± 11.72	2	27,418.07 ± 9.07–41,779.20 ± 39.96
Almonds	2	-	-	-	2	3,535.53 ± 5.27–4, 219.64 ± 11.98	2	717.40 ± 39.96–741.70 ± 7.09

Abbreviations: BEA = beauvericin; FA = fusaric acid; FB1 = fumonisin B1.
[a] Reference limit: Commission Regulation (EC) No. 11.