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Evaluation of fungal hazards associated with dried fig processing

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ABSTRACT

The processing of dried figs in the industry involves a number of stages that present a significant risk of filamentous fungal infection of the fruit and subsequent mycotoxin contamination, due to the changes in temperature and water activity (a_w) to which dried figs are exposed. In this study, the environmental conditions and the physicochemical parameters of dried figs at different processing stages were evaluated in 3 different industries, and were associated with fungal counts and the presence of toxigenic moulds and their mycotoxins. For this, dried figs at 5 relevant stages of industrial processing (curing, sizing, blanching, storage, and final product) in 3 industries located in Extremadura (Spain) were sampled. Changes in moisture content and a_w of dried figs during processing were observed and they influenced the mycological quality of figs. Among the fungal genera, *Aspergillus* spp. predominated in most stages except blanching, where *Penicillium* spp. prevailed. About 10% of the dried fig samples were contaminated with aflatoxins (AFs) and 6% with ochratoxin A (OTA). Based on findings, longer drying times are necessary after blanching to reduce a_w and to avoid the development of toxigenic moulds. In addition, all stages covering industry processing, final storage, and retailing of dried figs are advisable to be conducted at refrigeration conditions and controlled relative humidity to avoid mycotoxin production. The enumeration of AFs- and OTA- producing moulds by real-time PCR seems to be a good indicator for integration into prevention strategies to control filamentous fungal hazards and subsequent mycotoxin synthesis during the processing of dried figs.

1. Introduction

Commercial fig production takes place in areas with a predominantly Mediterranean climate (Mat Desa et al., 2019) such as California, Australia, South America, and the Mediterranean countries (Ersoy et al., 2017). Spain is the leader in European production being the region of Extremadura the main national producer of dried figs (MAPA, 2020). Calabacita is the most cultivated variety in this area due to its sweet taste and small size, allowing a uniform drying (Galván et al., 2021). Calabacita's characteristics favours a lower moisture content, and this is essential to minimize the fungal contamination and spoilage of dried figs. Traditional figs drying starts with the fruit senescence in the tree and continues to the ground, being harvested by hand or mechanically partially dried. Once harvested, the drying process is completed in solar dehydrators or in domestic storage rooms until the fig reaches a moisture content of ≤26%, in accordance with Standard DDP-14 on the commercial and marketing quality control of dried figs (UNECE, 2016). At industrial level in Extremadura, dried figs are first cured with aluminium or magnesium phosphide (phosphine) for pest control, and the stored at room temperature without humidity control until they are processed. Dried figs are usually blanched, hot-air dried, stored at room temperature and then placed onto the market to be commercialized with a shelf-life of around 6 months at room temperature.

The temperature and humidity conditions during the processing and storage of dried figs can favour the proliferation of toxigenic fungi and further mycotoxin production. In fact, mycotoxins are the most hazardous compounds that can occur in dried figs, throughout both pre- and postharvest (Bircan, 2009), being aflatoxins (AFs) and ochratoxin A (OTA) the most frequent mycotoxins found (Bircan, 2009; Drusch and Aumann, 2005; Kabak, 2016; Scott and Trucksess, 2009; Senyuva et al.,

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2005). The incidence of these toxic fungal metabolites leads to economic losses of producers due to the rejection of exported contaminated products (Heperkan et al., 2012). In addition, other relevant mycotoxins reported in dried figs are, fumonisin B_1, kojic acid, fusaric acid, and tenuazonic acid (Di Sanzo et al., 2018; López et al., 2016b; Sulyok et al., 2020).

Due to their toxicity and frequency in figs, several countries have set up regulations for mycotoxins in order to protect the consumers' health (Trucksess and Scott, 2008). The limits for these toxin compounds may vary between countries, as well as the type of mycotoxin. The maximum levels established by European Union (EU) for AFs in dried figs are 6 μg/kg for aflatoxin B_1 (AFB_1) and 10 μg/kg for total AFs (AFB_1, aflatoxin B_2, aflatoxin G_1, aflatoxin G_2) (EU, 2012). In the case of OTA, although no limit has been yet established in dried figs, the EU has started to discuss the products that must be subjected to a maximum level of this metabolite, including dried figs (EFSA, 2020). In other products like dried figs such as dried vine fruits (currants, sultanas) the maximum level of OTA has been set up 10 μg/kg (EC, 2006).

Therefore, from a food safety perspective, it is of utmost importance to identify the hazards that may occur during the processing of dried figs in order to design control mechanisms if necessary (Ropkins and Beck, 2000). The different stages in the processing of dried figs in the industry, such as blanching and long storage periods, combined with inadequate temperatures and humidity, could favour the growth of toxigenic moulds and greater mycotoxin contamination of dried figs both at storage and at fruit retailing. De Mello and Scussel (2007) in a study on the assessment of mycotoxins in dried fruits observed that fungal contamination was due to their intrinsic characteristics of moisture and nutrient content, long storage time, and high water activity (a_w) values. The contamination of dried fruits starts on the tree and continues during storage as a result of poor drying and storage conditions, including both high temperatures and relative humidities. Besides, rehydration of the dried product under unsuitable conditions of storage may reactivate the fungal growth with subsequent mycotoxin contamination (Karaca et al., 2010). Once mycotoxins are produced, they cannot be removed from the product during processing. In most industries, bright greenish-yellow fluorescence is commonly used in a preliminary screening of AFs contaminated dried figs (Mat Desa et al., 2019) despite certain types of AFs could not be detected (Trucksess and Scott, 2008). In this sense, a study of the processing stages of dried figs would be useful to evaluate which are the critical stages in relation to their microbiological quality. Thus, an effective preventive system could be established leading to a safer production of dried figs. In order to implement a HACCP system, it is essential to have a good understanding of the processing of the product and to determine which critical steps need to be controlled. To the best our knowledge, there is no scientific literature that evaluates the safety and quality of dried figs throughout the manufacturing process. Within this framework, the aim of this study was to evaluate the impact that different processing stages of dried figs has on fungal growth and mycotoxin production in order to establish efficient control actions to ensure the production of safely dried figs.

2. Materials and methods

2.1. Sampling

Dried fig samples were collected along the industry's processing line from three different producers during two consecutive seasons (2018 and 2019) located at the main producing areas of Extremadura such as Almoharín (39°10′35″N; 6°02′39″O, 39°10′35″N; 6°02′39″O) (Industry A), Villar de Rena (−5.81178 39° 4′ 36″ N; 5° 48′ 42″ O) (Industry B) and Jerte Valley (40°08′23″N, 5°52′50″O) (Industry C) (Spain). Five kg of sample were collected from the main processing stages in each industry: curing, sizing, blanching, stored product, and final product (Table 1). Sampling was carried out by triplicate in each industry and each year of study from different random batches, taking two samples from each stage. The samples were immediately transported to the laboratory facilities and homogenized using a Mortar Grinder Pulverisette 2 (Fritsch, Germany). From these homogenates, instrumental triplicates were carried out for each analysis.

2.2. Physico-chemical determinations

Water activity was determined using an a_w meter (LabMASTER-a_w, Novasina AG, Lachen, Switzerland). Moisture content was determined according to the AOAC Official Method 934.01 (AOAC, 2005) by oven drying for 24 h at 104 °C and expressed as a percentage (%).

2.3. Mould and yeast counts

Ten grams of sample were homogenized in 90 mL of sterile peptone water. Then, serial dilutions were carried out with 0.1% (*w*/*v*) peptone water, and 0.1 mL aliquots were placed in acidified potato dextrose agar (PDA, Scharlab, Barcelona, Spain) to pH 3.5 and rose bengal plates with chloramphenicol (RBCA; Oxoid). Plates were incubated at 25 °C for 5 days, and the results were expressed as log cfu/g.

2.4. Isolation and identification of moulds

2.4.1. Isolation

Four-five isolates were randomly taken from each acidified PDA plate from the highest dilutions. Each colony was transferred onto a new acidified PDA plate for obtaining twice pure colonies. The isolates were stored in 50% sterile glycerol (*v*/v) at −80 °C until required.

Table 1
Production process of dried figs and differences between the three industries studied (A, B, C).

<table>
<tr><th rowspan="2">Industry</th><th colspan="11">Dried figs production process</th></tr>
<tr><th>Curing</th><th>Storage product</th><th>Sizing</th><th>Washing</th><th>Blanching</th><th>Cooling shower</th><th>Drying tunnel</th><th>BGY-F and metal detector</th><th>Stored product</th><th>Floured and Packing</th><th>Final product</th></tr>
<tr><td>A</td><td rowspan="3">Phosphine fumigation (1 g phosphine/ t) (~ 7 days of exposure at room temperature)</td><td rowspan="3">Room temperature until product processing (1–8 weeks)</td><td rowspan="3">Sorting dried figs by size using special equipment</td><td rowspan="3">Washing with tap water for 1 min</td><td>100 °C for 1 min.</td><td rowspan="3">Showers with running water for 1 min</td><td rowspan="3">Hot air drying of figs for 1 min</td><td>Detection of metals</td><td>Room temperature (± 25 °C)</td><td rowspan="3">Figs with flour and packed in bags</td><td rowspan="3">Dried figs with rice flour</td></tr>
<tr><td>B</td><td>70 °C for 1 min.</td><td>Detection of aflatoxins and metals</td><td>Refrigeration chamber (± 0 °C)</td></tr>
<tr><td>C</td><td>100 °C for 1 min.</td><td>Detection of aflatoxins and metals</td><td>Refrigeration chamber (± 8 °C)</td></tr>
</table>

2.4.2. Identification

2.4.2.1. DNA extraction. Genomic DNA from mould isolates was extracted using the quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, California, USA) according to the manufacturer's instructions. To measure the concentration (μg/μL) and purity (A_{260}/A_{280}) of DNA, a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used.

2.4.2.2. PCR. Inter-simple sequence repeat (ISSR)-PCR was performed using the primer $(GTG)_5$ as described by (Cabañas et al., 2020), following the PCR conditions described by (Gallardo et al., 2014). Amplification products were visualized after electrophoresis in 1.5% agarose gels, by staining with Midori Green Advance (Nippon, Japan). The GeneRuler 100 bp plus DNA ladder (Thermo Fisher Scientific, San Jose, CA, USA) was used as a reference. The fragment profiles obtained were grouped into operational taxonomic units (OTUs). Cluster analysis of the bands of each isolate was carried out using the unweighted pair group method with arithmetic averages (UPGMA). The analysis was performed using the software NTSYSpc version 2.0. Three representative isolates of each OUT were identified to species level by sequencing the ITS1–5.8 rDNA ITS2 region using the primers pairs ITS1 and ITS4 described by (White et al., 1990) and β- tubulin genes using the forward and the reverse primers Bt2a and Bt2b described by (Glass and Donaldson, 1995). PCR reactions were run in a T100™ thermal cycler (Bio-Rad, Hercules, CA, USA). The DNA obtained was purified using the GeneJET PCR purification kit (Thermo Fisher Scientific, USA) and sequenced by the Service of Bioscience Applied Techniques (STAB) at the University of Extremadura (Badajoz, Spain).

2.4.2.3. Sequence analysis. Sequences were analyzed using Bioedit version 7.2 and checked by nucleotide-nucleotide BLAST comparison at the NCBI database. The identities of the isolates were determined on the highest score and > 97% similarity was used as the criterion for species identification. For the confirmation of *Aspergillus* section *Flavi* species, specific PCR assays were carried out using primers FLA1/FLA2 for *A. flavus* as described by González-Salgado et al. (2008). *A. niger* and *A. welwitschiae* species belonging to *Aspergillus* section *Nigri* species could be distinguished using the *β-tubulin* gene as described (Perrone et al., 2011).

2.5. qPCR assays to quantify potentially toxigenic moulds

2.5.1. DNA extraction

DNA extraction from dried figs was carried out with the quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research) with some changes. Five grams of dried figs were weighed and homogenized in a Stomacher 400 (Lab Blender, Model 4001, Seward Medical, London, UK) with 20 mL of Tris Buffer Ph 8. Two mL of this mixture was then transferred to an Eppendorf and centrifuged in an Eppendorf Centrifuge 5424 R (Eppendorf AG, Germany) at 15,871*g*/10 min. The supernatant was discarded, and the pellet was resuspended in 300 mL of nuclease-free water (Thermo Fisher Scientific, USA). The protocol of the kit was then followed according to the manufacturer's instructions, except that just before beating with the 1600 MiniG mixer (Automated Tissue Homogenizer and Cell Lyser, SPEX, Metuchen) for 5 min and 1500 rpm, 10 μL of proteinase K were added (20 mg/mL, Thermo Fisher Scientific, USA), followed by an incubation step at 65 °C for 45 min. The DNA concentration and quality were assessed by the Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). DNA was stored at −20 °C until use.

2.5.2. qPCR reactions

The Applied Biosystems 7300 Fast real-time PCR system (Applied Biosystems, USA) was used for qPCR amplification and detection. To detect the presence of the AFs producers, primer pairs F-omT and R-omT (200 nM) were used to amplify the *aflP* gene of *Aspergillus* section *Flavi* (Rodríguez et al., 2012). The primers ANPKSFRT and ANPKSRRT (300 nM) were used to amplify the *pks* gene of *Aspergillus* section *Nigri* (Castellá and Cabañes, 2011). The SYBR Green methodology was used. Reaction mixtures were prepared in triplicate of 12.5 μl reaction mixture in 96-well optical MicroAmp reaction plates and sealed with optical adhesive caps (Applied Biosystems). The reaction mixture consisted of 7.5 μL NZY qPCR Green Master Mix 2× (NZYTech, Lisbon, Portugal), different concentrations of each primer, and 2.5 μL of DNA template. Three negative controls without DNA were included in the runs. An initial denaturation step of 10 min at 95 °C, was followed by 40 cycles performed according to the following temperature regime: 95 °C for 15 s and 60 °C for 30 s. After the last PCR cycle, melting curve analysis of the PCR products was carried out by heating to 72–95 °C and continuous measurement of the fluorescence to verify the PCR product. Quantitative cycle (Cq) determinations were automatically performed by the 7300 System Software (Applied Biosystems).

2.5.3. Potential aflatoxigenic and ochratoxigenic mould quantification

Quantification of potential OTA- and AFs- producing moulds was conducted by using standard curves relating Cq values and tenfold dilutions of potential ochratoxigenic and aflatoxigenic moulds (log cfu/g) of known standards, respectively. qPCR reactions were conducted as described in Section 2.5.2. Finally, the ochratoxigenic and aflatoxigenic mould counts were extrapolated from their respective standard curves.

2.6. Mycotoxin analyses

2.6.1. Extraction

For the extraction of AFs and OTA, 100 g of dried figs were mixed with 400 mL extraction solution (MeOH: H_2O, 8:2, *v*/v) and 5 g of NaCl, shaken in the dark for 30 min, and filtered through filter paper (Whatman no.4). Next, 36 mL of phosphate buffer solution (PBS) (Thermo Fisher Scientific, USA) was added to the 4 mL filtrate. The pH was adjusted to 7.4 with NaOH (2 M). The solution was eluted onto the immunoaffinity column AFLAOCHRA PREP® (R-Biopharm AG, Germany) at 1–2 drops/min, followed by washing with 20 mL PBS. The elution was carried out with 1 mL of HPLC grade methanol (Thermo Fisher Scientific, USA) followed by 1 mL of water.

2.6.2. Quantification

AFs and OTA were analyzed on an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with Agilent 1200 series fluorescence detector G1321A. The separation was achieved using a Supelcosil LC-18 column, 15 cm × 4.6 mm, 5 μm (Supelco, Bellefonte, USA). For analysis of AFs, a mobile phase containing a mixture of MeOH: ACN:water (20:20:60 *v*/v/v/) was used at an isocratic flow rate of 1 mL/min. In addition, post-column derivatization with 0.05% pyridinium bromide (*w/v*; Sigma) was pumped at 0.3 mL/min on an Agilent 1100 Series HPLC device. For the analysis of OTA, a mobile phase in an isocratic mode composed of water/acetonitrile/acetic acid (41:57:2 *v*/v/v) was used. Finally, 100 μL of extract were injected into HPLC for both AFs and OTA analyses. Fluorescence (FLD) detection was carried out using 333 nm (excitation) and 460 nm (emission). Calibration curves for AFs and OTA were built with a standard mixture (Sigma-Aldrich, St. Louis, MO). The detection limits were 0.07 μg/kg for AFB_1, 0.3 μg/kg for AFB_2, and 0.38 μg/kg for OTA.

2.7. Statistics

Microbiological count data were treated to a one-way analysis of variance (ANOVA) using SPSS for Windows, 25.0. Afterward, Tukey's test was applied to compare the mean values obtained and the level of significance was set at $p \leq 0.05$.

3. Results and discussion

To the best of our knowlewdge, this is the first study that in-depth analyzes the processing of dried fig to evaluate the filamentous fungal hazards that may suppose an important risk in consumer's health. In this work, the environmental conditions and physicochemical parameters of dried figs at different processing stages in 3 different industries were evaluated and associated with fungal counts and the presence of toxigenic moulds and their mycotoxins.

3.1. Moisture content and water activity

As shown in Fig. 1, a similar pattern of moisture content (%) and a_w values were observed in the industries studied, finding the main differences in the blanching and storage stages. The blanching temperature in industry B was 70 °C while in industries A and C was 100 °C (Table 1). Regarding storage temperatures, they were 25 °C in industry A, 8 °C in industry C and 0 °C in industry B (Table 1). Moisture content values ranged from 22.88 to 34.55% and their values increased slowly throughout the sequential stages of the dried fig processing (from curing to blanching) reaching the highest ones ($p \leq 0.05$) at the blanching stage, especially in industry C with a value of 34.55% (Fig. 1). Then, moisture content values dropped slightly until obtaining the final product values between 30.85% for industry C and 26.76% for industry B. According to the standard DDP-14 on the control of the commercial quality and marketing of dried figs (UNECE, 2016) the moisture content should be less than 26% in the final product. However, in this work, the moisture content of dried figs in the three industries studied was higher than 26%, probably due to the short time of drying (1 min) after the blanching stage for the three industries studied. In a study about the influence of blanching on the drying and rehydration of banana slices, the drying time was 6 h at 50, 60, and 80 °C and the moisture content losses ranged between 30% and 45%, depending on the temperature (Taiwo and Adeyemi, 2009). In another study focused on the effect of sun-drying on some quality parameters in dried figs, the moisture content in dried yellow-skinned figs was 17.3% (Kamiloglu and Capanoglu, 2015). Blanching is one of the most widely used methods (Kidmose and Martens, 1999), mainly to inactivate enzymes and reduce the microbial load (Xu et al., 2012). However, it has been described that changes in environmental conditions during processing or inadequate blanching can lead to the development of mycotoxigenic moulds (*Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp.) (Heperkan et al., 2012; López et al., 2016a) and further mycotoxin production such as AFs and OTA, which are the most frequently found in dried figs (Bircan, 2009; Gilbert and Senyuva, 2008; Rahimi and Shakerian, 2013; Turkoz Bakirci, 2020).

With respect to a_w values, their evolution was similar to the moisture content (Fig. 1). After a slow rise in the a_w values until the blanching stage, there was a steady decline until the final product was obtained, reaching a_w values between 0.65 a_w in industry B and 0.69 a_w in industry A. These a_w values are higher than those recommended by Jay et al. (2005) who reported that dried foods should be stored at a_w values <0.60, In addition, the relative humidity conditions of the chamber should be controlled in order to avoid increases in a_w value of the product favouring mould growth on the surface of the product. Again, the greatest a_w values in the three industries were observed at the blanching stage, ranging from 0.65 a_w for industry B to 0.72 a_w for industries A and C.

3.2. Mould and yeast counts

The mean values of mould and yeast counts (log cfu/g) at the different stages of dried fig processing revealed significant differences between stages and industries (Table 2). In dried fig processing, several factors can occur that lead to fungal or yeast proliferation (Gilbert and Senyuva, 2008). Regarding mould counts, significant increases ($p \leq 0.05$) were found at the curing and final product stages in the industries. Besides, a significant decrease ($p \leq 0.05$) in the counts from the sizing to the blanching stage in industries A and B was observed. The highest mould counts were encountered in the stored product, with values ranging from 2.88 to 3.13 log cfu/g, although no significant effect of the storage temperature on mould counts was observed. Neme and Mohammed (2017) showed that low or cold temperatures have fungistatic properties, not fungicides, so fungal growth and metabolism are enormously minimized. However, it should be noted that mould counts in the final product in industry B, with a storage temperature of 0 °C, were higher ($p \leq 0.05$) than in the other two industries. This may be because microbes surviving the drying process may remain inactive for long periods and become active once the food is rehydrated (Prabhakar and Mallika, 2014). Therefore, it seems clear that proper refrigeration conditions of dried figs are extremely necessary to avoid fungal hazards in this product. Mould counts of this work are similar or slightly lower than those found in other studies. Guirguis (2018) in a study based on the microbiological quality of commercial dried figs found mould and yeast counts of 4.6 log cfu/g. Villalobos et al. (2019) in a study about the influence of different fig drying methods reported similar mould counts in the control treatment (completely dehydrated figs). However,

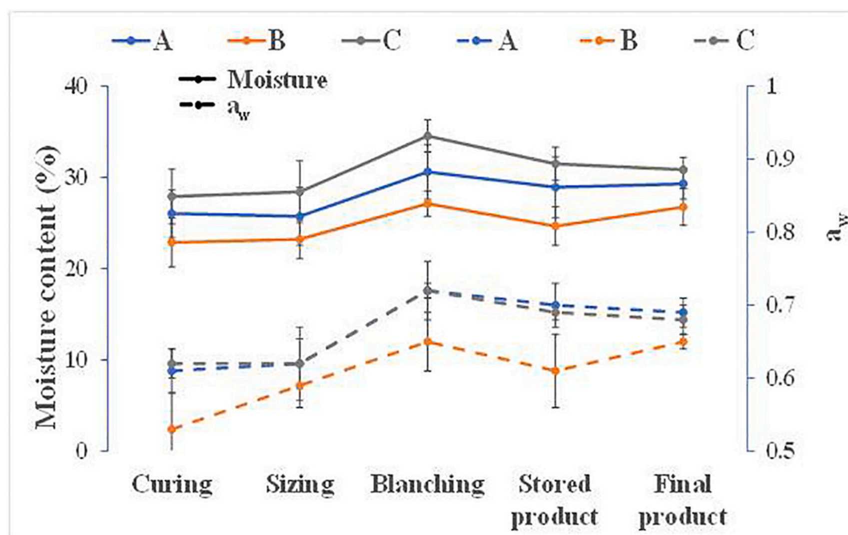

Fig. 1. Mean values of moisture content (%) and water activity (a_w) at the different stages of the dried fig production process in the three industries evaluated.

Table 2
Mean values of mould and yeast counts (log cfu/g) at the different stages of dried fig processing in the three industries evaluated.

	Industries	Stages					p
		Curing	Sizing	Blanching	Stored product	Final product	
Mould	A	3.05^{a}A	2.96^{a}	2.21^{b}	3.13^{a}	2.27^{b}B	0.000
	B	2.65^{ab}B	2.96^{a}	2.03^{b}	2.81^{a}	3.10^{a}A	0.000
	C	2.16^{ab}C	2.73^{ab}	2.40^{ab}	2.88^{a}	2.03^{b}B	0.030
	p	0.000	0.639	0.138	0.068	0.000	
Yeast	A	2.25B	2.71	2.54	2.57AB	2.44B	0.539
	B	2.11^{b}B	2.16^{ab}	3.08^{a}	2.00^{b}B	2.44^{ab}B	0.006
	C	3.26^{ab}A	2.46^{b}	2.83^{ab}	2.91^{ab}A	3.61^{a}A	0.008
	p	0.001	0.285	0.189	0.021	0.000	

Limit of detection: 2 log cfu/g.
Different capital letters (A,B) indicate significant differences between industries within the same stage **p** means statistical significance.
Different letters (a, b, c) indicate significant differences between stages within the same industry.

(Öztekin et al., 2006) encountered initial yeast and mould counts in dried figs around 1.46 log cfu/g.

Regarding yeast counts, significant differences were found between industries and processing stages at the curing, stored product, and final product stages ($p \leq 0.05$). In addition, significant differences between some of them were observed in both industries B and C ($p \leq 0.05$). The highest yeast counts were found in industry C in all stages studied where there were significant differences between industries. This fact may be because products from industry C possessed the highest moisture content at all stages.

3.3. Identification of moulds

The prevalence of mould species in the three studied industries and in the processing stages (curing, sizing, blanching, stored, and final products) are shown in Figs. 2 and 3. A total of 231 strains were identified, 88 belonging to industry A, 71 to industry B and 72 to industry C. The identification of moulds is especially important to provide information on which mycotoxins might be present. Identification was done by sequencing DNA regions amplified with the primers pairs ITS1 and ITS4 and Bt2a and Bt2b. Dried figs provide a suitable medium that favours the growth of a wide range of moulds. The predominant genus in industries A and B was *Aspergillus* spp. with prevalence rates of 54.6% and 76%, respectively. Next, *Penicillium* spp., *Cladosporium* spp. and *Alternaria* spp. were the most important ones with 25%, 13.6%, and 6.8%, respectively, in industry A and 18.3%, 4.3%, and 1.4% in industry B (Fig. 2). However, in industry C the genus *Penicillium* spp. was the predominant with a prevalence rate of 50%, followed by *Aspergillus* spp., *Alternaria* spp. and *Cladosporium* spp. with 33.3%, 11.1%, and 5.6%, respectively (Fig. 2). Regarding the processing stages of dried figs, the principal genus was *Aspergillus* spp. in all stages except in the blanching

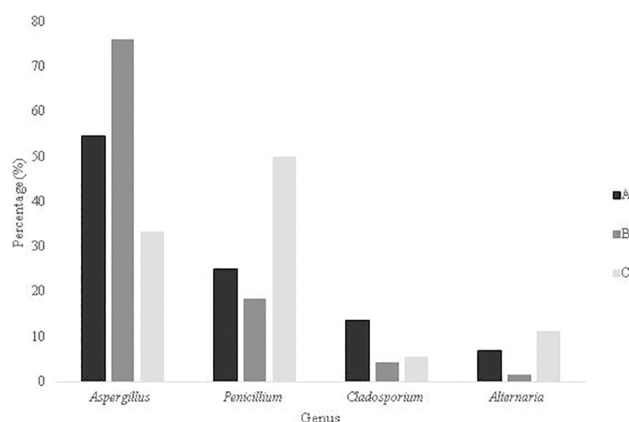

Fig. 2. Percentage of mould genus frequencies in the three industries (A, B and C) studied.

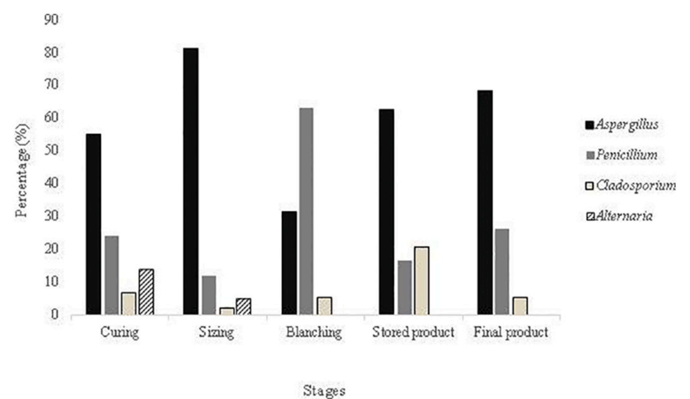

Fig. 3. Percentage of mould genus frequencies in the analyzed dried fig processing stages (Curing, Sizing, Blanching, Stored product, Final product) in the three industries (A, B and C). The values reflect the mean of the three sampled companies.

stage, where the genus *Penicillium* spp. prevailed (Fig. 3). The highest percentages of *Aspergillus* spp. were found in the sizing and final product stages with 81% and 68.4%, respectively. *Alternaria* spp. was only observed in the curing and sizing stage with 13.8% and 4.8%, respectively (Fig. 3). In dried date fruits collected in Perugia (Italy) the presence of mould species belonging to *Aspergillus*, *Penicillium* and *Cladosporium* had been previously described (Quaglia et al., 2020). Besides, Javanmard (2010) in a study regarding the occurrence of mould species on Iranian dried figs at different stages of production and processing, observed that *Aspergillus* spp. comprised 34.4% of the total fungal population.

The main mycotoxin-producing fungal genera found were *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp. (Table 3). In the case of *Aspergillus* spp., *A. tubingensis*, *A. niger*, *A. welwitschiae* and *A. flavus* were the prevailing mould species in the three industries. In industry A the predominant species of the genus *Aspergillus* spp. were *A. tubingensis* (36.8%) and *A. flavus* (7.9%). In industry B, *A. tubingensis* was the most predominant (42.6%) followed by *A. welwitschiae* and *A. flavus* (10.3%), while in industry C, *A. welwitschiae* and *A. flavus* were the main *Aspergillus* species with 11.8%. Heperkan et al. (2012) reported that *A. flavus* was the dominant species among dried fig samples. Senyuva et al. (2008) observed that *A. niger* is the most isolated fungus in Turkish dried figs. Out of these species, *A. flavus* produces AFB_1, AFB_2 and cyclopiazonic acid (CPA) (Vaamonde et al., 2003), *A. niger* and *A. welwitschiae* have been described as OTA producers (Perrone et al., 2011). In the case of *A. tubingensis,* this species seems to produce OTA in some experimental conditions (Chiotta et al., 2011; Lasram et al., 2012); however, Storari et al. (2012) reported that such species is not a mycotoxin producer. Although in California, the main source of OTA contamination in

Table 3
Identification of the mould species isolated in the three industries and the different stages of dried fig processing studied.

Number of isolates (%)													
			Industries				Stages					GeneBank accession numbers	
Genus	Species	Primers	A	B	C	TOTAL	Curing	Sizing	Blanching	Stored product	Final product	ITS	Btub
	A. welwitschiae	2*	5.3	10.3	11.8	8.9	3.7	9.8	–	5.3	27.7	MH374611.1	MG832179.1
	A. niger	2*	5.3	57.4	5.9	6.5	–	12.3	–	10.5	5.6	MT447518.1	LC573662.1
	A. tubingensis	2*	36.8	42.6	5.9	35.8	33.3	51.3	16.6	42	16.5	LC573618.1	LC387873.1
	A. ochraceus	2*	2.6	–	–	0.8	3.7	–	–	–	–	MH864932.1	KJ775051.1
Aspergillus	*A. flavus*	1*	7.9	10.3	11.8	9.8	3.7	7.3	16.6	10.5	16.5	CP044617.1	CP059871.1
	A. tamarii	2*	5.3	2.9	–	3.3	3.7	2.4	–	10.5	–	MH865259.1	EF661474
	A. calidoustus	4*	–	1.5	–	0.8	3.7	–	–	–	–	–	MN735972.1
	A. foetidus	2*	–	1.5	–	0.8	3.7	–	–	–	–	MH854624.1	DQ768454.1
	A. transmontanensis	2*	–	1.5	–	0.8	–	–	–	–	5.6	JF412771	HM803101
	P. citrinum	2*	10.5	4.4	5.9	6.5	14.8	–	11.1	–	11.1	MH864240.1	GU944545.1
	P. expansum	2*	–	8.8	11.8	6.5	–	–	33.3	5.3	5.6	DQ339562.1	AY674399.1
	P. corylophilum	2*	2.6	1.5	–	1.6	–	2.4	5.6	0	–	MF475922.1	GU944519.1
	P. glabrum	2*	7.9	–	11.8	4	7.5	2.4	5.6	5.3	–	MH854998.1	GQ367502.1
Penicillium	*P. crustosum*	2*	5.3	–	11.8	3.3	–	2.4	5.6	5.3	5.6	MH857883.1	AY674351.1
	P. chrysogenum	3*	2.6	2.9	–	2.5	3.7	2.4	–	5.3	–	MH865982.1	–
	P. menonorum	2*	–	–	5.9	0.8	–	–	5.6	–	–	HQ646591.1	HQ646573.1
	P. janczewskii	3*	–	1.5	–	0.8	–	2.4	–	–	–	MK179261.1	–
	P. bilaiae	2*	0	–	5.9	0.8	–	–	–	–	5.6	AF033402.1	KC773834.1
Alternaria	*A. alternata*	2*	7.9	1.5	11.8	4.9	14.8	4.9	–	–	–	MW326089.1	KY814627.1

1*ITS1F/ITS4, Bt2a/Bt2b, FLA1/FLA2 2* ITS1F/ITS4, Bt2a/Bt2b 3* ITS1F/ITS4 4* Bt2a/Bt2b.

dried figs was *Aspergillus alliaceus* (Bayman et al., 2002), this mould has not been isolated in any of the three industries studied in Extremadura.

Regarding the *Penicillium* genus, the most predominant species found in dried figs were *P. citrinum*, *P. expansum*, *P. glabrum* and *P. crustosum* with differences between industries. *P. citrinum* (10.5%) and *P. expansum* (8.8%) were the main species encountered in industries A and B, respectively; whereas in industry C, *P. expansum* (11.8%), *P. glabrum* (11.8%) and *P. crustosum* (11.8%) were the most prevailing ones. *P. citrinum* produces citrinin (Perrone and Susca, 2017), *P. expansum* is the most important source of patulin and can also produce citrinin (Perrone and Susca, 2017; Watanabe, 2008) and *P. crustosum* produces roquefortine C (Perrone and Susca, 2017).

With respect to *Alternaria* spp. genus, only *A. alternata* was found, being more found in industry C (11.8%), followed by industries A and B with 7.9% and 1.5%, respectively. Heperkan (2006) reported that the dominant mould population on Turkish dried figs belonged to *Aspergillus* section *Nigri*, *Aspergillus* section *Flavi*, *Fusarium* spp., and *Penicillium* spp.

Regarding the predominant moulds in the different stages studied, *A. tubingensis* was the prevailing species of *Aspergillus* genus in all stages except in the final product stage, where *A. welwitschiae* was predominant. Concerning *Penicillium* genus, the species *P. citrinum* was the most frequently encountered at the curing and final product stages while *P. expansum* was at the blanching stage. *A. alternata* was only identified at the curing (14.8%) and sizing (4.9%) stages. In the last years, some reports have also informed about the presence of *Alternaria* spp. in dried figs (López et al., 2016a). The differences between the prevalence and the heterogenicity of mould species found in the three industries studied could be due to the different geographical areas of dried fig production as reported by Ozer (2008). In addition, differences in blanching and storage temperatures with respect to the prevalence of mould species between industries may be associated with the temperature changes to which dried figs are exposed and storage times (Kesho et al., 2019). In fact, there was no treatment during processing able to remove the presence of moulds on the product surface.

3.4. Detection and quantification of aflatoxin- and ochratoxin A-producing moulds by qPCR

Results regarding detection and quantification of AFs- and OTA-producing moulds by qPCR by using SYBR Green methodology are shown in Table 4. The analysis revealed the high co-occurrence of samples contaminated by AFs- and OTA- producing *Aspergillus* species. AFs-producing moulds were detected in the three industries studied at the curing, blanching and storage stages. In addition, in industry C, they were also detected at the sizing stage. In general, *A. flavus* were found in samples where AFs-producing moulds were detected. The counts determined by qPCR ranged between <LOD and 5 log cfu/g, obtaining the highest values (3–5 log cfu/g) in industry C. This fact may be due to a higher moisture content of dried figs at all stages in industry C, which can influence their a_w, promoting fungal growth. In addition,

Table 4
Detection and quantification of aflatoxin- and ochratoxin A- producing moulds (log cfu/g) at different stages of dried fig processing with the specific primers F-omt/R-omt and ANPKSFRT/ANPKSRRT in the three industries studied.

	Industries					
	A		B		C	
Stages	Aflatoxin	Ochratoxin A	Aflatoxin	Ochratoxin A	Aflatoxin	Ochratoxin A
Curing	[c]12.5%	[b]37.50%	[b]25%	[b]25%	[c]37.5%	[c]37.5%
Sizing	[a]<LOD	[a]<LOD	[a]<LOD	[c]25%	[c]12.5%	[a]<LOD
Blanching	[c]12.5%	[a]<LOD	[c]50%	[c]12.5%	[c]25%	[a]<LOD
Stored product	[b]25%	[a]<LOD	[c]25%	[a]<LOD	[c]25%	[c]12.5%
Final product	[a]<LOD	[b]12.5%	[a]<LOD	[a]<LOD	[a]<LOD	[a]<LOD

[a] LOD = 1 cfu/g (0 log cfu/g).
[b] Aflatoxigenic mould counts (1–3 log cfu/g) and ochratoxigenic mould counts (0–1.5 log cfu/g).
[c] Aflatoxigenic mould counts (3–5 log cfu/g) and ochratoxigenic mould counts (1.5–3 log cfu/g).

environmental conditions during processing and storage are well known to influence the growth and biosynthesis of toxins (Hope and Magan, 2003). High relative humidity and temperature values can favour mould growth in dried figs (Rodríguez et al., 2012).

Regarding the detection of OTA-producing moulds in the dried fig samples, the counts quantified by qPCR ranged from <LOD to 3 log cfu/g. The incidence of OTA producers was higher at the curing stage in the three industries studied. Additionally, the counts were higher in industry B in the sizing and blanching stages and in industry C in the curing and stored product stages. Industry A had the lowest incidence of both OTA- and AFs- producing moulds. Overall, our results indicated a high incidence of toxigenic *Aspergillus* in dried figs. Similarly, other studies have also observed high counts of these mycotoxin-producing mould species (Iamanaka et al., 2005; Saadullah and Abdullah, 2015).

3.5. Aflatoxin and ochratoxin A detection

The percentage of samples contaminated with AFs and OTA at the different processing stages of the dried fig in the three industries studied are shown in Table 5. Although the presence of mould does not always indicate the presence of mycotoxins, it does indicate a potential mycotoxin hazard. In this context, approximately 10% of the dried fig samples were contaminated with AFs and 6% with OTA. AFs concentrations ranged from < LOD to 75 μg/kg for AFB_1 and < LOD to 22 μg/kg for AFB_2. About OTA concentrations, they varied from < LOD to 50 μg/kg. In the EU, the legislation setting limits for mycotoxins in food is Regulation (EC, 2006). The maximum allowed content for total AFs (B_1, B_2, G_1 and G_2) in dried figs is 10 μg/kg while for aflatoxin B_1 is 6 μg/kg. However, although other dried fruits such as dried grapes and nuts are covered by this European Regulation with regard to OTA, maximum amounts of this mycotoxin in dried figs are currently not regulated. Nevertheless, some countries such as Germany have set OTA limits in dried figs at 8 μg/kg (Bundesgesetzblatt Jahrgang, 2004).

The highest amounts of AFs (AFB_1 50–75 μg/kg and AFB_2 6–12 μg/kg) were detected at the blanching and final product stages in industry B. OTA amounts were mainly detected at the curing stage in industries A (37,5%) and C (12,5%), and at the sizing (25%) and blanching (12,5%) stage in industry B. The highest amounts of OTA (25–50 μg/kg) were found at the curing stage in industry A and at the sizing stage in industry B. The incidence of AFs and OTA observed in this study in dried figs agrees with the results of recent studies. Bircan (2009) analyzed the presence of AFs in dried figs destined for exportation from Turkey and found that 32% of the samples contained AFs with levels ranging from 0.2 to 260 μg/kg. In a similar study, dried fig samples collected from the Aegean region of Turkey were found to be contaminated with AFs with a frequency similar to that found in this study (10%). Özay and Alperden (1991) detected OTA in only 3% of the dried fig samples from the Aegean Region at levels 5.2–8.3 μg/kg in the harvest during 1988. A high incidence of OTA contamination was reported by Karbancıoğlu-Güler and Heperkan (2008), 44.3% of the samples contained OTA within the range of 0.12–1 μg/kg, while 3.5% had levels above 8 μg/kg. Bircan (2009))detected that 18% of dried fig samples collected from different exporting companies prior to the packaging were contaminated with detectable levels of OTA in the range of 0.87–24.37 μg/kg. Senyuva et al. (2005) reported the presence of 14–15% OTA contaminated samples with a maximum concentration of 26 μg/kg. In industry A, OTA had a higher incidence than AFs. The optimum temperature for OTA production by *Aspergillus* section *Nigri* is 15 °C–30 °C in peanut seeds, and the maximum concentration was observed at 25 °C (Astoreca et al., 2010).

In the curing stage, dried figs were kept approximately for 4–5 days at environmental temperature. This could result in the development of moulds and consequently the production of mycotoxins at later stages. In general, AFs occurred with a relatively greater incidence than OTA in industry B, whereas both AFs and OTA had the same incidence in industry C. However, industry A had a higher incidence of OTA than AFs (Table 5).

Table 5
Percentage of samples contaminated with aflatoxins (AFs) and ochratoxin A (OTA) and the concentration (μg/kg) in the different processing steps of dried figs studied in the three industries.

	Industries					
	A		B		C	
Stages	AFs	OTA	AFs	OTA	AFs	OTA
Curing	[a]<LOD	[c]37.5%	[b]25%	[a]<LOD	[a]<LOD	[b]12.5%
Sizing	[a]<LOD	[a]<LOD	[a]<LOD	[c]25%	[a]<LOD	[a]<LOD
Blanching	[c]12.5%	[a]<LOD	[d]50%	[b]12.5%	[b]12.5%	[a]<LOD
Stored product	[a]<LOD	[a]<LOD	[b]25%	[a]<LOD	[a]<LOD	[a]<LOD
Final product	[a]<LOD	[a]<LOD	[d]12.5%	[a]<LOD	[a]<LOD	[a]<LOD

[a] LOD = AFB_1 (0.07 μg/kg), AFB_2 (0.3 μg/kg) and OTA (0.38 μg/kg).
[b] Aflatoxin B_1 values (0–25 μg/kg) and B_2 values (0–6 μg/kg)/ochratoxin A values 0–10 μg/kg.
[c] Aflatoxin B_1 values (25–50 μg/kg) and B_2 values (6–12 μg/kg)/ochratoxin A values of 25–50 μg/kg.
[d] Aflatoxin B_1 values (50–75 μg/kg) and B_2 values (12–22 μg/kg).

AFs were found in all three industries at the blanching stage. This fact could be justified because mycotoxins are heat resistant (Bullerman and Bianchini, 2007) and this stage was conducted during a short time (1 min). High heat treatments would have to be applied for longer periods of time and this may lead to alterations in the organoleptic characteristics of the products (Kabak et al., 2006). Therefore, short blanching periods would favour the production of mycotoxins since fungi can resist this treatment and can activate secondary metabolic routes in order to favour its growth and survival by synthesizing these secondary metabolites (Patriarca et al., 2019). In addition, in industry B, AFs were also detected at the stored product and final product stages. Blanching and storage are two important critical points in the processing of dried figs for mycotoxin production. Blanching can lead to the breakdown of structures and disposal of nutrients, and in the absence of rapid cooling, it leads to the development of toxigenic moulds. In addition, during the storage of dried figs, there are favourable relative humidity conditions for the development of toxigenic moulds.

4. Conclusions

The results indicate that the changes in a_w and temperature that dried figs undergo during the different stages of industrial processing have great relevance to the mycological quality of dried figs. A high incidence of *Aspergillus* spp. capable of producing AFs and OTA was found in most of the processing stages studied regardless of the industry evaluated. Based on findings, blanching should be homogeneous and efficient with a longer subsequent drying stage to reduce the dried fig moisture content below 26% in order to minimize or even avoid the development of toxigenic moulds. In addition, postharvest of dried figs during all stages covering industry processing, final storage and retailing are advisable to be conducted at refrigeration conditions and controlled relative humidity to avoid mycotoxin production. The early detection of AFs- and OTA- producing moulds by qPCR can be considered as a good indicator for integration into prevention strategies to control filamentous fungal hazards and subsequent mycotoxin synthesis during the processing of dried figs.

Declaration of competing interest

None.

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References

AOAC, 2005. Official Method of Analysis of the AOAC International, 18th edition. In: Method 934.01. Association of Official Analytical Chemists, Gaithersburg, MD.

Astoreca, A.L., Magnoli, C.E., Dalcero, A.M., 2010. Ecophysiology of Aspergillus section nigri species potential ochratoxin A producers. Toxins 2, 2593–2605. https://doi.org/10.3390/toxins2112593.

Bayman, P., Baker, J.L., Doster, M.A., Michailides, T.J., Mahoney, N.E., 2002. Ochratoxin production by the Aspergillus ochraceus Group and Aspergillus alliaceus. Appl. Environ. Microbiol. 68, 2326–2329. https://doi.org/10.1128/AEM.68.5.2326-2329.2002.

Bircan, C., 2009. Incidence of ochratoxin a in dried fruits and co-occurrence with aflatoxins in dried figs. Food Chem. Toxicol. 47, 1996–2001. https://doi.org/10.1016/j.fct.2009.05.008.

Bullerman, L.B., Bianchini, A., 2007. Stability of mycotoxins during food processing. Int. J. Food Microbiol. 119, 140–146. https://doi.org/10.1016/j.ijfoodmicro.2007.07.035.

Bundesgesetzblatt Jahrgang, 2004. Verordnung zur Änderung der Mykotoxin-Höchstmengenverordnung und der Diätverordnung, Teil 1, Nr.5. Bundesanzeiger Verlag.

Cabañas, C.M., Hernández, A., Martínez, A., Tejero, P., Vázquez-Hernández, M., Martín, A., Ruiz-Moyano, S., 2020. Control of Penicillium glabrum by indigenous antagonistic yeast from vineyards. Foods 9, 1864. https://doi.org/10.3390/foods9121864.

Castellá, G., Cabañes, F.J., 2011. Development of a real time PCR system for detection of ochratoxin A-producing strains of the Aspergillus niger aggregate. Food Control 22. https://doi.org/10.1016/j.FoodControl.2011.02.014.

Chiotta, M.L., Susca, A., Stea, G., Mulè, G., Perrone, G., Logrieco, A., Chulze, S.N., 2011. Phylogenetic characterization and ochratoxin A - fumonisin profile of black Aspergillus isolated from grapes in Argentina. Int. J. Food Microbiol. 149, 171–176. https://doi.org/10.1016/j.ijfoodmicro.2011.06.002.

De Mello, F.R., Scussel, V.M., 2007. Characteristics of in-shell Brazil nuts and their relationship to aflatoxin contamination: criteria for sorting. J. Agric. Food Chem. 55 https://doi.org/10.1021/jf071392x.

Di Sanzo, R., Carabetta, S., Campone, L., Bonavita, S., Iaria, D., Fuda, S., Rastrelli, L., Russo, M., 2018. Assessment of mycotoxins co-occurrence in Italian dried figs and in dried figs-based products. J. Food Saf. 38, 1–8. https://doi.org/10.1111/jfs.12536.

Drusch, S., Aumann, J., 2005. Mycotoxins in fruits: microbiology, occurrence, and changes during. Fruit Process. https://doi.org/10.1016/S1043-4526(05)50002-0.

EFSA, 2020. European Food Safety Authority. Risk Assessment of Ochratoxin A in Food, 18, Issue 5. https://doi.org/10.2903/j.efsa.2020.6113.

Ersoy, N., Gozlekci, S., Gok, V., Yilmaz, S., 2017. Fig (Ficus carica L.) fruit: some physical and chemical properties. Acta Hortic. 1173. https://doi.org/10.17660/ActaHortic.2017.1173.57.

European Commission (EC), 2006. Commission regulation N° 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364, 5–24.

European Union (EU), 2012. Commission Regulation N° 1058/2012 of 12 November 2012, amending Regulation (EC) N° 1881/2006 as regards maximum levels for aflatoxins in dried figs. Off. J. Eur. Union L313, 14–15.

Gallardo, G., Ruiz-Moyano, S., Hernández, A., Benito, M.J., Córdoba, M.G., Pérez-Nevado, F., Martín, A., 2014. Application of ISSR-PCR for rapid strain typing of Debaryomyces hansenii isolated from dry-cured Iberian ham. Food Microbiol. 42 https://doi.org/10.1016/j.fm.2014.03.022.

Galván, A.I., Serradilla, M.J., Córdoba, M.G., Domínguez, G., Galán, A.J., López-Corrales, M., 2021. Implementation of super high-density systems and suspended harvesting meshes for dried fig production: effects on agronomic behaviour and fruit quality. Sci. Hortic. 281 https://doi.org/10.1016/j.scienta.2021.109918.

Gilbert, J., Senyuva, H., 2008. Fungal and mycotoxin contamination of dried figs-a review. JSM Mycotoxins 58 (2), 73–82. https://doi.org/10.2520/myco.58.73. In this issue.

Glass, N.L., Donaldson, G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61, 1323–1330.

González-Salgado, A., González-Jaén, T., Vázquez, C., Patiño, B., 2008. Highly sensitive pcr-based detection method specific for Aspergillus flavus in wheat flour. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 25, 758–764. https://doi.org/10.1080/02652030701765715.

Guirguis, E.A.H., 2018. Assessment of the microbiological and mycotoxins quality of selected dried fruits with special reference to microwave treatment. IOSR-JESTFT 12, 48–55. https://doi.org/10.9790/2402-1210014855.

Heperkan, 2006. In: The Importance of Mycotoxins and a Brief History of Mycotoxin Studies in Turkey, 54. ARI Bulletin of the Istanbul Technical University, pp. 18–27.

Heperkan, Z., Moretti, A., Daskaya Dikmen, C., Heperkan, D., Moretti, A., Dikmen, C.D., Logrieco, A.F., 2012. Toxigenic fungi and mycotoxin associated with figs in the Mediterranean area mycotoxins and biosynthetic genes View project prevention of Cronobacter sakazakii in baby foods view project Toxigenic fungi and mycotoxin associated with figs in the Mediterran. Phytopathol. Mediterr. 51 https://doi.org/10.14601/Phytopathol_Mediterr-9467.

Hope, R., Magan, N., 2003. Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by Fusarium culmorum on a wheat-based substrate. Lett. Appl. Microbiol. 37, 70–74. https://doi.org/10.1046/j.1472-765X.2003.01358.x.

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 Addit. Contam. 22, 1258–1263. https://doi.org/10.1080/02652030500260447.

Javanmard, M., 2010. Occurrence of mould counts and Aspergillus species in Iranian dried figs at different stages of production and processing. J. Agric. Sci. Technol. 12, 331–338.

Jay, J.M., Loessner, M.J., Golden, D.A., 2005. Modern Food Microbiology, 7th edition. Springer, New York.

Kabak, B., 2016. Aflatoxins in hazelnuts and dried figs: occurrence and exposure assessment. Food Chem. 211 https://doi.org/10.1016/j.foodchem.2016.04.141.

Kabak, B., Dobson, A.D.W., Var, I., 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. Crit. Rev. Food Sci. Nutr. 46 https://doi.org/10.1080/10408390500436185.

Kamiloglu, S., Capanoglu, E., 2015. Polyphenol content in figs (Ficus carica L.): effect of sun-drying. Int. J. Food Prop. 18 https://doi.org/10.1080/10942912.2013.833522.

Karaca, H., Velioglu, Y.S., Nas, S., 2010. Mycotoxins: contamination of dried fruits and degradation by ozone. Toxin Rev. 29, 51–59. https://doi.org/10.3109/15569543.2010.485714.

Karbancıoğlu-Güler, F., Heperkan, D., 2008. Natural occurrence of ochratoxin a in dried figs. Anal. Chim. Acta 617. https://doi.org/10.1016/j.aca.2008.01.009.

Kesho, A., Chala, A., Shikur, E., 2019. Determination of major factors associated with fungal contamination of wheat under storage conditions. Int. J. Photochem. Photobiol. 3, 21. https://doi.org/10.11648/j.ijpp.20190302.12.

Kidmose, U., Martens, H.J., 1999. Changes in texture, microstructure and nutritional quality of carrot slices during blanching and freezing. J. Sci. Food Agric. 79 https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1747::AID-JSFA429>3.0.CO;2-B.

Lasram, S., Oueslati, S., Mliki, A., Ghorbel, A., Silar, P., Chebil, S., 2012. Ochratoxin A and ochratoxigenic black Aspergillus species in tunisian grapes cultivated in different geographic areas. Food Control 25, 75–80. https://doi.org/10.1016/j.foodcont.2011.10.006.

López, P., Venema, D., de Rijk, T., de Kok, A., Scholten, J.M., Mol, H.G.J., de Nijs, M., 2016a. Occurrence of Alternaria toxins in food products in the Netherlands. Food Control 60, 196–204. https://doi.org/10.1016/j.foodcont.2015.07.032.

López, P., Venema, D., Mol, H., Spanjer, M., de Stoppelaar, J., Pfeiffer, E., de Nijs, M., 2016b. Alternaria toxins and conjugates in selected foods in the Netherlands. Food Control 69, 153–159. https://doi.org/10.1016/j.foodcont.2016.04.001.

MAPA, 2020. Ministerio De Agricultura, Alimentacion y Medio Ambiente. https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/ (accessed on 1 September 2021).

Mat Desa, W.N., Mohammad, M., Fudholi, A., 2019. Review of drying technology of fig. Trends Food Sci. Technol. 88, 93–103. https://doi.org/10.1016/j.tifs.2019.03.018.

Neme, K., Mohammed, A., 2017. Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies.A review. Food Control 78. https://doi.org/10.1016/j.foodcont.2017.03.012.

Özay, G., Alperden, I., 1991. Aflatoxin and ochratoxin - a contamination of dried figs (Ficus carina L.) from the 1988 crop. Mycotoxin Res. 7, 85–91. https://doi.org/10.1007/BF03192171.

Ozer, 2008. In: Ar ch of of. Phytopathol. Mediterr, 51, pp. 148–157.

Öztekin, S., Zorlugenç, B., Zorlugenç, F.K., 2006. Effects of ozone treatment on microflora of dried figs. J. Food Eng. 75 https://doi.org/10.1016/j.jfoodeng.2005.04.024.

Patriarca, A., da Cruz Cabral, L., Pavicich, M.A., Nielsen, K.F., Andersen, B., 2019. Secondary metabolite profiles of small-spored Alternaria support the new phylogenetic organization of the genus. Int. J. Food Microbiol. 291, 135–143. https://doi.org/10.1016/j.ijfoodmicro.2018.11.022.

Perrone, G., Susca, A., 2017. Penicillium species and their associated mycotoxins. Methods Mol. Biol. 1542, 107–119. https://doi.org/10.1007/978-1-4939-6707-0_5.

Perrone, G., Stea, G., Epifani, F., Varga, J., Frisvad, J.C., Samson, R.A., 2011. Aspergillus niger contains the cryptic phylogenetic species A. awamori. Fungal Biol. 115, 1138–1150. https://doi.org/10.1016/j.funbio.2011.07.008.

Prabhakar, K., Mallika, E.N., 2014. Dried foods. In: Batt, C.A., Lou Tortorello, M. (Eds.), Encyclopedia of Food Microbiology, Second edition. Elsevier, pp. 574–576.

Quaglia, M., Santinelli, M., Sulyok, M., Onofri, A., Covarelli, L., Beccari, G., 2020. Aspergillus, Penicillium and Cladosporium species associated with dried date fruits collected in the Perugia (Umbria, Central Italy) market. Int. J. Food Microbiol. 322, 108585 https://doi.org/10.1016/j.ijfoodmicro.2020.108585.

Rahimi, E., Shakerian, A., 2013. Ochratoxin a in dried figs, raisings, apricots, dates on iranian retail market. Health 05. https://doi.org/10.4236/health.2013.512282.

Rodríguez, A., Rodríguez, M., Luque, M.I., Martín, A., Córdoba, J.J., 2012. Real-time PCR assays for detection and quantification of aflatoxin-producing molds in foods. Food Microbiol. 31, 89–99. https://doi.org/10.1016/j.fm.2012.02.009.

Ropkins, K., Beck, A.J., 2000. Evaluation of worldwide approaches to the use of HACCP to control food safety. Trends Food Sci. Technol. 11 https://doi.org/10.1016/S0924-2244(00)00036-4.

Saadullah, A., Abdullah, S., 2015. Mycobiota and incidence of toxigenic fungi in dried fruits from Duhok markets, North Iraq. Egypt. Acad. J. Biol. Sci. G Microbiol. 7, 61–68. https://doi.org/10.21608/eajbsg.2015.16486.

Scott, P.M., Trucksess, M.W., 2009. Prevention of mycotoxins in dried fruit, other fruit products, and botanicals. In: ACS Symposium Series, 1031. American Chemical Society, pp. 17–35. https://doi.org/10.1021/bk-2009-1031.ch002.

Senyuva, H.Z., Gilbert, J., Ozcan, S., Ulken, U., 2005. Survey for co-occurrence of ochratoxin a and aflatoxin B1 in dried figs in Turkey by using a single laboratory-validated alkaline extraction method for ochratoxin A. J. Food Prot. 68 https://doi.org/10.4315/0362-028X-68.7.1512.

Senyuva, H., Gilbert, J., Samson, R., Özcan, S., Öztürkoğlu, Ş., Önal, D., 2008. Occurrence of fungi and their mycotoxins in individual Turkish dried figs. World Mycotoxin J. 1 https://doi.org/10.3920/WMJ2008.x009.

Storari, M., Bigler, L., Gessler, C., Broggini, G.A.L., 2012. Assessment of the ochratoxin A production ability of Aspergillus tubingensis. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 29, 1450–1454. https://doi.org/10.1080/19440049.2012.698656.

Sulyok, M., Krska, R., Senyuva, H., 2020. Profiles of fungal metabolites including regulated mycotoxins in individual dried Turkish figs by LC-MS/MS. Mycotoxin Res. 36, 381–387. https://doi.org/10.1007/s12550-020-00398-5.

Taiwo, K.A., Adeyemi, O., 2009. Influence of blanching on the drying and rehydration of banana slices. Afr. J. Food Sci. 3, 307–315.

Trucksess, M.W., Scott, P.M., 2008. Mycotoxins in botanicals and dried fruits: a review. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 25, 181–192. https://doi.org/10.1080/02652030701567459.

Turkoz Bakirci, G., 2020. Investigation of aflatoxins levels in commercial dried figs from western Turkey. Int. Food Res. J. 27, 245–251.

UNECE, 2016. Standard DDP 14. https://unece.org/fileadmin/DAM/trade/agr/standard/dry/dry_e/14DriedFigs_E2016.pdf.

Vaamonde, G., Patriarca, A., Fernández Pinto, V., Comerio, R., Degrossi, C., 2003. Variability of aflatoxin and cyclopiazonic acid production by Aspergillus section flavi from different substrates in Argentina. Int. J. Food Microbiol. 88, 79–84. https://doi.org/10.1016/S0168-1605(03)00101-6.

Villalobos, M.C., Serradilla, M.J., Martín, A., Ruíz-Moyano, S., Casquete, R., Hernández, A., Córdoba, M.G., 2019. Use of efficient drying methods to improve the safety and quality of dried fig. J. Food Process. Preserv. 43 https://doi.org/10.1111/jfpp.13853.

Watanabe, M., 2008. Production of mycotoxins by Penicillium expansum inoculated into apples. J. Food Prot. 71, 1714–1719. https://doi.org/10.4315/0362-028X-71.8.1714.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal Rna genes for phylogenetics. PCR Protoc. 315–322 https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

Xu, Y., Sismour, E.N., Pao, S., Rutto, L., Grizzard, C., Ren, S., 2012. Textural and microbiological qualities of vegetable soybean (edamame) affected by blanching and storage conditions. J. Food Sci. Technol. 3 https://doi.org/10.4172/2157-7110.1000165.