Natural co-occurrence of mycotoxins in wheat grains from Italy and Syria

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ABSTRACT

This article describes the application of an analytical method for the detection of 25 mycotoxins in wheat grain based on simultaneous extraction using matrix solid-phase dispersion (MSPD) followed by liquid chromatography coupled to tandem mass spectrometry, a hybrid triple quadrupole-linear ion trap mass spectrometer (QTrap®). Information Dependent Acquisition (IDA), an extra confirmation tool for samples that contain the target mycotoxins, was used. The analysis of 40 Syrian and 46 Italian wheat grain samples interestingly showed that Syrian samples were mainly contaminated with ochratoxin A and aflatoxins, whereas Italian samples with deoxynivalenol and 15-acetyldeoxynivalenol. Emerging Fusarium mycotoxins were predominant in Italian samples compared to the Syrian. Among the analysed samples, only one was found containing zearalenone with level above the maximum European recommended concentration (100 ppb). These results confirm that climatic differences between Syria and Italy, both in Mediterranean basin, play a key role in the diversity of fungal genera and mycotoxins in wheat grains. © 2014 Elsevier Ltd. All rights reserved.

1. Introduction

FAO estimation for global wheat production in 2011 was at a record 676 million tonnes, while for the European Union (EU), the world’s largest producer, was 142 million tonnes (FAO, 2011). In Syria, wheat is considered to be the main strategic crop with an annual production that ranged from 4 to 5 million tonnes in the last years (FAO, 2011; NAPC, 2009; Sadiddin & Atyia, 2009). In Syria wheat two main products, bread and bulgur, are common food commodities (Haydar, Benelli, & Brera, 1990) with an average consumption of bread of 12.9 kg per capita per month (FAO, 2003). The importance of wheat has been mainly attributed to its ability to be ground into flour and semolina, which form the basic ingredients for bread and other bakery products, and pasta (Chandraka & Shabidi, 2006).

Wheat can be easily grown in different climatic regions, and then it is exposed to many pathogenic fungi, of which some are toxigenic fungi, such as Fusarium, Aspergillus and Penicillium. These fungal genera are producers of mycotoxins, which are a hazard for both human and animal health (Mankeviciene, Butkutė, Dabkevičius, & Suproniene, 2007). Wheat kernels can be contaminated during pre or/and post-harvest with mycotoxins. The knowledge of the occurrence of specific fungal genera in different growing areas will help to predict mycotoxin content in harvested grains.

The most well-known mycotoxins, aflatoxins (AFs), ochratoxin A and some Fusarium toxins (deoxynivalenol, zearalenone, fumonisins), have been classified by IARC (1993) and regulated by European Union (EU, 2006a, 2010). On the other hand, there are other mycotoxins, such as enniatins and beauvericin, which have not been classified nor legislated yet.

Mycotoxin existence depends on several factors, such as fungal strains present, climatic and geographical conditions, cultivation technique and susceptibility level of host plants and crop management practices (Logrieco, Bottalico, Mulé, Moretti, & Perrone, 2003; Pancaldi et al., 2010; Rubert, Soriano, Mañes, & Soler, 2013). Several surveys have been conducted on the levels of mycotoxins in wheat all over the world such as USA, Canada, Serbia, Italy or Jordan (Gallo, Lo Bianco, & Bognanni, 2008; Jelinek, Pohland, & Wood, 1989; Roscoe et al., 2008; Salem & Ahmad, 2010; Skribic, Zivancev, Miladenovic, & Godula, 2012), while only one survey on aflatoxins (AFs) presence has been carried out on different Syrian’s food commodities, in Lattakia (Syria) (Haydar et al., 1990).

Since toxicity has not been evaluated for all the mycotoxins found in food, the total effect of these naturally occurring...
contaminants on human health cannot be estimated. In fact, for the most well-documented toxins, the tolerable daily intakes (TDI), established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA), remain temporary (t-TDI), provisional (PTWI) or provisional maximum levels (PMTDI), due to the lack of information on toxicology and exposure (Leblanc, Tard, Volatier, & Verger, 2005).

The analysis of mycotoxins is challenging as they are usually present in low concentrations in complex matrices, and they may occur in various combinations produced by a single or by several fungal species. During the last decade, liquid chromatography–mass spectrometry (HPLC–MS/MS) has been established as a powerful tool for unambiguous identification of mycotoxins in food (Turner, Subrahmanyan, & Piletsky, 2009). In addition, alternative extraction methods have recently emerged, such as QuEChERS and MSPD (Rubert, James, Mañes, & Soler, 2012a; Rubert, Soler, & Mañes, 2011; Rubert et al., 2012b; Shephard et al., 2012). A recent review, focused on recent trends in matrix solid phase dispersion (MSPD), has described the key factors for the success of MSPD and its application in food and animal tissues, highlighting its feasibility, flexibility, versatility, low costs and rapidity (Capriotti, Cavaliere, Laganà, Piovesana, & Samperi, 2013). To our knowledge, MSPD–HPLC–QTRAP® is still scarcely used as a routine analytical technique for mycotoxin analysis.

The aims of this work were to obtain the first report on the incidence and levels of mycotoxins in Syrian and Italian wheat grains for human and animal consumption by the application of a mycotoxin analytical method based on MSPD extraction method followed by HPLC–MS/MS using a 3200 QTRAP® instrument and to validate the method to analyze mycotoxins in wheat grain.

2. Materials and methods

2.1. Chemical and reagents

Acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Solid-phase used for matrix solid-phase dispersion (MSPD) extraction was Sepra C18–E (50 μm, 65 Å) endcapped silica-based C18 from Phenomenex (Torrance, USA). Deionized water (>18 MΩ cm−1 resistivity) was purified using Milli-Q® SP Reagent water system plus from Millipore Corp. (Bedford, USA). All solvents were passed through a 0.45 μm cellulose filter purchased from Scharlab (Barcelona, Spain). Analytical grade reagent formic acid (purity > 98%), and ammonium formate were obtained from Panreac Quimica S.A.U. (Barcelona, Spain).

The standards of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), sterigmatocystin (STER), α-zearalenol (αZOL), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), fusarenon X (FUX), neosolaniol (NEO), diacetoxyscirpenol (DAS), fumonisin B1 (FB1), fumonisin B2 (FB2), beauvericin (BEA) were purchased from Sigma Aldrich (Madrid, Spain). T-2 toxin (T-2) and HT-2 toxin (HT-2) stock solutions (in acetone) were purchased from Biopure referenzsubstanzen GmbH (Tulln, Austria). Fumonisin B1 (FB1) was supplied by the PROMEC unit (Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa). Enniatins (ENS): ENA3, ENA, ENB and ENB1 were purchased by Enzo Life Science (Lausen, Switzerland).

The standard solutions were prepared and kept at −20°C (Rubert et al., 2012a,b). All working standard solutions were prepared before the use by diluting the stock solution with acetonitrile.

2.2. Sampling

A total of 86 wheat grain samples collected during 2009 and 2010 seasons were analysed for the presence of mycotoxins. Forty samples (40) of durum and soft wheat were collected from different areas of Syria including Deir Ezzor (11), Damascus rural (19), Daraa (3) and Al Hassakeh (7). Forty-six samples (46) of durum wheat were collected from different Italian areas, Emilia-Romagna (12), Toscana (5), Marche (12), Umbria (7), Lazio (3), Basilicata (3) and Sicilia (4). Fig. 1 shows the regions in Italy and Syria where the wheat samples were collected.

According to EU guidelines (EU, 2006b), three incremental samples of at least 1 kg were collected to obtain an aggregate sample of 3 kg total weight. After homogenization, samples were packed in a plastic bag and kept at −20°C in a dark and dry place until analysis. Just before analysis, a subsample of 200 g was mixed thoroughly using an Oster® food processor (Professional Series Blender model BPST02-B00) to obtain wheat flour.

2.3. Extraction procedure

Sample preparation was optimized in a previous study (Rubert, Soler, & Mañes, 2012c). A MSPD extraction method was applied to wheat grain. Samples (200 g) were prepared using an Oster® food processor (Professional Series Blender model BPST02-B00), mixing the sample thoroughly. Portions of 1 g were weighed and placed into a glass mortar (50 mL) and were gently blended with 1 g of C18 for 5 min using a pestle, to obtain a homogeneous mixture. This mixture was introduced into a 100 mm × 9 mm i.d. glass column, and eluted dropwise with 15 mL of elution solvent which was a mixture of acetonitrile/methanol (50/50, v/v) with 1 mM ammonium formate by applying a slight vacuum. Then, the extract was transferred to a 25 mL conical tube and evaporated to dryness at 35°C with a gentle stream of nitrogen using a multi-sample Turbo-ovap LV Evaporator (Zymark, Hoptkinton, USA). The residue was reconstituted to a final volume of 1 mL with methanol/water (50/50, v/v) and filtered through a 13 mm/0.22 μm nylon filter purchased from Membrane Solutions (Texas, USA) before the injection into the liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) system.

For spiked samples (EU, 2002), 1 g of “blank” sample (sample in which it was corroborated that none of the analytes were present) was spiked with 0.1 mL of a working mixture of the mycotoxins at the appropriate concentration. Spiked samples were then left to stand for 3 h at room temperature before the extraction to allow the solvent to evaporate and to establish equilibration between the spiked mycotoxins and wheat flour samples. Ten replicates were prepared at each spiking level.

2.4. HPLC–MS/MS method

The 3200 QTRAP® mass spectrometer (AB Sciex, Foster City, CA, USA) was coupled to Agilent 1200 chromatograph (Agilent Technologies, Palo Alto, CA, USA). Separation of analytes was performed with a reversed-phase analytical column (Gemini C18, 150 mm, 2 mm i.d., 5 μm; Phenomenex) maintained at 35°C. As mobile phase, 5 mM ammonium formate and 0.1% formic acid in water (A) and 5 mM ammonium formate in methanol (B) were used. The gradient was as follows: at the start 5% of solvent B and after 10 min the percentage of solvent B was linearly increased to 95% in 10 min. The percentage of solvent B was kept for 5 min. Finally, the column was equilibrated to initial conditions for 10 min. The flow rate was 250 μL min−1 and the injection volume was 10 μL. The 3200 QTRAP® mass spectrometer was equipped with a Turbo V™ Ion Spray (ESI) interface. The QTRAP® analyzer combines a fully functional triple-quadrupole and ion trap mass spectrometer within the same instrument. The analyses were performed using Turbo V™ Ion Spray in positive ionization mode (ESI+). The operation conditions were previously optimised (Rubert et al., 2012a,c). The operation conditions for ESI+ were as follow: ion spray voltage...
5500 V, probe temperature 450 °C, curtain gas 20 (arbitrary units) and GS1 and GS2, 50 and 55 psi, respectively. Nitrogen served as nebulizer and collision gas. Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of target molecules. The optimization of MS parameters as declustering potential (DP), collision energy (CE) and collision cell entrance potential (CEP) were performed by flow injection analysis for each compound; entrance potential (EP) and collision cell exit potential (CXP) were set 10 and 4 V, respectively for all analytes. The mass spectrometer was operated in SRM mode and with a unit resolution for Q1 and Q3. For LC–MS/MS analysis, scheduled SRM (sSRM) was used at 50 s of SRM detection window and 1 s of target scan time, in this form was obtained more than 12 data points for all selected mycotoxins.

In order to compare the performance distinctive of two operational modes of the QTRAP\textsuperscript{®} triple quadrupole and triple quadrupole linear ion trap, IDA method was developed. The monitoring of the sSRM ratio and the enhance product ion (EPI) scan (as an extra-information tool) were used. Analyst\textsuperscript{®} version 1.5.2 software (AB Sciex) was used to control and also for data collection and analysis.

2.5. Validation of the method

Validation of the method was performed according to previous studies (Rubert et al., 2012a,c). The criteria for mycotoxin identification were applied according to the EU (2002): precursor ion and two transitions were monitored; four identification points, moreover the measured retention time of the suspected peak had to correspond to the measured retention time of the standard and the area ratio between the two monitored SRM transitions had to be equal in the sample and in the standard or matrix-matched. In parallel, the EPI scan (as an extra-information tool) was carried out for positives samples.

The matrix effect (ME) for each analyte is defined as the percentage of the matrix-matched calibration slope (B) divided by the slope of the standard calibration in solvent (A) and it was calculated for wheat. The ratio $\frac{B}{A} \times 100$ is defined as the absolute ME\%. A value of 100% indicates that there is no absolute matrix effect. There is signal enhancement if the value is >100% and signal suppression if the value is <100%. Matrix-matched calibration was used for effective quantification. The linearity in the response was calculated using matrix-matched curves prepared by spiking one “blank” wheat sample and analysing it in triplicate at eight concentration levels within the analytical range: from the limit of quantification (LOQ) to 100 times this LOQ.

The recovery experiments were conducted by spiking the blank wheat sample in ten replicates at two concentration levels (LOQ and 100 times LOQ).

The recovery study was carried out spiking wheat at LOQ and 100 times LOQ levels ($n = 10$). The precision of the method (% RSD), was estimated by the repeated analysis ($n = 10$) of a spiked wheat at LOQ and 100 times LOQ during the same day (intra-day) and on different five days (inter-day). The limits of detection (LODs) were determined as the lowest mycotoxin concentration whose qualified transition (q) presented a signal-to-noise ratio (S/N) $\geq 3$. The LOQs were determined as the minimum detectable amount of analyte with a S/N $\geq 10$ for the quantified transition (Q). LOD and LOQ were calculated using Analyst\textsuperscript{®} version 1.5.2 software (AB Sciex).

2.6. Estimation of daily intake

To estimate the toxin uptake by consumers, a mean concentration of each found mycotoxin was calculated, considering: only positive samples, both positives and negative samples and the high concentration level found. For the exposure assessment based on a deterministic approach, the estimated daily intake (EDI) were obtained by an integration of mycotoxin analysis data obtained from the samples analysed combined with the food consumption assumption of adult population with a body weight of 60 kg. The EDI of each mycotoxin was calculated as indicated in the following equation: \[\text{EDI}_m = \left(\frac{C_m \times K}{\text{bw}}\right)\text{bw},\] where $\text{EDI}_m$ is the estimated daily intake (µg kg\textsuperscript{-1} bw d\textsuperscript{-1}) for each mycotoxin $m$; $C_m$ is the mean content of a mycotoxin (µg kg\textsuperscript{-1}); $K$ is the average consumption of the commodity (g d\textsuperscript{-1}) and bw is the body weight used in each population group. (Nakanishi, Gamo, Iwasa, & Tanaka, 2003; Rodriguez-Carrasco, Ruiz, Font, & Berrada, 2013).

3. Results and discussion

3.1. Validation of the method
Precision was studied by performing intra-day and inter-day precision experiments and the results are shown in Table 1. Repeatability, expressed as RSD was lower than 16% for intra-day experiments and for inter-day precision, RSDs were always lower than 19% for both spiked levels.

The MEs were calculated for each mycotoxin in wheat grain (Table 1). NIV and fumonisins showed signal enhancement and AFs and enniatins showed matrix suppression. Thereby, matrix-matched calibration was used for matrix effect compensation. The presented method is characterised by good linearity. In all cases, the coefficient of determination \( r^2 \) was higher than 0.99. All compounds were linear for the linear dynamic range studied, LOQ to 100 times LOQ level.

The MSPD–HPLC–MS/MS method was successfully validated according to the criteria specified in Commission Decision 2002/657/EC for quantitative confirmation method (EU, 2002).

### 3.2. Occurrence of mycotoxins in Italian and Syrian wheat grains

There are not many studies focused on the presence of mycotoxins in wheat grain in Syria; in addition the occurrence of mycotoxins in this particular matrix from Syria and Italy has not been commonly studied. For this reason, this research evaluated twenty-seven mycotoxins in wheat grains from Italy and Syria: levels of aflatoxins, fumonisins, type A and B trichothecenes, ZEN, αZOL, OTA, STER and emerging mycotoxins were evaluated and compared, as well as, an estimation of consumer risk was calculated.

The identification of positive samples is described in the Section 2.5 according to the European Commission (EU, 2002). Following these criteria, a total of 61 samples (71%) were contaminated by mycotoxins. Going into details, a total of 37 Italian and 24 Syrian samples were unambiguously confirmed as contaminated samples.

#### 3.2.1. Italian wheat samples

Forty-six Italian wheat grain samples were analysed and the results are summarised in Table 2. At least 80% of samples were contaminated with one mycotoxin. Furthermore, during this study the co-occurrence of mycotoxins in wheat grain was observed. For example, two mycotoxins were found in 27% of Italian contaminated samples and 38% of them were contaminated with three or more mycotoxins.

DON showed the highest incidence (59%), and their precursors 3-ADON and/or 15-ADON were found in 24% of samples (Table 2). Regarding the co-occurrence of DON, 3-ADON and 15-ADON, in five samples were detected only 3-ADON and 15-ADON, but no DON presence. This is in contrast with the finding that in highly contaminated grains, a positive correlation occurred between levels of DON and its acetyl derivatives (Edwards, 2009). DON levels were always higher than those of 3-ADON and 15-ADON, with ratios ranging from 2.9 to 155 (Placinta, D'mello, & Macdonald, 1999). Higher concentrations of DON in comparison with 3-ADON and 15-ADON were also detected in wheat samples (Berthiller et al., 2005).

These results are in accordance with Joint FAO/WHO Expert Committee on Food Additives (JECA, 2001), which showed that DON was the most abundant trichothecenes in Italian cereals. The Italian durum and soft wheat samples collected from different areas were contaminated with DON. Furthermore, 12 EU member States indicated that DON was the most frequently detected mycotoxin in wheat grains (EU, 2003).

The low co-occurrence of DON and other type B trichothecenes, such as NIV and FUSX has been highlighted (Table 2). NIV was detected in three samples, in which only one presented co-occurrence of DON, while FUSX was found in three samples, of which two of them showed the co-presence of DON.

On the other hand, type A trichothecens, T-2 and HT-2, were detected in 26% of samples and the co-occurrence of T-2 and HT-2

### Table 1
Validation results for MSPD–HPLC–MS/MS method. LODs and LOQs, MEs, percentage of recovery and repeatability (% RSD) at two levels used for validation, and Inter-day precision (% RSD) at LOQ level 100.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LOD (µg/kg)</th>
<th>LOQ (µg/kg)</th>
<th>ME</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIV</td>
<td>20</td>
<td>60</td>
<td>108</td>
<td>67 (8)</td>
<td>65 (11)</td>
</tr>
<tr>
<td>DON</td>
<td>5</td>
<td>15</td>
<td>79</td>
<td>71 (7)</td>
<td>75 (6)</td>
</tr>
<tr>
<td>3-ADON</td>
<td>3</td>
<td>10</td>
<td>81</td>
<td>70 (11)</td>
<td>69 (9)</td>
</tr>
<tr>
<td>15-ADON</td>
<td>3</td>
<td>10</td>
<td>84</td>
<td>69 (14)</td>
<td>68 (9)</td>
</tr>
<tr>
<td>FUSX</td>
<td>15</td>
<td>45</td>
<td>75</td>
<td>70 (8)</td>
<td>75 (9)</td>
</tr>
<tr>
<td>NEO</td>
<td>2</td>
<td>6</td>
<td>68</td>
<td>72 (10)</td>
<td>77 (8)</td>
</tr>
<tr>
<td>DAS</td>
<td>2</td>
<td>5</td>
<td>73</td>
<td>79 (6)</td>
<td>82 (7)</td>
</tr>
<tr>
<td>HT-2</td>
<td>2</td>
<td>6</td>
<td>77</td>
<td>72 (12)</td>
<td>76 (11)</td>
</tr>
<tr>
<td>T-2</td>
<td>1</td>
<td>3</td>
<td>72</td>
<td>70 (16)</td>
<td>72 (11)</td>
</tr>
<tr>
<td>ZEA</td>
<td>3</td>
<td>9</td>
<td>89</td>
<td>69 (8)</td>
<td>73 (6)</td>
</tr>
<tr>
<td>αZOL</td>
<td>1</td>
<td>3</td>
<td>79</td>
<td>74 (9)</td>
<td>77 (3)</td>
</tr>
<tr>
<td>FB1</td>
<td>5</td>
<td>15</td>
<td>102</td>
<td>89 (6)</td>
<td>91 (7)</td>
</tr>
<tr>
<td>FB2</td>
<td>8</td>
<td>25</td>
<td>98</td>
<td>87 (8)</td>
<td>85 (5)</td>
</tr>
<tr>
<td>FB3</td>
<td>8</td>
<td>25</td>
<td>109</td>
<td>85 (7)</td>
<td>88 (9)</td>
</tr>
<tr>
<td>BEA</td>
<td>0.4</td>
<td>1</td>
<td>67</td>
<td>72 (18)</td>
<td>71 (16)</td>
</tr>
<tr>
<td>ENA</td>
<td>0.1</td>
<td>0.4</td>
<td>68</td>
<td>69 (11)</td>
<td>71 (10)</td>
</tr>
<tr>
<td>ENA1</td>
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<td>0.4</td>
<td>69</td>
<td>70 (17)</td>
<td>72 (15)</td>
</tr>
<tr>
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<td>0.6</td>
<td>73</td>
<td>75 (9)</td>
<td>74 (12)</td>
</tr>
<tr>
<td>ENB1</td>
<td>0.2</td>
<td>0.6</td>
<td>69</td>
<td>70 (12)</td>
<td>71 (13)</td>
</tr>
<tr>
<td>AFB1</td>
<td>0.2</td>
<td>1</td>
<td>66</td>
<td>77 (7)</td>
<td>82 (8)</td>
</tr>
<tr>
<td>AFB2</td>
<td>0.25</td>
<td>1</td>
<td>71</td>
<td>73 (2)</td>
<td>76 (6)</td>
</tr>
<tr>
<td>AFB3</td>
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<td>69</td>
<td>71 (10)</td>
<td>79 (8)</td>
</tr>
<tr>
<td>AFB4</td>
<td>0.25</td>
<td>1</td>
<td>73</td>
<td>72 (11)</td>
<td>77 (12)</td>
</tr>
<tr>
<td>STER</td>
<td>0.25</td>
<td>1.5</td>
<td>75</td>
<td>76 (12)</td>
<td>80 (8)</td>
</tr>
<tr>
<td>OTA</td>
<td>0.4</td>
<td>1</td>
<td>82</td>
<td>79 (4)</td>
<td>80 (5)</td>
</tr>
</tbody>
</table>

* ME*: (slope matrix matched calibration/slope standard in solvent) × 100.
* Number of replicates: 10.
* Different days: 5.
was found in 18% of samples. The samples contaminated with T-2 and HT-2 came from Marche region.

ZEN was present in 35% of Italian samples while its metabolite, αZOL, was present in three samples. The co-occurrence of T-2, HT-2 and ZEN in wheat samples and derived products was demonstrated in a previous work conducted in the European Union (Schothorst and van Egmond, 2004). In spite of the fact of the high incidence of type A and B trichothecenes and ZEN, only in one sample from Marche exceeded MLs established by the EU for ZEN (Table 2).

The geographical area has been related to the presence of fungal isolates and it is well known that depends on different factors such as climatic, agronomic practices, as well as the competition with other species (Doohan, Brennan, & Cooke, 2003; Logrieco et al., 2003; Pancaldi et al., 2010; Parry, Jenkinson, & McLeod, 1995; Rutherford et al., 2013; Saremi, Burgess, & Backhouse, 1999). The variability of these factors may strongly alter the mycophora composition from season to season; therefore, this relation is important in order to establish a monitoring plan for fungal diseases and the evaluation of mycotoxins in food and feed.

3.2.2. Co-occurrence of mycotoxins in Syrian wheat grains

Forty Syrian samples were evaluated, and 24 samples (60%) were contaminated by mycotoxins (Table 3). In this case, AFs exhibited the highest incidence. Regarding the co-occurrence of AFs, 14 samples (58%) were contaminated with AFB2 and AFG2. In this study none of the tested samples were contaminated with AFB1 while STER was present in four samples. By contrast, Haydar et al. (1990) estimated AFs contamination in sixty-three samples of nineteen food commodities of Syrian origin (no levels were reported) and found that AFB2 was present only in one sample whilst AFG1 and AFG2 were not detected in any commodity. The absence of AFB1 could be explained by two hypotheses: first, it was present at concentration levels lower than LOQ; secondly AFB1 rapidly decomposes to AFB2, a much less toxic form (Carvajal & Arroyo, 1997), and AFB2 degrades more slowly.

STER, detected in 10% of analysed samples, has been described as a precursor of AFs (Wilkinson, Ramaswamy, Sim, & Keller, 2004). In this research, the occurrence of STER was lower than AFs; it could therefore attribute to the transformation of this toxin to AFs owing to long periods of storage. These results indicate that more attention should be paid to post-harvest conditions to minimize the content of these toxins (Frenich, Vidal, Romero-Gonzalez, & Aguiler-Luiz, 2009). In fact, storage fungi are frequent in Syrian wheat kernels (Alkadri et al., 2013) due to not appropriate storage conditions.

The second most abundant mycotoxin was OTA; 12 samples (50%) of a total of 24 were contaminated with OTA. Six samples were contaminated only by OTA while the other 6 by OTA and AFs.

The incidence and levels of Fusarium toxins in Syrian samples were lower than Italian ones. Approximately, Fusarium toxins were detected in half of positive samples, 11 out of 24. ZEN was detected in 10 samples (42%) and its metabolite αZOL was detected in 3 samples. Type A trichothecenes, such as DON was found in 6 samples (25%) and fumonisins (FB1 and FB2) were found in 4 samples.

3.2.3. Emerging mycotoxins in Italy and Syria wheat

The occurrence of emerging mycotoxins is summarized in Table 4. Usually, Fusarium spp. and Alternaria spp. are responsible for emerging mycotoxin production. In this study, the low incidence of emergent mycotoxins in Syrian samples comparing to Italian ones could be to the low contamination of Fusarium or the presence of non mycotoxigenic Alternaria strains (Alkadri et al., 2013). Moreover, the occurrence of these mycotoxins was different: While ENB was the most common mycotoxin in Italian samples (49%), BEA was the most common mycotoxin (21%) in Syrian samples.

According to Italian results, co-occurrence of the 5 emerging mycotoxins was only verified in one sample and the four enniatins were simultaneously present in 4 samples. ENB and ENB1 were simultaneously detected in 8 samples and ENB was detected together with ENA in 4 samples. Six samples were contaminated...
with only one mycotoxin. BEA was detected alone in only one sample.

Regarding to Syrian samples, the co-occurrence of BEA and ENA and ENA1 was confirmed in 3 samples. ENB was detected in one sample, while ENB1 was not detected in any analyzed sample.

Climatic differences between Syria and Italy can explain this diversity. Syria has an arid and dry climate, very hot in the summer, in winter cold, with an average maximum inland summer temperatures between 33 and 40 °C. On the other hand, the climate of Italy is mainly temperate, and it slightly varies according to the areas. The northern Italian regions have warm humid summers. The Mediterranean climate of Syria is characterized by shorter rainy periods and longer periods of high temperatures, with occasional rains compared with the southern part, while the areas. The northern Italian regions have warm humid summers, with only one mycotoxin. BEA was detected alone in only one sample.

Regarding to Syrian samples, the co-occurrence of BEA and ENA and ENA1 was confirmed in 3 samples. ENB was detected in one sample, while ENB1 was not detected in any analyzed sample.

Climatic differences between Syria and Italy can explain this diversity. Syria has an arid and dry climate, very hot in the summer, in winter cold, with an average maximum inland summer temperatures between 33 and 40 °C. On the other hand, the climate of Italy is mainly temperate, and it slightly varies according to the areas. The northern Italian regions have warm humid summers, with occasional rains compared with the southern part, which is hot and dry. Hence, the existence of Fusarium spp. and their mycotoxins is more abundant in the northern regions.

3.3. Estimation of daily intake

The results of estimate dietary intake (EDI) were compared with temporary TDI (tTDI) of the respective mycotoxins, in order to evaluate the possible health risk associated with the wheat intake (Table 5). Data on wheat consumption were mainly obtained from FAO statistical study (http://faostat.fao.org/).

Regarding Italian samples, the highest exposure to the investigated mycotoxins is attributed to trichothecenes. This fact is in accord to data collection on the occurrence of Fusarium toxins in food in the European Union that showed 57% incidence of positive samples for DON and 16% for NIV out of several thousands of samples analysed (SCOOP, 2003). The European Scientific Committee on Food (SCF) evaluated the Fusarium toxins (EC, 2002), including DON, NIV, T-2 and HT-2 and established a fully tolerable daily intake (TDI) for DON at 1 mg kg⁻¹ body weight day⁻¹, a temporary tolerable dietary intake (t-TDI) for NIV at 0.7 mg kg⁻¹ body weight day⁻¹, and a combined t-TDI for T-2 and HT-2 at 0.06 mg kg⁻¹ body weight day⁻¹. Estimated dietary intake (EDI) of samples with the highest concentration level found (worst case) for DON mycotoxin (DON+3-ADON+15-ADON), NIV, T-2, HT-2 and ZEN exceeded the set TDI. However, in our opinion mean toxin level of total samples (EDI2) represent an estimate of the long-term exposure, and in this study, all EDI2 calculations were lower than TDI established.

Focusing on Syrian samples, no sample exceeded the TDI established, however, it exists AFs intake. Aflatoxins, in particular aflatoxin B1, are considered to be genotoxic and carcinogenic and there is evidence that they can cause liver cancer in humans. In accordance with expert scientific panels, it is not possible to identify an intake without risk. Then the limits set for certain foodstuffs for direct human consumption represent those that are considered to be as low as reasonably achievable (ALARA).

At this point, it is necessary to highlight that the processing of wheat and the exact form of the product is used as food has an important impact on the exposure assessment. The fate of mycotoxins during various processing stages has been the subject of various research papers and published results indicate that large reductions in contamination levels can be achieved (Cano-Sanchez, Sanchis, Ramos, & Marín, 2013; Vaclavikova et al., 2013). For this reason, although the incidence of several mycotoxins was high in raw wheat, it is expected that it would be low for processed
products. For example, bread, and pasta samples would be less often contaminated than wheat samples and with less than half the value in comparison to wheat grains after to cleaning, milling or fermentation steps.

4. Conclusions

There are few studies regarding the presence of different groups of mycotoxins simultaneously in wheat grain samples. In this way, this is the first study, which evaluates a broad spectrum of mycotoxins in wheat grains from Italy and Syria. In this regard, the level of contamination was evaluated in these countries. Type A and B trichothecenes, ZEN, and emergent mycotoxins were commonly detected in Italy. On the other hand, AFs and OTA were detected in Syria. In parallel, the estimation of consumer risk was calculated showing a certain risk in both countries.

In recent years the consumption of wheat around the world has increased. Because of the simultaneous occurrence of different mycotoxins in the analysed samples, there should be a continuous monitoring of wheat and more studies attempting to understand the dynamics involving mycotoxin production in grains need to be carried out with the aim of reducing the presence of these mycotoxins in grain and its cereal-based products.

In general, the concentrations of mycotoxins analysed in our survey were below the maximum levels established by EU. However, one sample exceeded the maximum levels of ZEN in Italy.

The present study demonstrated evidences of a frequent mycotoxin contamination in wheat grains, although the analysis revealed significant differences in occurrence and concentration. The presence of trichothecenes has been mainly detected in samples produced in Italy while AFs and OTA were identified in wheat samples from Syria. These evidences highlighted that the production of mycotoxins are different according to climatic conditions. In this way, the climate change could be an important factor in the future due to temperature increases, which could change fungi distribution.

In light of EDI results, it appears to be low the risk of mycotoxins to consumers, moreover, after the grain processing. However, the presence of some mycotoxins at relative high levels in wheat have evidenced that there is a potential health risk and the necessity of monitoring programs.

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