Deliverable **3.2**

# FOODSAFER

## Report on data about fungal and plant toxins collected from selected geographical regions

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# Deliverable D3.2: Report on data about fungal and plant toxins collected from selected geographical regions


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| Work package number and title | WP3: Chemical Risks Emergence |
| Lead-beneficiary | WU – Wageningen University |
| Work package Leader | BAR – Barilla |
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| Participants | FFoQSI, BAR, IRIS, WU, QUB, BSI |
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# Contents





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# 1. Introduction

Despite the high level of food safety in the European Union, there are still some challenges that need to be addressed in this field. These include foodborne zoonotic diseases caused, for example, by *Campylobacter* species, *Salmonella*, bacterial toxins, and noroviruses[1]. Additionally, the presence of naturally occurring toxins such as mycotoxins, marine biotoxins, and cyanogenic glycosides; environmental pollutants like persistent organic pollutants; and heavy metals such as lead poses a challenge[2]. The FoodSafeR project aims to identify emerging microbiological and chemical risks related to food safety. The definition of an emerging risk is "a risk resulting from a newly identified hazard to which a significant exposure may occur, or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard"[3,4]. Emerging chemical risks include mycotoxins, for which the exposure is changing due to climate change, resulting from modifications in the occurrence of fungal species or their mycotoxin biosynthesis behaviour[5,6]. In Europe, the altered environmental conditions will favour, for example, the growth of *Aspergillus* species such as *A. flavus*[7]. A model projecting a realistic temperature increase of +2 °C above pre-industrial levels over the next 100 years predicts that aflatoxin $B_1$ contamination will become a significant food safety concern in maize in Europe, particularly in Eastern Europe, the Balkan Peninsula, and the Mediterranean regions[8]. Furthermore, a potential shift of the primary fungal species responsible for Fusarium head blight in cereals from *Fusarium culmorum* to *F. graminearum* has been reported for Central and Northern Europe, which could alter mycotoxin contamination[7,9]. Therefore, regular monitoring of natural toxin occurrences and predicting changes is of great importance. Some data on the occurrence of mycotoxins is available, such as the World Mycotoxin Survey, which has been monitoring the presence of mycotoxins in feed since 2004[10]. However, there is still a lack of monitoring data including additionally plant metabolites. To address this gap, wheat and tomato samples were collected from selected regions within the European Union as part of the FoodSafeR project. Then, the samples were analysed using the published multiclass method which enables the simultaneous identification and quantification of more than 1200 different mycotoxins, plant toxins, and pesticides to get a broad spectrum of metabolites, including less well-known mycotoxins and precursors of mycotoxins[11]. The aim with this data is to enhance current prediction tools for the presence of fungal and plant metabolites in food through a big data and machine learning approach, leading to more accurate forecasting of these natural metabolites. In the future, additional data from multiple sources such as satellite images, field and weather records, as well as thermal and hyperspectral imagery will be integrated into a big data model. This report, however, focusses only on the presentation of fungal and plant metabolites in the analysed wheat and tomato samples. In the past, wheat has been reported to be primarily contaminated with the *Fusarium* metabolites deoxynivalenol, nivalenol, fumonisins, enniatins, HT-2 toxin, and zearalenone within the European Union[12–14]. Tomatoes are primarily infected by *Alternaria* species and, thus, tomatoes and tomato-based products are the main contributor to the dietary exposure of the *Alternaria* toxins tenuazonic acid (except for infants) and tentoxin within the European Union[15].

# 2. Material & Methods

## 2.1 Material

Grain and tomato samples from various regions of the European Union were analysed for this project task. The samples were collected in the summer and autumn of 2023 under the coordination of Barilla. The 116 durum wheat samples were sourced from Italy (71 samples, Table 1), Greece (30 samples, Table 2), and Turkey (15 samples, Table 3). The 51 common wheat samples originated from Italy (38 samples, Table 4), France (12 samples, Table 5), and Germany (1 sample, Table 6). In addition, a rye sample from Germany was analysed (Table 6). Wheat and rye samples were collected in some circumstances directly at the harvesting field on the farm but in the majority, they were collected at reception points of storage centres (this explain why they do not cover a single geolocalized field but instead an area of a few square kilometres).The 50 tomato samples originated from various regions of Italy (

Table 7) and in this case, they were all collected at the farm level with precise correlation to GPS geolocalization coordinates.

## 2.2 Sample preparation and LC-MS measurements

The grain and tomato samples were homogenized and toxins were extracted as follows:

The complete grain samples provided (approx. 200–800 g per sample) were ground using a mill (Laboratory Mill 3610, Perten Instruments, Stockholm, Sweden). Subsequently, the ground wheat/rye was mixed by stirring, and 5.00 g (± 0.05 g) were weighed in. For the extraction of toxins, 20 mL of extraction solvent (acetonitrile/water/acetic acid, 79:20:1, v/v/v) was added, and the samples were shaken on a rotary shaker under horizontal conditions for 90 min.

The tomatoes (approx. 4–8 per sample) were cut into pieces and shredded using a mill (GM200, Retsch GmbH, Haan, Germany). Toxins were extracted from 5.00 g (± 0.05 g) of the resulting tomato juice using 20 mL of extraction solvent (acetonitrile/acetic acid, 99:1, v/v) by shaking the samples for 90 min under horizontal conditions on a rotary shaker.

Next, 0.5 mL of the extract of each grain and tomato sample was diluted with 0.5 mL of a dilution solution (acetonitrile/water/acetic acid, 20:79:1, v/v/v). The samples were measured in the positive and negative ionization mode on a QTrap 5500 MS/MS system (SCIEX, Foster City, CA, USA) coupled to a 1290 series UHPLC system (Agilent Technologies, Waldbronn, Germany) following the published multiclass method[11]. The software MultiQuant version 3.0.3 (SCIEX, Foster City, CA, USA) was used for the subsequent data analysis. The samples were manually screened for the presence of toxins and chromatograms were manually integrated. Metabolite concentrations were calculated using the concentrations of the individual standards. Results were corrected for individual recoveries. For metabolites without a standard available, the area under the curve is provided in the results section instead of the concentration. The final data evaluation and calculation were performed using Microsoft Excel version 2108.

# 3. Results

## 3.1 Quantification of fungal and plant metabolites

The data is given in the supplemental excel file and a summery showing mean values is given in Appendix 2: Average concentration of fungal and plant metabolites

Table 8. For further use of the data, it will be accessible on the FoodSafeR Open Digital Hub.

### 3.1.1 Durum wheat from Italy

A total of 71 durum wheat samples from Italy were analysed. In particular, *Fusarium* metabolites were detected among the relevant fungal metabolites analysed. Among these, enniatins A, A1, B, B1, and B2 were detected in all samples, and these metabolites were found to have the highest levels among all analysed grain samples in individual Italian durum wheat samples. The highest concentrations of the various enniatins were found in sample 322059 from Tuscany and samples 315691, 317603, and 317604 from Campania. Deoxynivalenol, a further *Fusarium* metabolite, was detected in all durum wheat samples. Further, HT-2 toxin was detected in 68%, HT-2 glucoside in 31%, T2-toxin in 45%, zearalenone in 52%, and zearalenone sulfate in 58% of the samples. Among these metabolites, sample 310246 from Puglia contained the highest levels of HT-2 toxin (91.9 µg/kg), HT-2 glucoside (82.9 µg/kg), and T2 toxin (33.8 µg/kg) among all the analysed grain samples. Additionally, fumonisin $B_1$ was detected in 4 samples. This metabolite was found in these durum wheat samples at the highest concentrations among all the analysed grains. The highest level was measured in the sample 312311 from Molise (26.3 µg/kg).

In addition, metabolites of *Alternaria* such as altersetin (in all samples), tenuazonic acid (in 99% of the samples), alternariol methyl ether (83%), altertoxin I (72%), and alternariol (69%) were detected in numerous samples. In individual samples, ergot alkaloids such as ergometrine (31%), ergosin (27%), ergocristine (17%), ergocryptine (17%), and ergotamine (10%) were additionally detected.

The cyanogenic glycoside prunasin was detected in 62% of the samples of the relevant plant metabolites.

### 3.1.2 Durum wheat from Greece

A total of 30 samples of durum wheat from Greece were analysed. Metabolites of *Alternaria* species were particularly detected. Among these, tenuazonic acid was measured in all samples, altersetin (in 70% of the samples), alternariol methyl ether (77%), and alteichin (93%) were detected in most, and alternariol (47%) as well as altertoxin I (13%) in some samples, representing only a selection of the metabolites detected. Among all the analysed grain samples, individual Greek durum wheat samples (numbers 3, 4, 5, 6, 14, 16) contained the highest levels of tenuazonic acid (1.0–4.3 mg/kg). Also, the sample with the highest concentration of alternariol (52.2 µg/kg) and alternariol methyl ether (248 µg/kg) was from Megalokampos in Greece (number 17).

In addition, the *Fusarium* metabolites moniliformin and deoxynivalenol, as well as enniatins were detected in all samples. Further, HT-2 toxin, T-2 toxin, and zearalenone were found in

30%, 27%, and 23% of the samples. Hardly any ergot alkaloids were detected in the Greek samples.

Of the analysed plant toxins, xanthotoxin was measured in 77% of the samples, while prunasin was detected in two individual samples and atropine and hyoscine were each detected in only one sample. Sample 17 was the only one among all the analysed grains in which atropine and hyoscine were detected.

### 3.1.3 Durum wheat from Turkey

*Fusarium* metabolites were measured in extracts from all 15 durum wheat samples from Turkey. Of these, enniatin B1 was detected in all samples, enniatin A in 73%, enniatin A1 in 87%, enniatin B in 73%, moniliformin in 93%, deoxynivalenol in 60%, 15-acetyldeoxynivalenol in 20%, and HT-2 toxin in 20% of the samples. Further, the *Alternaria* metabolites tenuazonic acid and infectopyrone were also detected in all samples, while, among others, tentoxin was found in 93%, alternariol in 13%, and alternariol methyl ether in 20% of the samples. In two samples, some ergot alkaloids, including ergotamine, ergometrine, ergometrinine, and ergocristinine, were also detected.

Of the analysed plant metabolites, prunasin and xanthotoxin were detected in 33% and 7% of the samples.

### 3.1.4 Common wheat from Italy

A total of 38 common wheat samples from Italy were analysed. Among the *Fusarium* metabolites, HT-2 toxin (in 13% of the samples), deoxynivalenol (82%), deoxynivalenol-3-glucoside (68%), the enniatins A, A1, B, B1, B2, and B3 (at least three different ones in each sample), (epi-)equisetin (42%), zearalenone (24%), and fumonisin $B_1$ (5%) were, among others, detected. Among the *Alternaria* metabolites, tenuazonic acid, altersetin, and alteichin were additionally detected in all or nearly all samples, and alternariol and alternariol methyl ether in 24% and 34% of the samples. A total of 18% of the samples were positive for various ergot alkaloids (e.g., ergocryptine, ergocornine, ergocorninine, and ergosin).

Plant metabolites, such as lotaustralin (3%) and xanthotoxin (21%), were detected in some of the samples.

### 3.1.5 Common wheat from France

12 common wheat samples from France were analysed. *Fusarium* metabolites were detected in all samples. As the most frequently found one, moniliformin was detected in all samples. Further, deoxynivalenol (in 58% of the samples), deoxynivalenol-3-glucoside (58%), nivalenol (25%), the enniatins A (58%), A1 (92%), B (92%), B1 (100%), and B2 (42%) were detected most frequently. Several *Alternaria* toxins, including tenuazonic acid (33%), altersetin (17%), alteichin (83%), and infectopyrone (100%), were detected in individual wheat samples from France. In addition, a range of ergot alkaloids such as ergometrine (33%), ergometrinine (33%), ergosin (25%), ergotamine (25%), ergocristine (25%), ergocristinine (17%), and ergocornine (17%) were detected.

### 3.1.6 Common wheat and rye from Germany

Only one rye and one wheat sample from Germany were analysed. In the rye sample, *Fusarium* metabolites such as deoxynivalenol, aurofusarin, and the enniatins B and B1, as well as *Alternaria* metabolites such as tenuazonic acid, altersetin, altertoxin II, and alteichin, were detected. In the wheat sample, we detected the *Fusarium* metabolites aurofusarin, and the enniatins A, A1, B, and B1, as well as the *Alternaria* metabolites tenuazonic acid, altersetin, altertoxin II and III, alteichin, and alternariol methyl ether.

The cyanogenic glycoside lotaustralin was detected in the extract of the wheat sample.

### 3.1.7 Tomatoes from Italy

Small spots of fungal growth, most likely from *Alternaria* species, were visible on only a few tomatoes, and only a small variety of different mycotoxins was detected in extracts from the 50 tomato samples. Among the *Alternaria* metabolites, altersetin was detected in 32% of the samples, tenuazonic acid in 30%, alternariol methyl ether in 6%, alternariol in 2%, and tentoxin in 2%. Particularly, one sample from Mucinasso contained relatively high concentrations of alternariol (43.8 µg/kg), alternariol methyl ether (19.6 µg/kg), and tenuazonic acid (1.95 mg/kg). Further, the *Penicillium* metabolite chrysogin was detected in 94% of the samples. Enniatin B, enniatin B1, and Siccanol were each detected in one sample. No other *Fusarium* metabolites were detected and also no ergot alkaloids were found. Furthermore, the metabolite cordycepin was detected in all samples, tryptophol in 74%, and the antibiotic chloramphenicol in 2% of the samples.

In the case of plant metabolites, the cyanogenic glycosides prunasin and amygdalin were detected in 86% and 80% of the samples, respectively, and the plant hormone abscisic acid was detected in all samples.

### 3.1.8 Comparison of average metabolite concentrations between samples from different countries

The mean concentrations of selected fungal and plant metabolites in the samples from each country were calculated (Table 8). The samples from Germany were not included, as we only had two samples. When comparing the mean concentrations, it was found that the durum wheat samples from Italy and from Greece showed the highest levels of contamination, while the common wheat samples from France and also the tomato samples from Italy were the least contaminated. However, the range of concentrations of most metabolites in individual samples from all countries was very high. Among the *Fusarium* metabolites, the highest levels of deoxynivalenol and nivalenol were measured, on average, in the common and durum wheat samples from Italy, as well as in the durum wheat samples from Greece. The deoxynivalenol concentrations of the durum wheat sample 322071 from Tuscany in Italy and of the durum wheat samples 17 and 28 from Megalokampos and from Theva in Greece even exceeded the European maximum level of 1750 µg/kg for unprocessed durum wheat grains[16]. In contrast, the mean concentrations of these metabolites were relatively low in the samples from France. Among the different enniatins, enniatin B and B1 were found to have the highest concentrations in most grain samples from all the different countries. The highest average levels of all analyzed enniatins were measured in the samples of Italian durum wheat. Fumonisin $B_1$ was only detected in six Italian wheat samples at relatively low concentrations,

while other fumonisins were not detected at all. For both T-2 and HT-2 toxins, the mean levels were highest in the durum wheat samples from Italy, followed by those from Greece. In contrast, both toxins were measured at clearly lower levels in the common wheat samples, particularly from France and Italy. Zearalenone and zearalenone sulfate were also measured in the highest concentrations on average in the durum wheat samples from Italy and Greece. The highest zearalenone concentrations were, however, 6 times lower than the European maximum level of 100 µg/kg for unprocessed grains[16]. Among the *Alternaria* toxins, alternariol, alternariol methyl ether, and tenuazonic acid were found to have the highest average concentrations in the durum wheat samples from Greece, followed by those from Italy. In contrast, low concentrations of *Alternaria* metabolites were detected in the common wheat samples from France, with the exception of infectopyrone. Among the various ergot alkaloids, the samples from France contained the highest average levels of ergotamine, ergosin, and ergocristine, while the common wheat samples from Italy contained the highest levels of ergocryptine and ergometrine. Interestingly, different ergot alkaloids were measured in the highest concentration in the samples from different countries. In the samples from France and Turkey, the highest levels of ergotamines were measured among the different ergot alkaloids. In common wheat samples from Italy, ergocryptine was predominant, while in the durum wheat samples from Italy, ergocornine was prevalent. In the samples from Greece, ergometrine was predominant among the different ergot alkaloids. However, hardly any ergot alkaloids were detected in the Greek wheat. Among the further major mycotoxins, aflatoxins were not detected in any sample. Among the plant toxins analysed, prunasin was measured in the Italian durum wheat samples at the highest levels.

## 4. Conclusion and outlook

The analysis of 168 grain and 50 tomato samples from Italy, Turkey, Greece, France, and Germany using a multiclass LC-MS method showed that the concentrations of the fungal and plant metabolites of particular interest were in most samples rather low, except for some samples with high concentrations of particularly deoxynivalenol, tenuazonic acid, enniatin B and B1, infectopyrone or altersetin. Most grain samples contained the *Alternaria* metabolites tenuazonic acid, alteichin, altersetin, and infectopyrone, and some samples additionally metabolites like alternariol, alternariol methyl ether, altertoxins, and tentoxin. Deoxynivalenol and enniatins were detected in most grain samples of the *Fusarium* metabolites and further metabolites of this species like HT-2 toxin, HT-2 glucoside, T-2 toxin, and zearalenone were detected in some samples. Ergot alkaloids were also detected in some grain samples. Of these, the most frequently detected ones were ergotamine, ergosine, ergometrine, ergometrinine, ergocryptine, ergocornine, ergocristine, and ergocristinine. The *Alternaria* toxins altersetin, tenuazonic acid, and alternariol methyl ether were detected in some tomato samples. Except for these, hardly any mycotoxins were detected in the tomato samples. The analysis of the plant toxins focussed on cyanogenic glycosides. Of these, especially prunasin was detected in some grain samples, and prunasin and amygdalin in many tomato samples.

Within the course of the FoodSafeR project, further samples will be collected and analysed. As next steps of Task 3.1 of the project, currently available prediction models for fungal infection and mycotoxin production in grains and tomatoes will be extended by this

comprehensive data to improve the models. Further, for plant toxins, for which no models exist at the moment, the data will be used to develop first models for their occurrence in Europe. Further data like crop phenology models, satellite data, the Copernicus climate database, global soil maps, elevation data, and historical monitoring data on mycotoxin occurrences will be used and combined with the models with data fusion methods. Then, machine learning models will be applied to find hidden dependencies between the external factors and the fungal infections. Developed models for mycotoxins and plant toxins will be tested and validated in the last year for the selected geographical regions in cooperation with Barilla.

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# 6. Appendix

*The related excel-based database is available on the FoodSafeR Zenodo Community site:*

*https://zenodo.org/communities/foodsafer*

## Appendix 1: Lists of samples

*Table 1: List of the durum wheat samples from Italy*

| **Province** | **Region** | **Sample code** |
|---|---|---|
| Foggia | Puglia | 310242 |
| Foggia | Puglia | 310243 |
| Foggia | Puglia | 310245 |
| Foggia | Puglia | 310246 |
| Foggia | Puglia | 310247 |
| Foggia | Puglia | 310248 |
| Parma | Emilia Romagna | 310253 |
| Piacenza | Emilia Romagna | 310259 |
| Parma | Emilia Romagna | 310263 |
| Parma | Emilia Romagna | 310273 |
| Brescia | Lombardia | 310277 |
| Mantova | Lombardia | 310281 |
| Mantova | Lombardia | 310283 |
| Mantova | Lombardia | 310285 |
| Mantova | Lombardia | 310289 |
| Foggia | Puglia | 310846 |
| Chieti | Abruzzo | 310847 |
| Macerata | Marche | 311859 |
| Ancona | Marche | 311861 |
| Ancona | Marche | 311862 |
| Macerata | Marche | 311863 |
| Macerata | Marche | 311864 |
| Ancona | Marche | 311865 |

| | | |
|---|---|---|
| Campobasso | Molise | 312311 |
| Campobasso | Molise | 312312 |
| Campobasso | Molise | 312313 |
| Modena | Emilia Romagna | 312691 |
| Modena | Emilia Romagna | 312692 |
| Modena | Emilia Romagna | 312693 |
| Mantova | Lombardia | 312694 |
| Mantova | Lombardia | 312695 |
| Mantova | Lombardia | 312696 |
| Padova | Veneto | 312697 |
| Padova | Veneto | 312698 |
| Padova | Veneto | 312699 |
| Rovigo | Veneto | 312700 |
| Rovigo | Veneto | 312701 |
| Rovigo | Veneto | 312702 |
| Rovigo | Veneto | 312703 |
| Rovigo | Veneto | 312704 |
| Verona | Veneto | 312706 |
| Verona | Veneto | 312707 |
| Ferrara | Emilia Romagna | 313703 |
| Ferrara | Emilia Romagna | 313705 |
| Ferrara | Emilia Romagna | 313706 |
| Ferrara | Emilia Romagna | 313707 |
| Ferrara | Emilia Romagna | 313709 |
| Pescara | Abruzzo | 313714 |
| Teramo | Abruzzo | 313715 |
| Benevento | Campania | 313716 |
| Benevento | Campania | 316486 |
| Caserta | Campania | 315691 |
| Benevento | Campania | 317603 |
| Avellino | Campania | 317604 |

| | | |
|---|---|---|
| Cosenza | Calabria | 318949 |
| Grosseto | Tuscany | 322056 |
| Grosseto | Tuscany | 322068 |
| Pisa | Tuscany | 322064 |
| Pisa | Tuscany | 322066 |
| Grosseto | Tuscany | 322053 |
| Grosseto | Tuscany | 322055 |
| Grosseto | Tuscany | 322059 |
| Grosseto | Tuscany | 322071 |
| Pisa | Tuscany | 318951 |
| Arezzo | Tuscany | 322069 |
| Siena | Tuscany | 319526 |
| Livorno | Tuscany | 318952 |
| Terni | Umbria | 319536 |
| Perugia | Umbria | 319525 |
| Perugia | Umbria | 319522 |
| Perugia | Umbria | 319524 |

*Table 2: List of durum wheat samples from Greece*

| **Area** | **Region** | **Sample code** |
|---|---|---|
| Kileler | Larisa | Greece 1 |
| Chalkidiki | Chalkidiki | Greece 2 |
| Almyros | Magnesia | Greece 3 |
| Kilkis | Kilkis | Greece 4 |
| Kilkis | Kilkis | Greece 5 |
| Koilada | Larisa | Greece 6 |
| Swthrio | Larisa | Greece 7 |
| Plasia | Larisa | Greece 8 |
| Falanh | Larisa | Greece 9 |
| Sitochoro | Farsala | Greece 10 |
| Orchomenos | Voiwtia | Greece 11 |

| | | |
|---|---|---|
| Prodromos | Larisa | Greece 12 |
| Karditsa | Karditsa | Greece 13 |
| Kotani | Kozani | Greece 14 |
| Sterea Ellada | Sterea Ellada | Greece 15 |
| Hrakleia | Serres | Greece 16 |
| Megalokampos | Drama | Greece 17 |
| Mavrolefki | Drama | Greece 18 |
| Gazwros | Serres | Greece 19 |
| Agchilos | Thessaloniki | Greece 20 |
| Drama | Drama | Greece 21 |
| Drama | Drama | Greece 22 |
| Drama | Drama | Greece 23 |
| Axioupoli | Kilkis | Greece 24 |
| Aiginio | Pieria | Greece 25 |
| Leptokarya | Pieria | Greece 26 |
| Anthochori | Drama | Greece 27 |
| Theva | Theva | Greece 28 |
| Theva | Theva | Greece 29 |
| Komotini | Komotini | Greece 30 |

*Table 3: List of durum wheat samples from Turkey*

| **Province** | **Region** | **Sample code** |
|---|---|---|
| Ankara | Central Anatolia | Turkey 1 |
| Usak | Aegean | Turkey 2 |
| Afyonkarahisar | Aegean | Turkey 3 |
| Konya | Central Anatolia | Turkey 4 |
| Mardin | South East Anatolia | Turkey 5 |
| Konya | Central Anatolia | Turkey 6 |
| Sanliurfa | South East Anatolia | Turkey 7 |
| Mardin | South East Anatolia | Turkey 8 |

| | | |
|---|---|---|
| Corum | Central Anatolia | Turkey 9 |
| Gazientep | South East Anatolia | Turkey 10 |
| Kutahya | Aegean | Turkey 11 |
| Mardin | South East Anatolia | Turkey 12 |
| Mardin | South East Anatolia | Turkey 13 |
| Mardin | South East Anatolia | Turkey 14 |
| Corum | Central Anatolia | Turkey 15 |

*Table 4: List of the common wheat samples from Italy*

| **Province** | **Region** | **Sample code** |
|---|---|---|
| Novara | Piemonte | 317620 |
| Mantua | Lombardia | 317621 |
| Alessandria | Piemonte | 317622 |
| Alessandria | Piemonte | 317623 |
| Alessandria | Piemonte | 317781 |
| Alessandria | Piemonte | 317782 |
| Alessandria | Piemonte | 317783 |
| Milan | Lombardia | 317784 |
| Ferrara | Emilia Romagna | 317785 |
| Ferrara | Emilia Romagna | 317786 |
| Rovigo | Veneto | 317787 |
| Ravenna | Emilia Romagna | 317788 |
| Rovigo | Veneto | 320308 |
| Rovigo | Veneto | 320310 |
| Cremona | Lombardia | 320311 |
| Cremona | Lombardia | 320312 |
| Alessandria | Piemonte | 320313 |
| Alessandria | Piemonte | 320314 |
| Parma | Emilia Romagna | 320771 |
| Parma | Emilia Romagna | 320772 |
| Parma | Emilia Romagna | 320773 |

| | | |
|---|---|---|
| Lodi | Lombardia | 320775 |
| Bologna | Emilia Romagna | 320776 |
| Cuneo | Piemonte | 321848 |
| Cuneo | Piemonte | 321849 |
| Cuneo | Piemonte | 321850 |
| Rovigo | Veneto | 321851 |
| Modena | Emilia Romagna | 322431 |
| Reggio Emilia | Emilia Romagna | 322432 |
| Reggio Emilia | Emilia Romagna | 322433 |
| Modena | Emilia Romagna | 322434 |
| Turin | Piemonte | 324054 |
| Turin | Piemonte | 324055 |
| Rovigo | Veneto | 324056 |
| Rovigo | Veneto | 324057 |
| | | AS1 |
| | | AS2 |
| | | AS3 |

*Table 5: List of common wheat samples from France*

| **Sample code** |
|---|
| 325101 |
| 325102 |
| 325104 |
| 325105 |
| 325106 |
| 325107 |
| 326012 |
| 326014 |
| 326015 |
| 326016 |
| 326017 |

| |
|---|
| 326018 |

*Table 6: List of grain samples from Germany*

| Grain | Region | Sample code |
|---|---|---|
| rye | Uetze | R1 |
| wheat | Uetze | W1 |

*Table 7: List of tomato samples from Italy*

| Sample code | GPS data |
|---|---|
| Capra | 45°01'17.9"N 9°46'58.9"E |
| Prada | 44°42'59.5"N 10°22'41.4"E |
| Guideo | 45°03'09.7"N 9°46'50.6"E |
| Prada Quirino | 44°41'12.1"N 10°21'35.1"E |
| Mucinasso | 45°00'21.0"N 9°42'40.1"E |
| Bellica | 44°83'16.85"N 10°15'83.82"E |
| Forzani 6 | 44°91'20.13"N 10°14'90.03"E |
| Aschieri | 44°71'41.92"N 10°32'68.17"E |
| Troni | 44°90'60.38"N 10°78'90.50"E |
| Brambilla Spigarolo | 44°59'08.8"N 10°04'31.2"E |
| Barabaschi Bersano | 44°59'06.1"N 10°00'32.2"E |
| Bernazzoli Busseto | 45°00'21.0"N 10°03'01.3"E |
| Torreciani Villanova | 45°01'15.0"N 9°59'06.3"E |
| Calza | 44°98'80.63"N 10°10'70.84"E |
| Sfolcini | 45°02'22.52"N 9°51'67.68"E |
| RoncoleVerdi-Bassa MaJ | 44°95'94.96"N 10°05'91.00"E |
| Passavanti | 44°93'90.86"N 10°40'55.79"E |
| Forzani 8 | 44°88'50.41"N 10°19'31.11"E |
| Pantera | 44°89'19.14"N 10°42'68.85"E |
| Boselli | 44°73'58.35"N 10°27'25.37"E |
| Varani | 44°88'35.24"N 9°90'25.66"E |
| Benassi | 44°80'62.56"N 10°24'12.3"E |
| Donelli | 44°76'85.72"N 10°46'25.30"E |
| Freddi | 44°91'34.96"N 10°79'20.97"E |
| Bartoli | 44°88'20.35"N 10°71'69.26"E |
| Minara | 44°86'54.81"N 10°72'35.30"E |
| BLAB6202LC50 | 44°93'07.08"N 9°63'18.52"E |
| BLAB6630CC80 | 45°03'26.3"N 10°04'32.0"E |
| 6428LS40 | 45°09'49.08"N 9°53'29.99"E |

| | |
|---|---|
| 6712_IMM_50 | 45°01'17.69"N 10°79'19.37"E |
| BLAB5752MR39 | 44°56'01.6"N 9°58'02.0"E |
| BLAB6358 | 44°56'01.6"N 9°58'02.0"E |
| 5935LS41 | 45°02'71.99"N 9°44'99.67"E |
| 6684_IMM_51 | 45°03'91.67"N 10°29'11.12"E |
| BLAB6615LC50 | 45°01'44.65"N 9°73'44.75"E |
| BLAB6564MR41 | 44°56'13.9"N 9°52'47.7"E |
| BLAB6630CC79 | 45°03'02.6"N 10°03'48.5"E |
| BLAB1429LC49 | 44°94'38.21"N 9°76'64.65"E |
| Alseno1301 | 44°55'40.9"N 9°55'51.2"E |
| PC-1 | 44°79'73.83"N 10°41'34.32"E |
| PC-2 | 44°78'90.06"N 10°22'97.18"E |
| Fiorenzuola Sala BIO | 44°53'38.0"N 9°57'30.5"E |
| Fiorenzuola Bufari BIO | 44°55'40.9"N 9°55'51.2"E |
| 64685B40 BIO | 44°68'83.45"N 10°35'58.35"E |
| 7435B36 BIO | 44°79'97.01"N 10°41'93.65"E |
| 6468M526 BIO | 44°71'56.03"N 10°34'88.04"E |
| 64675B50 BIO | 44°83'29.23"N 10°28'23.35"E |
| 66125B39 BIO | 44°67'04.12"N 10°34'56.09"E |
| 743SB37 BIO | 44°78'38.87"N 10°41'68.76"E |
| AINPO 6679 BIO | 44°78'50.26"N 10°64'35.53"E |

## Appendix 2: Average concentration of fungal and plant metabolites

*Table 8: Mean [µg/kg] ± standard deviation of fungal and plant metabolites. The substitution method was chosen and all values < LOQ were set to zero (except for those metabolites which were not detected in any sample of one country) to calculate mean vales.*

| Metabolite | Durum wheat Italy | Durum wheat Greece | Durum wheat Turkey | Common wheat Italy | Common wheat France | Tomatoes Italy |
|---|---|---|---|---|---|---|
| Deoxynivalenol | 385 ± 407 | 312 ± 559 | 103 ± 167 | 220 ± 209 | 22.0 ± 38.3 | < LOD |
| DON-3-glucoside | 136 ± 118 | 118 ± 163 | 56.6 ± 89.7 | 54.4 ± 55.9 | 26.0 ± 46.0 | < LOD |
| Enniatin A | 5.33 ± 5.70 | 0.761 ± 1.327 | 1.63 ± 4.52 | 0.523 ± 0.620 | 0.106 ± 0.164 | < LOD |
| Enniatin A1 | 39.8 ± 38.2 | 5.12 ± 7.90 | 9.31 ± 26.25 | 4.27 ± 4.72 | 0.865 ± 1.228 | < LOD |
| Enniatin B | 215 ± 181 | 37.6 ± 46.9 | 12.7 ± 30.1 | 40.7 ± 44.3 | 3.28 ± 4.50 | 0.0065 ± 0.0455 |
| Enniatin B1 | 156 ± 155 | 18.3 ± 22.4 | 17.7 ± 47.1 | 19.0 ± 19.6 | 2.74 ± 3.71 | < LOD |
| Fumonisin B1 | 0.702 ± 3.689 | < LOD[a] | < LOD | 0.392 ± 2.386 | < LOD | < LOD |
| HT-2 toxin | 10.4 ± 13.6 | 4.08 ± 7.59 | 3.13 ± 7.31 | 0.872 ± 2.372 | 0.673 ± 2.231 | < LOD |
| Moniliformin | 349 ± 451 | 50.7 ± 84.3 | 14.9 ± 16.4 | 40.9 ± 49.0 | 1.03 ± 2.32 | < LOD |
| Nivalenol | 56.9 ± 77.3 | 17.4 ± 36.5 | < LOD | 5.45 ± 18.82 | 9.35 ± 18.76 | < LOD |
| T-2 toxin | 2.35 ± 4.75 | 0.469 ± 0.899 | 0.101 ± 0.270 | < LOD | 0.124 ± 0.411 | < LOD |
| Zearalenone | 1.43 ± 3.11 | 1.62 ± 3.60 | < LOD | 0.238 ± 0.666 | 0.0823 ± 0.2729 | < LOD |
| Zearalenone-sulfate | 11.5 ± 20.7 | 9.08 ± 22.15 | < LOD | 1.26 ± 3.27 | 1.30 ± 4.31 | < LOD |
| Alternariol | 3.76 ± 5.95 | 6.08 ± 11.63 | 0.629 ± 1.711 | 0.894 ± 2.261 | < LOD | 0.875 ± 6.125 |



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|---|---|---|---|---|---|---|
| Alternariol methyl ether | 9.17 ± 19.86 | 12.4 ± 44.6 | 1.02 ± 2.36 | 0.567 ± 1.393 | < LOD | 0.470 ± 2.770 |
| Altersetin | 130 ± 146 | 74.4 ± 100.6 | 1.64 ± 6.15 | 97.8 ± 189.1 | 2.50 ± 6.32 | 15.1 ± 91.0 |
| Altertoxin-I | 3.29 ± 3.39 | 1.18 ± 4.26 | < LOD | 0.785 ± 2.244 | < LOD | < LOD |
| Infectopyrone | 780 ± 475 | 1116 ± 562 | 476 ± 535 | 509 ± 278 | 1065 ± 294 | < LOD |
| Tenuazonic acid | 267 ± 216 | 744 ± 863 | 127 ± 89 | 91.4 ± 53.3 | 4.42 ± 9.34 | 91.0 ± 345.7 |
| Ergocornine | 12.0 ± 62.3 | < LOD | < LOD | 8.15 ± 26.62 | 0.499 ± 1.654 | < LOD |
| Ergocristine | 5.87 ± 24.86 | < LOD | 0.419 ± 1.570 | 2.42 ± 9.73 | 20.0 ± 64.2 | < LOD |
| Ergocryptine | 4.72 ± 19.89 | < LOD | < LOD | 12.1 ± 55.6 | 0.417 ± 1.385 | < LOD |
| Ergometrine | 2.72 ± 7.29 | 0.267 ± 1.439 | 2.54 ± 9.50 | 7.73 ± 33.30 | 6.19 ± 16.76 | < LOD |
| Ergosin | 4.77 ± 16.89 | 0.101 ± 0.349 | 0.0765 ± 0.2862 | 9.56 ± 43.45 | 11.1 ± 27.0 | < LOD |
| Ergotamine | 1.74 ± 8.97 | < LOD | 31.0 ± 114.9 | 4.34 ± 22.25 | 45.2 ± 108.5 | < LOD |
| Atropine | < LOD | 8.69 ± 46.79 | < LOD | < LOD | < LOD | < LOD |
| Prunasin | 6.71 ± 23.66 | 0.222 ± 0.860 | 0.554 ± 0.833 | < LOD | < LOD | 10.7 ± 6.6 |

[a]LOD, limit of detection