

Review

# Malting and Brewing Industries Encounter *Fusarium* spp. Related Problems

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Received: 30 November 2017; Accepted: 4 January 2018; Published: 9 January 2018

**Abstract:** Versatile microbiota are inevitably naturally present on cereals. Fungi, yeasts and bacteria and their metabolites all contribute to the quality and safety of the final products derived from most common beer cereals—barley and wheat. The microorganisms that are most often associated with the safety and quality of cereals for beer production belong to the *Fusarium* spp. They greatly influence yields from the field, and can modify and diminish economic success for farmers. However, the real problem is their harmful metabolites—mycotoxins—that affect the health of humans and animals. In the era of emerging analytical methodologies, the spectrum of known toxins originating from microorganisms that can pose a threat to humans has grown tremendously. Therefore, it is necessary to monitor microflora throughout the productive “barley to beer” chain and to act suppressive on the proliferation of unwanted microorganisms, before and during malting, preventing the occurrence of mycotoxins in final products and by-products. Multi-mycotoxin analyses are very advanced and useful tools for the assessment of product safety, and legislation should follow up and make some important changes to regulate as yet unregulated, but highly occurring, microbial toxins in malt and beer.

**Keywords:** barley; wheat; *Fusarium*; malt; beer; mycotoxins

## 1. Introduction

Cereals, such as wheat and barley, have always been a valuable source of food for humans and animals. Wheat is mostly used as a raw material for the baking industry, but malting and brewing industries take up a significant portion of cereal production. These industries are very conscious of *Fusarium* infections of cereals intended for malting. Wheat and barley intended for malting have to fulfil strict limits and recommendations on the quality of raw material used for malting and brewing. Some of the main quality parameters for malt are: protein content,  $\beta$ -glucan content, Kolbach index, malt extract, extract difference, saccharification time, wort colour, viscosity FAN (free amino nitrogen) [1]. Protein content is one of the most important parameters and ranges between 11 and 12% [2]. Protein content higher than 12% results in heightened soluble protein content in wort, causing beer to exhibit undesirable flavours. On the other hand, lower protein content is usually correlated with low carbohydrate levels and lower extract values [3]. This causes adverse effects in fermentation, due to the poor amino acid content available for yeast nutrition. Lower amounts of  $\beta$ -glucans (<4%) are considered useful in brewing cereals, because they stabilize beer foam and improve beer organoleptic properties [1,4], but in higher amounts they cause serious problems during both malting and brewing.

*Fusarium* spp. causes damage to producers and consumers on several levels. Namely, the noxious effect of mycotoxins to humans and animals is well known, and the detrimental damage of mycotoxins is well described in many scientific papers cited in this review. Some of the mycotoxins produced by *Fusarium* spp. (trichotecenes, zearalenone (ZEA), fumonisins) have been studied for many

years and are included in legislative regulations. However, multi-mycotoxin analyses have shown a tremendous advance and are more and more accurate in determining novel mycotoxins in very low concentrations [5,6]. They appear to be a useful tool for assessment of final product safety, and in exposure assessment, through the analysis of mycotoxin biomarkers [7,8]. The legislation is, on the other hand, a bit slow when it comes to defining levels of these new emerging mycotoxins: enniatins, beauvericin, moniliformin and many others, as reported by Bolechová et al. [9] and Juan et al. [10]. All this indicates that the legislation needs some changes, including beer and brewing by-products regulations and/or expanding the regulated mycotoxins list, according to their occurrence and toxicity. The European Commission (EC) has established a range of maximum limits for deoxynivalenol (DON) and ZEA in cereals and cereal-based foods [11], but maximum amounts for these mycotoxins in beer are yet to be set.

Gushing is also one of the side effects of *Fusarium* fungi presence [12]. The over-foaming of beer can cause great economic losses for breweries and most likely will drive away consumers.

The aim of this work was to present some of the main problems related to *Fusarium* infections in malting and brewing industries.

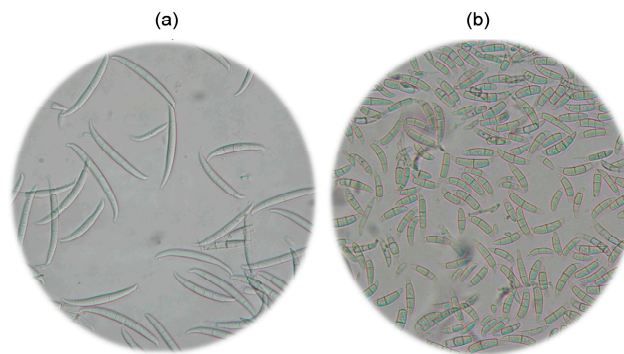
## 2. *Fusarium* Fungi and Its Effect on Barley Grains

Good quality malt requires a good quality raw material, usually barley or wheat. *Fusarium* fungi are the most common fungal species that invade grains in our region [13]. Genus *Fusarium* includes the following species: *F. acuminatum*, *F. anthophilium*, *F. avanceum*, *F. cerealis* (*crookwellense*), *F. chlamydosporum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. heterosporum*, *F. nygamai*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. sambucinum*, *F. semitectum*, *F. sporotrichoides*, *F. subglutaminans*, *F. tricintum* and *F. verticillioides*. They reproduce asexually through conidiospores, but many of them have the ability to sexually reproduce. The asexual phase (anamorphic form) is referred to as *Fusarium*, and the sexual phase (teleomorphic form) is known as *Gibberella* or *Nectria*. *F. graminearum*, *F. culmorum*, *F. cerealis* are described as aggressive pathogens and cause *Fusarium* head blight (FHB) [14]. Other species that are also commonly found on barley and wheat, shown in Table 1 are called “weak parasites” or “opportunists”.

**Table 1.** *Fusarium* species division on weak and aggressive pathogens [14].

Species	Weak Pathogens	Aggressive Pathogens
<i>F. graminearum</i>	+	—
<i>F. culmorum</i>	+	—
<i>F. cerealis</i>	+	—
<i>F. avanceum</i>	—	+
<i>F. tricintum</i>	—	+
<i>F. sporotrichoides</i>	—	+
<i>F. poae</i>	—	+
<i>F. acuminatum</i>	—	+
<i>F. oxysporum</i>	—	+
<i>F. equiseti</i>	—	+
<i>F. sambucinum</i>	—	+

They attack only the already damaged grains. According to Bottalico [15], *F. graminearum* and *F. culmorum* (Figure 1) are the most widespread pathogenic species in the Central European climate zone. Most found species causing FHB in Central and Northern Europe are *F. avanceum*, *F. graminearum*, *F. poae* and *F. culmorum* (WG Smith) Sacc. [16–18].



**Figure 1.** Microphotography of (a) *F. graminearum* and (b) *F. culmorum* macrospores ( $\times 1000$ ).

There are several *Fusarium* species in Croatia: *F. nivale*, *F. avanceum*, *F. culmorum* and *F. graminearum* [19]. Jurković et al. [13], Krstanović et al. [16], Ivić et al. [20] and Poštić et al. [21] reported that the most prevalent species in Eastern Croatia is *F. graminearum*, being present in more than 20% of cereals. In colder climatic areas, for example in NorthWestern Europe, the prevalent species is *F. culmorum* [22]. However, there are many reports about how climatic changes influence the adaptation of *Fusarium* spp. and cause a shift in their current distribution. Global warming gave its contribution to this modification, and according to Parikka et al. [18] *F. culmorum* is not the prevalent species in Nordic countries any more. *F. graminearum* in cereal grain is already proliferating in Central Europe and is expected to eventually populate the Northern European areas. This is most likely a consequence of warmer weather conditions, reduced tillage and the increase in maize cultivation in Nordic countries. This is reported all over the world, not only in Europe. Moreover, Ward et al. [23] reported on toxicity upgrade of *F. graminearum* in North America. Similar research was conducted in China, and the results indicated that more aggressive isolates are taking over [24]. The term “more aggressive” implies that they produce a wider variety of mycotoxins that end up in human or animal food.

Although, according to MEBAK (Mittleuropäische Brautechnische Analyskomommision), microbiological barley control upon admission to the malt factory includes determining the degree of infection with *F. graminearum* and *F. culmorum* fungi, the European Brewing Convention (EBC) requires barley to be controlled only for the presence of total number of fungi from the genus *Fusarium* [25,26]. *Fusarium* fungi are held responsible for a small grains cereal disease called FHB (Figure 2).



**Figure 2.** Example of FHB on wheat.

This disease classifies the grains as unacceptable and unsuitable for malting, even though all other quality parameters are acceptable for malting [27]. *Fusarium* spp. are known soil borne pathogens, but in some cases, they can be dispersed by wind and rain [28]. They can survive for more than a year in the soil's surface layer and remain active [29]. The most convenient period for infection is 2–3 days before flowering. Air humidity during wheat anthesis has a huge impact on the FHB development [16,30]. According to Bai and Shaner [31] and McMullen et al. [32], optimal conditions for infection are temperatures (20–25 °C), accompanied by high relative air humidity (>80%). FHB symptoms become visible about two weeks after the infection (green ears become yellow). In rainy periods, barley grains can be colorized with pink or red stains (Figure 3). The consequences of *Fusarium* infection can be seen in yield decrease, lower average seed dimensions and a decrease in nutritive value—lower starch and proteins mass fractions, loss of colour, and changes in smell and taste of the grain [17,33–35].



**Figure 3.** *Fusarium*-infected wheat malt grain.

In cases of severe infection, the grain does not detach from the hull during threshing. Microbial grain colonization is mostly limited to the outer layer of the grain, to the hull and to the space between the hull and the pericarp, but sometimes the fungus penetrates into the endosperm [36]. The type and degree of infection varies in relation to climate conditions, the location of breeding and the genetic susceptibility of barley/wheat varieties. Higher barley genotypes are not so susceptible to infection, and their grain accumulates less mycotoxins [37–39].

Magan et al. [40], in their work, described the impact fungicides and *Fusarium* spp. have on mycotoxin production. According to several authors cited in their paper, some fungicides, even though they suppressed the growth of *Fusarium* mycelium, actually contribute to formation of some mycotoxins (T-2, DON, etc.).

### 3. *Fusarium* Proliferation during Malting

*Fusarium* fungi are considered the most important pathogens in the malting and brewing industries, because they proliferate during the malting process and extensively degrade endosperm, as they integrate into the grain. Fungi-thriving, favourable process conditions (available nutrients, lower temperatures, good aeration, high air humidity and grain moisture during germination and first phase of kilning) contribute to the infection by supporting microbial growth and synthesis of secondary metabolites. The standard malting procedure comprises of several stages with different moisture contents (45% during steeping, to circa 4% after the kilning phase). Temperatures at which the malting process usually takes place range from 10–80 °C and this too helps the proliferation of fungi throughout the whole malting process. During steeping, grain is in direct contact with water in order for the grain to achieve a moisture content of about 45%. High moisture content in combination with

lower temperatures (10–14 °C) enables fungal spores to germinate and start mycelial growth. Drying is the final phase of malting, where grain moisture lowers to about 4%. Drying represents unfavourable conditions for the fungi and the mycelium dies out. *Fusarium* proliferation during malting is mostly caused by the initial infection rate and nutritive characteristics of the grain.

Niessen et al., [41] described that symptoms of *Fusarium* spp. infection become evident during the malting process, particularly during steeping and germination phases, when white, airy mycelium develops, giving grains a fluffy, hairy look. The most intense mycelium growth is in the germ, which dies out. As a result, these grains do not show any ability to germinate. The white mycelium gets destroyed when turning the green malt during the process. In its place, the grain gets an intense purple colour that is permanently retained on the grain.

According to several authors [35,42] *Fusarium* fungi cause a reduction of 1000 kernels, a reduction in test weight, an increase in grain moisture content, prolongation of saccharification time and speed of filtration and an increase in total/soluble protein and total/soluble nitrogen content. Wort colour, colour after cooking, viscosity, free amino nitrogen (FAN) and pH value are also susceptible to changes due to *Fusarium* infection. *Fusarium* infection affects other malt quality parameters as well: lower grain weight and germinative capacity, yield loss, endosperm protein and starch degradation, etc. [43,44]. *Fusarium* fungi contribute to strong beer aromas and flavours, which can vary from the burnt molasses flavour to the sharp taste of wine [45].

#### 4. Fungal Toxins in Malting and Brewing

Mycotoxins are secondary metabolites of *Fusarium* fungi and are also considered quality and safety indicators of cereals. It is important to determine their concentrations prior to the cereals entering into the malting factory, and after brewing, if they are used as feed. Wide varieties of *Fusarium* fungi produce these toxic compounds that represent health hazards not only for humans, but for animals too. Every day, new methods are being developed, in order to detect and quantify lower and lower concentrations of different mycotoxins and their derivatives. This has resulted in the classification of several hundred mycotoxins [46] currently. Circa 200 of these mycotoxins are representatives of the trichothecenes group [47].

Mycotoxins are known to be very resilient to high temperatures [48–50] that are used in malting and brewing production. Most studies in professional and scientific literature, consider trichothecenes as the most relevant mycotoxins in the malt-beer chain. This is mainly a consequence of their water-soluble property, which allows them to dissolve in wort and end up in the final product: the beer. Other, equally important, mycotoxins recognized in the barley-to-beer chain are aflatoxins, fumonisins, ochratoxin A (OTA) and ZEA [51–53]. Mycotoxins affect human and animal health in many ways (nephropathy, infertility, induce immunosuppression and reproductive problems in animals, cancer or even death) [43,54,55] and lessen the food quality and safety.

Yeasts are the main microorganism carrying out the brewing process. Inoue et al. and Pfliegler et al. [56,57] connect the bio-transformations of mycotoxins to less toxic forms—so called masked or modified mycotoxins with yeast metabolism. The transformation is reversible, meaning that some microorganisms and unit operations cause deconjugation of masked forms present in raw materials [58]. Plants also have a detoxifying mechanism, which can transform some of the mycotoxins to less toxic products. In order to minimize the toxic effects of a mycotoxin, plants have developed two mechanisms. They can chemically modify mycotoxins or compartmentalize them to a less toxic form [58]. For example, deoxynivalenol-3-glucoside (D3G) is a product of a detoxifying plant mechanism, where DON is subjected to glycosylation via a different conjugation reaction [58,59]. The plant modification system has been thoroughly described in a review by Berthiller et al. [58].

Nowadays, brewing comprises industrial (cosmopolitan) breweries and breaching home and craft breweries. The storage of raw materials in such (small) breweries can cause fungal proliferation and consequently, the production of mycotoxins that may result in health and safety issues [60]. The PMTDI (provisional maximum tolerable daily intake) for DON (1 µg/kg bw/day) can be exceeded in so-called



casual beer-drinkers—people consuming two beers or 1 L a day—according to Warth et al. [61]. According to this research, similar intake can be expected for other mycotoxins related to beer. In comparison to industrial beers, craft beers are more prone to being contaminated, given that they are defined as non-filtered and unpasteurized products. Piacentini et al. [62] reported that such beer can be contaminated with microbes and mycotoxins, and its sensorial characteristics can be clouded by undesired tastes and aromas. Encouraged by the recent craft expansion, many studies were carried out in order to compare the occurrence of mycotoxins in commercial and craft beers [62–66]. According to this research, DON, nivalenol (NIV), T-2, HT-2, diacetoxyscirpenol (DAS), ZEA, aflatoxins, OTA, and fumonisins can be found in beers, but generally in quantities of <1 µg/L. Peters et al. [67] carried out a research profiling 1000 beers, comparing those produced on craft and industrial scales, and the different mycotoxin contents in beers. The results showed that 27 craft beers contained DON and D3G (deoxynivalenol-3-glucoside) above (or at) the Tolerable Daily Intake (TDI).

Legislation on mycotoxin content in cereals and cereal products, mainly referring to the major mycotoxin, DON, is set at 1250 µg/kg for unprocessed cereals and 750 µg/kg for cereals intended for direct human consumption. ZEA concentrations in the same products, according to the European Union legislation, are set at much lower levels: 100 µg/kg (EC, 1881/2006) [11]. However, no regulations are set for malt and beer, so this gives maltsters and brewers an opportunity to determine their own limits [60]. Further, emerging mycotoxins should also be of a high priority for legislation, since many of them (D3G, 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), fusarenon-X-glucoside, 3-O-glucosides of T-2 toxin and HT-2 toxin, etc.) are found in malt and beer [58,68–72].

Hops can also be a contributor of mycotoxins. According to several authors cited in Lancova et al. [68], hops can also be contaminated with *Fusarium* spp. [73], causing *Fusarium* cone tip blight [74]. However, the literature on mycotoxins in hops is very limited and the research on it being a possible source of *Fusarium* mycotoxins has been neglected [75]. Also, the amount of hops added to beer is very small, compared to the amount of malt, which is the major mycotoxin contributor in beer.

## 5. Gushing—An Important Economic Factor

*Fusarium* infected grains represent a serious problem in the malting and brewing industries. *Fusarium* species are known producers of mycotoxins, but they also excrete hydropobins that cause gushing [35,76–81]. Gushing can be described as uncontrolled, eruptive foaming of beer after a bottle has been opened, without previous shaking or any agitation (Figure 4). This phenomenon affects breweries worldwide, causing great economic losses for the malting and brewing industries. Beer gushing causes consumer suspicion in the quality of the selected beer [82], and breweries must invest more money in repairing the impression such beer leaves. However, it is important to emphasise that not only beer is subjected to gushing, as other products, such as sparkling water, sparkling fruit sodas, ciders, fruit spritzers and sparkling wines, with even higher levels of carbonation, can show signs of gushing (over-foaming) [83–85].

According to Gjertsen et al. [86,87], there are two types of gushing: primary and secondary. Primary gushing is a direct result of fungal activity, mostly belonging to *Fusarium* sp., but it has also been linked to species of genera *Aspergillus*, *Rhizopus*, *Penicillium* and *Nigrospora* [79,88,89]. In general, gushing factors are surface-active molecules and studies point that hydrophobins, small fungal proteins characteristic for filamentous fungi, are responsible for gushing [35,79,90–98]. Manufacturing factors, such as coarse bottle surface, CO<sub>2</sub> oversaturation, increased oxalate concentrations, components of hops, etc., may cause secondary gushing [4,85].

Not only hydrophobins, but also fungispumins [95,98,99] and elevated levels of pathogenesis-related proteins (PRs) may be connected with the appearance of gushing [95].



**Figure 4.** Example of beer gushing.

Gushing was also related with the mycotoxin content in malt, since Schwarz et al. [100], reported that approximately 90% of all malts containing DON are inclined to gush. Sarlin et al. [80], however, reported that hydrophobins and DON in malts do not show any correlation with gushing. According to this, the DON content and gushing potential of malt are not connected.

Hydrophobins act as surfactants and stabilize the CO<sub>2</sub> bubbles in beer by forming a layer around the micro-bubbles, which prevents them bursting [80,101]. This causes excessive and sudden over-foaming [90]. Fungal hydrophobins help fungi cohere with a better grip on different surfaces, by reducing the surface tension of water [102]. By nature, they are thermostable and Müller et al. [103] reported that 10% of hydrophobins go through the processes and temperatures applied during brewing process unchanged, transfer to beer and can cause gushing. A similar claim was made by Sarlin et al. [79]. Kleemola et al. [92] and Sarlin et al. [80] demonstrated that the addition of purified fungal hydrophobins to beer causes gushing. In 2007, Sarlin et al. [79] found that even small concentrations of hydrophobins added to beer (only 3 µg/L) cause gushing. However, fungi used in the aforementioned studies were tagged as not relevant for gushing in Central Europe (*T. reesei*, *F. poae* and *Nigrospora* sp.) [98]. Sarlin et al. [79] also reported that 250 µg/g in malt can induce gushing.

The influence that applied fungicides have on the gushing potential of malt has also been investigated in many research studies. Some fungicides can even instigate, rather than prevent, the gushing potential of malt, according to Havlova et al. [4]. This is because in order to survive on the grain surface, the fungicide treatment stimulated the fungus to produce more hydrophobins. Early detection of gushing potential in raw materials for beer production (barley, malt, wheat, corn) is, strictly speaking, the only method to reduce the risk of gushing in breweries.

Currently, no safe and reliable procedure is known to prevent gushing. All methods that are currently in use require additional or modified process steps, which make malting and brewing more expensive, or significantly affect the aroma profile of the beer product [103].

Gushing is a complex phenomenon, comprised of many factors, and demands high levels of modern analytical methods and molecular insight into fungal and plant biochemistry. However, hydrophobins, produced by species of the genus *Fusarium*, occur depending on weather conditions, making great fluctuations in the raw material quality from year to year. There is still not enough data on how the brewing process influences hydrophobins. More detailed research is needed, to evaluate the effects of different process steps on the gushing potential of wheat and barley malt.

## 6. Malting and Brewing By-Products and Toxic Metabolites

Malting and brewing by-products represent a nutritious and very low-cost source of feed for livestock. Studies even describe them as additives in the food industry [104,105]. However, little research has been focused on mycotoxins in malting and brewing by-products: germ/rootlets, spent grains and spent yeast. In order to improve sensory and nutritional characteristics, some industries use germ/rootlets, spent grains or spent yeast for their products. Spent grains can be utilized in snack production, according to Mussatto et al. [104], and spent yeast is a great source of  $\beta$ -glucan [106]. Nevertheless, these by-products can be contaminated with mycotoxins [49,68,107]. Research conducted by Caupert et al. [108] indicated that mycotoxin concentrations were within acceptable levels in the majority of analysed DGS (DDSG-dried or WDSG-wet distiller grains). Since DGS is not given to animals as 100% of their feed, but it is mixed with other nutritive compounds, the possibility of contamination is actually very low. Also, factories reject grains infected with *Fusarium* or contaminated with mycotoxins [109]. Aflatoxin B1, deoxynivalenol, zearalenone, fumonisins, ochratoxin A, T-2 and HT-2 toxins in animal feed are regulated by European Union (EU) legislation [110–112].

## 7. What to Do with Contaminated Cereals and By-Products?

In the field, the adverse effects of mycotoxins can be reduced by genetic selection (choosing more resistant varieties) and the use of proper agronomic measures (fungicide application, tillage, crop rotations). After the infection, it is still possible to obtain acceptable grains by diluting the contaminated grains (only for feed), removing the outer layer of contaminated grain and applying chemical and physical decontamination. This involves alkaline treatments, the use of binders that reduce the bioavailability of mycotoxins in the intestinal tract, detoxifiers and enzymes. Biocontrol is also a possible method. However, the question remains: What to do with heavily infected *Fusarium* grains? Handling the infected grains demands appropriate equipment, and, during storage, such grains should be excluded healthy ones [113]. According to the EC 1881/2006 [11], badly infected grains should be burnt. Another option is to use it in biogas production but the reuse of DDGS is not possible, due to an increase in mycotoxin concentration in the final product.

## 8. Analytical Methods for Mycotoxin Detection

Mycotoxins have been studied for decades, and subsequently, analytical methods have been developed in order to ensure more accurate and precise compound measurement. Thin-layer chromatography (TLC), gas chromatography (GC), liquid chromatography (LC), LC coupled with mass spectrometry (LC/MS) or immunochemical methods, such as ELISA (enzyme-linked immunosorbent assay), can be used for mycotoxin determination [58]. The ELISA method is best used as a screening method before the actual LC analysis. Due cross-reactivity with masked mycotoxins, ELISA results usually show an over-estimation of results [49,114,115]. Some of the most significant methods in mycotoxin detection are presented in Table 2.

However, the newer methods rely on multi-mycotoxin analysis [116]. These are time- and money-saving methods, in which you can determine more mycotoxins at once, but, according to Zhang et al. [115], the accuracy and efficiency of analytical performances of LC-MS multi-toxin methods still need to be improved, and matrix effects and interference need to be reduced to a minimum. Since this is a very important topic in the scientific and professional sphere, many researchers are currently investigating improvements in analytical methods for mycotoxin determination in cereals, cereal-based foods and beer, and some of them have been described in recent papers [58,116–120].

A 2018 update review was published by Freire and Sant'Ana [121], giving a detailed overview on modified mycotoxin and detection methods. For now, the LC-MS/MS method is the most used and most reliable method in mycotoxin detection, including emerging, modified mycotoxins.

Smaller samples and a short analysis time are also appealing for scientific and professional purposes. Fewer reagents and a short-time analysis save time and money when it comes to mycotoxin



detection. Man et al. [122] published a review describing mycotoxin determination in foods, using microchip technology. Various kinds of detection methods, incorporated into the microchip (optical detection, electrochemical detection, photo-electrochemical detection and label-free detection method), can be used and are described in the review paper by Man et al. [122]. Microchips are innovative, automatic, integrative, portable, require small amounts of sample and display a rapid sensing time. However, there are some downsides of using microchips in mycotoxin detection, and one of them is the complex food matrix that requires a sample clean up before the actual analysis. The diversity and polarity of mycotoxins are also an important factor in sample preparation and analysis and can present a problem during the implementation of this revolutionary method.

**Table 2.** Some of the most used methods in mycotoxin detection and quantification.

Method	Advantage	Disadvantage	Source
Chromatographic Techniques	TLC	low cost; simple; rapid;	lack of automation
	GC		for volatile compounds;
	HPLC	high resolution; low limit of detection; can be coupled with a multiple detection automated system; specific;	expensive; time-consuming; expensive equipment and clean-up procedures;
	LC-MS/MS	high selectivity; high sensitivity; relatively easy sample clean up; multi-mycotoxin determination;	costly; expensive; time-consuming; expensive equipment and clean-up procedures;
Immunological	ELISA	screening method for different matrices; sensitive, specific, rapid, relatively low cost and simple; low detection limit;	due to the cross-reactivity with masked mycotoxins, ELISA results usually show an overestimation of results; enzyme stability;
Biological	Biosensors	rapid; sensitive; practical;	regeneration of the receptor surface; specificity; sensitivity; reproducibility; stability;

Novel techniques have contributed to the revealing of new emerging toxins that are not metabolites from only *Fusarium* spp., but originate from the raw material itself, as plant toxins (cyanogenic glucosides), other bacterial, yeast and fungal toxins, or pesticides [121].

## 9. Conclusions

Today, the demands for safe and healthy food is increasing, and the numerous consumers who are willing to educate themselves on this subject have set the bar high for good food on the market. Institutions monitor every aspect of the food industry, and, every day, new analytical techniques and methods are being developed, in order to determine lower and lower levels of harmful or nutritive compounds that directly or indirectly—via animal products—affect human health in a negative manner. Today's industries are prone to preventing every possibility for economic loss and health safety issues, before raw material acceptance into the malting or brewing factory. Big breweries have the ability to mix and blend different batches, in order to minimize the losses caused by *Fusarium* infections, but smaller, craft breweries do not have this convenience. Growing healthy and marketable cereals is dependent on choosing a suitable genotype that is less susceptible to *Fusarium* infection in cultivation agro-climatic conditions. Fungicide application also plays an important role in preventing infection and in overall damage control. According to some authors—Homdork et al. [125] and Španić and Drezner [126]—*Fusarium* infections can best be controlled using tebuconazole based fungicides. However, the application time, type of a fungicide and pathogen and the plant's ability to resist the pathogen are key elements in determining the successfulness of fungicide against *Fusarium* related diseases [127].

**Author Contributions:** Kristina Mastanjević, Vinko Krstanović, Krešimir Mastanjević and Bojan Šarkanj contributed equally.

**Conflicts of Interest:** The authors declare no conflict of interest.

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