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Impact of fungal contamination of wheat on grain quality criteria

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Ergosterol (PubChem CID: 247705)
Deoxynivalenol (PubChem CID: 40024)
Zearalenone (PubChem CID: 5281576)
3-Acetyl deoxynivalenol (PubChem CID: 104759)
Deoxynivalenol 3-glucoside (PubChem CID: 71312510)
Gliadin (PubChem CID: 17787981)
Sucrose (PubChem CID: 3036169)
Maltose (PubChem CID: 6255)
Glucose (PubChem CID: 5793)
Arabinose (PubChem CID: 66308)

ABSTRACT

The aim of this study was to investigate the spread of minimal, field born *Fusarium* infections in wheat during storage and the resulting impact on grain quality. Therefore, *Fusarium culmorum* was chosen as the representative strain. Wheat grains were artificially infected and stored for 6 weeks in a model system. To estimate the fungal growth, the ergosterol content was determined as this correlates with the fungal biomass. Ergosterol levels revealed a rapid spread of the infection during storage conditions. Furthermore, analysis of nine mycotoxins showed that Deoxynivalenol and Zearalenone occurred in concentrations exceeding the maximum residue limits. Scanning electron microscopy illustrated the penetration of the fungus into the endosperm and showed the degradation of important seed constituents, such as starch and storage proteins. This is mainly due to the increased activity of proteases and amylases by the fungal metabolism. The results of this study show how small levels of field contamination can easily spread during storage and so lead to significant losses in grain quality and present a potential consumer health hazard. Thus, it demonstrates the need to develop efficient methods for crop protection during storage, without compromising the quality.

1. Introduction

Cereals have made an essential contribution to human nutrition for centuries. Wheat, in particular, is one of the most important cereals worldwide. Nowadays, with a production of 713 million tons per year wheat is, after maize and rice, the third most produced cereal worldwide (FAOSTAT, 2014). It is grown on an area of 216 million hectares globally. In the 2013/2014 season 697 million tons of wheat were consumed worldwide (USDA, 2014). Its high prominence is mainly because it is the basis for a large variety of products. By far the most important is its use in the baking industry, mainly for bread, but also for cakes, biscuits etc. In addition, breakfast cereals, pasta and alcoholic beverages, like beer, are often based on wheat and it is also used for animal feed (USDA, 2013).

However, crop losses due to fungal contamination represent a significant problem for many cereals all over the world. Especially for cereals like wheat, different toxigenic *Fusarium* spp. are frequently found as contaminants. A very common disease due to mould contamination in the field is *Fusarium* Head Blight, mainly

Abbrevations: 3-ADON, 3-acetyldeoxynivalenol; DAS, desoxyscirpenol; DON3G, deoxynivalenol-3-glycoside; EFSA, European Food Safety Agency; *Fusarium* spp., *Fusarium* species; FUS-X, fusarenone-X; MS, mass spectrometry; RP(U)HPLC, reverse phase (ultra) high performance liquid chromatography; SDS, sodiumdodecylsulfate; s/n, signal-to-noise-ratio; USDA, United States Data Agency; UV, ultra violet light.

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caused by *Fusarium culmorum* (Parry et al., 1995). Grain colonization with these moulds causes significant quality losses due to the fungus exploiting the grains nutrient resources. Furthermore, from the consumer health safety point of view, the production of mycotoxins is a major problem. Mycotoxins frequently produced by *F. culmorum* are type B trichothecenes, such as deoxynivalenol (DON) and nivalenol (NIV), type A trichothecenes, like T-2, and zearalenone (ZON) (Llorens et al., 2006; Wagacha and Muthomi, 2007). The predominant mycotoxin is DON, as it was found in 90% of all cereal samples analysed, but it can also indicate the presence of other mycotoxins (Sobrova et al., 2010). Due to its heat stability and water solubility, it is easily transferable into processed food (Lancova et al., 2008). Consequently, mycotoxins are well known as a substantive health hazard in the baking and brewing industries (Scudamore et al., 2009).

However, due to the permanent and ubiquitous presence of microorganisms and fungal spores in the environment, it is not possible to avoid contamination completely. Consequently, methods for reduction and control of fungal contaminations are of major interest.

Although *Fusarium* spp. are field fungi, infection can also occur post-harvest if the conditions are favourable. In the USA alone the post-harvest economic loss on wheat due to fungal spoilage and mycotoxins exceeds $300 million annually (Pitt and Hocking, 2009). Furthermore, if wet grains are harvested and not dried immediately *Fusarium* spp. can grow post-harvest (Champeil et al., 2004). Due to this fact it is likely that i.e. during storage, small amounts of *Fusarium* spp. infected grains can contaminate larger amounts of healthy grains. Consequently, the mycotoxins produced could cause a health hazard for the consumer. Additionally, the technological and nutritional quality of the grains would be reduced significantly, due to the fungal metabolism. Therefore, a small field infection has the potential to cause immense economic damage.

Thus, the aim of this study was to investigate the spread of a *F. culmorum* infection, of minor degree of initial infection, under storage conditions generally suitable for fungal growth. Although this does not resemble industrial storage practices it allows the study of the interactions of the fungus with the grains. Therefore, the behaviour of the fungus was characterised, regarding the development of biomass and the production of mycotoxins. Analysis of the grain ultrastructure was used to visibly illustrate the changes occurring to all important components due to the infection. Further information about the pathway of fungal infection was obtained by determining selected enzymatic activities. Finally, the fungal impact on major grain quality parameters, such as starch content and storage proteins was evaluated to estimate the reduction of marketability, leading to economic losses. Hereby, the results of this study provide essential information for the understanding of fungal proliferation during storage, which is of great interest to cereal science and industry.

2. Materials and methods

2.1. Materials

Commercial hard winter wheat (*Triticum aestivum*), harvested in 2013, was supplied by Doves Farm Foods Ltd. (Hungerford, UK). Wheat grains were stored in barrels under cool conditions (<15 °C) and were aerated regularly.

F. culmorum TMW 4.2043 originally isolated from brewing barley was provided by the culture collection of Lehrstuhl für Technische Mikrobiologie, TU-München Weihenstephan.

All reagents used in the following analysis were at least analytical grade.

2.2. Grain surface disinfection

The grains were disinfected according to the method described by Oliveira et al. (2012). Briefly, grain portions of 600 g were disinfected in 4 L 10% (w/v) hydrogen peroxide (H_2O_2) solution for 10 min with continuous stirring. Subsequently, the grains were washed for 5 min in 4 L distilled water. This procedure was repeated once, but with only 5 min of disinfection. Immediately, the grains were moved to sterile plastic boxes and dried at room temperature for 24 h under vertical sterile laminar flow. Finally, the grains were exposed to ultraviolet light (10 min) and collected aseptically for further use.

2.3. Preparation of fungal spore suspension and grain infection

The spore solution of *F. culmorum* was prepared according to the method described by Oliveira et al. (2012). Briefly, fungus was cultivated at 25 °C for 5 days on potato-dextrose-agar (PDA) plates. After cultivation six small fragments of inoculated PDA were transferred to 800 mL synthetic nutrient-poor bouillon (Nirenberg, 1976). Fungal suspensions were kept at room temperature under continuous stirring to induce spore production and filtered through 30 μm filter paper. The concentration of spores was determined to be 10^5 spores/mL, using a haemocytometer.

Infected wheat grains were prepared using the following procedure. Disinfected wheat grains were mixed with 2% (v/w) sterile filtered spore suspension of *F. culmorum*. Subsequently, grains were incubated for 10 days at 25 °C with 75% relative humidity to allow fungal growth. The infected grains produced were defined as 100% infected. The infected grains were homogenised and analysed for moisture. Uncontaminated wheat was incubated in the same way and used as a negative control.

2.4. Mixing and storage trials

Infected and disinfected grains were mixed to samples of 4.5 kg (dry matter) with specific infection levels of 0, 5, 10 and 20% and stored in model systems under conditions generally suitable for fungal growth. Each mixture and the control sample was divided into nine portions and filled into sterile plastic bags. Every bag was sealed and perforated by two pipette tips with barrier filter to allow gas exchange. The bags were stored for 6 weeks at room temperature. After 0, 3 and 6 weeks, 3 portions of each sample were taken, milled to a whole grain flour (particle size < 0.5 mm), homogenised and stored at −20 °C until further use.

2.5. Determination of ergosterol

Ergosterol content (free and ester bound) was determined using the method of Jedličkova et al. (2008) with the following modifications. The RPHPLC column used was a Nova-Pak C_{18} (300 × 3.9 mm, 4 μm). Peak-identity was verified using the UV-spectra recorded by the DAD. The limit of detection (LOD) and the limit of quantification (LOQ) were determined from the signal/noise (s/n) ratio. The LOD was set for s/n of 3:1 and the LOQ was set for s/n of 10:1. For calibration, ergosterol standards between 1.0 and 200 μg/mL in methanol were prepared and analysed. The recovery was determined by spiking an ergosterol free sample with a standard solution and found to be 95 ± 1%.

2.6. Mycotoxin analysis

The analysis of mycotoxins was carried out by UHPLC-MS/MS according to the method of DeColli et al. (2014). In brief, 2 g milled, homogenised sample was extracted by shaking by hand in the