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Fungi in malting barley grain and malt production

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Contamination of malting barley grain with microscopic fungi during different periods was investigated from 2012 to 2014. Grain samples of malting barley were taken directly from the field before harvesting and from a storage silo; malted grain was taken from two malt producers in Lithuania. The fungi were isolated and identified in barley grain before the malting and after steeping and germination during the malting process. The total count of fungi was done in these barley samples during the mentioned phases. Most attention was given to widely distributed fungi known as producers of toxins: *Aspergillus, Penicillium*, and *Fusarium*. Abilities of some of these fungi to produce secondary toxic metabolites cause a possible hazard to people consuming the contaminated products.

The aim of this work was to investigate changes in fungi composition and in their total count in barley grain, the raw material for the production of malt.

Keywords: malting barley, grain, fungi, mycotoxins

INTRODUCTION

Barley is the raw material for the production of malt, and malted barley is the major raw material used in brewing of most beers and kvass. Due to its nutritive value, barley boosts the growth of various microorganisms, first of all different fungi (Angelino, Bol, 1990; Moss, 1991). Microbes greatly affect malting performance and malt quality and have a significant impact on beer quality. Depending on the nature and extent of the microbes present, their effects may be either beneficial or disadvantageous to the process and/or the final product (Flannigan et al., 1992; Jay et al., 2005). Barley may be contaminated by fungi during its growth in the field, storage, and malting (Flannigan, 2003). The activity and growth of the fungi mostly depend on initial contamination of barley, moisture content, temperature, and aeration (Christensen, Meronuck, 1986; Moss, 1991; Pitt, Hocking, 1999).

Fungi often damage grain while it is still ripening in ears. Fungi of the *Alternaria* and *Fusarium* genera are most frequent on various grain in fields. They perish if the humidity of the nutritious substrate does not exceed 12–13% (water activity, a_w = 0.65) for a longer period of time. In many European countries of temperate climate, contamination of grain with the abovementioned fungi reaches 100% at the moment of harvesting. In field, fungi of the *Cladosporium*, *Bipolaris*, *Botrytis*, *Ulocladium*, *Acremonium* genera are recorded on ripe grain (Chelkowski, Grabarkiewicz-Szczesna, 1991; Moss, 1991).

In recent years, small grain such as barley has been greatly affected by *Fusarium*, primarily caused by *Fusarium graminearum*, leading to significant

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yield losses and quality reduction. Flannigan (Flannigan, 2003) estimated the total losses for barley and wheat due to *Fusarium* between 1991 and 1996 as being in the order of $3 billion dollars in the United States alone. Another *Fusarium* species that causes kernel blight is *Fusarium culmorum*. Both *F. graminearum* and *F. culmorum* may also cause root rot, crown rot, foot rot, and stem rot in wheat and barley. Two other *Fusarium* species, *F. poae* and *F. avenaceum*, may also cause kernel blight in barley.

A difference as little as 0.5% in grain moisture content can be significant in microbial growth and the composition of the microbial community (Jay et al., 2005). Temperature is another important factor as the a_w, which is inherently linked to microbial growth and increases with temperature at constant moisture content of the grain. Also, *Absidia*, *Rhizopus* and *Mucor* fungi may develop at elevated moisture content (Flannigan et al., 1992; Moss, 1991).

Generally, storage fungi are saprophytes with low specificity, whose ability to grow and persist is mainly determined by a_w and temperature. They comprise xerophilic members of such genera as *Aspergillus*, *Eurotium*, *Micropolyspora*, *Penicillium*, *Rhizomucor* (Moss, 1991).

The conditions of malting may promote activation of fungal spores that are present on/in barley seed and the growth of fungi, resulting in the synthesis of highly toxic secondary metabolites – mycotoxins (Briggs, McGuinness, 1993). During the development of barley grain, the toxigenic fungal species may synthesize mycotoxins that are accumulating in the grain. Due to their thermal stability, these toxins may be transmitted into the final product – beer, over the malting process, worth production and pasteurization (Scott, 2006).

Fusarium species have been reported as the most toxigenic fungi in northern temperate regions (Salas et al., 1999). Among storage fungi such as *Aspergillus* and *Penicillium*, *Fusarium* species produce mycotoxins such as trichothecenes, fusarins, moniliformin, zearalenone, and fumonisins with varying toxicological properties. Some of these mycotoxins have been associated with human and animal diseases and are known to survive the malting and brewing process. Contamination of barley with mycotoxigenic fusaria is of particular concern to both maltsters and brewers (Salas et al., 1999; Scott, 2006).

Gushing of beer is regarded as one of the most negative consequences of moulds with respect to the quality of malt and beer (Flannigan, 2003).

MATERIALS AND METHODS

Contamination of barley grain with micromycete propagules (cfu g^{-1}) during different periods was investigated from 2012 to 2014. Grain samples of malt barley were taken directly from the fields of the farmers who grew barley for malt producers and from a silo; malted material was obtained from two malt producers in Lithuania. Dilution plating (surface-spread method) (Pitt, Hocking, 1999) was used for colony counting. Ten grams of each milled grain sample were homogenized in 90 ml of sterile water. Serial decimal dilutions up to 10^{-4} were made and 0.1 ml were inoculated in triplicate onto Petri dishes with media. Sabouraud glucose agar medium with chloramphenicol (0.5 g l^{-1}) for moulds and OGY agar for yeasts were used. The dishes were kept in thermostat at the temperature of 26 ± 2°C. The growing colonies of fungi were counted on days 3, 5, and 7. Fungi were identified according to morphological and microscopic characteristics (Leslie, Summerell, 2006; Pitt, Hocking, 1999; Watanabe, 2002; Nelson, Toussoun, Marasas, 2006). The DM750 optical microscope system with the ICC50 HD camera from Leica Microsystems was used.

RESULTS AND DISCUSSION

The results indicate that fungal contamination of barley grain before and during storage may be diverse and change during the process. Additionally, the community may continue to change when malting steps change or the malt is stored and finally transported to the breweries. Analysis of microbiological contamination of grain showed that the quantity of fungi in

grain conversion technology from the field to the malting process was increasing. The study of microbiological contamination of barley grain revealed 19 species of fungi in the grain before harvest, ten species in the grain from the storage silo, and eight species during malting.

Field fungi in barley

Field fungi invade the seeds before harvest while the crop is still in the field. Field fungi may affect the appearance and quality of seed or grain. In barley grain before storage, the number of fungi propagules was from 6.2 up to 9.0 cfu g^{-1}.

The following fungi were isolated from barley grain before storage: *Acremonium strictum*, *Alternaria* spp., *Aspergillus niger*, *A. flavus*, *Botrytis cinerea*, *Cladosporium herbarum*, *Drechslera sorokiniana*, *Fusarium culmorum*, *F. poae*, *F. equiseti*, *Mucor* spp., *Nigrospora sphaerica*, *Penicillium* spp., *Rhizoctonia* spp., *Rhizopus nigricans*, *Stemphylium* spp., *Trichoderma* spp., and *Verticillium albo-atrum*.

The isolation frequency (IF) of the most frequent fungal species (IF > 40%) is shown in Table. *Alternaria* spp. were the dominant species on samples of barley grain, followed by *Drechslera sorokiniana*, *Fusarium* spp., and *Cladosporium herbarum*.

The dominance of certain species depended on whether the kernels were of a darker colour. While *Alternaria* spp. were predominant and *Aspergillus* spp. occurred less in darker kernels, the reverse was observed on lighter kernels where *Aspergillus* spp. occurred in higher frequency.

The IF of other most frequent species (*Alternaria* spp., *Aspergillus* spp., *Fusarium* spp. and *Geotricum* spp.) depended mostly on the locality the barley samples were taken from. The most frequent species associations (several fungi isolated from one sample) in grain were *Alternaria* spp., + *Fusarium* spp., *Alternaria* spp. + *Fusarium* spp. + *Drechslera* spp. and *Geotrichum* spp. + *Mucor* spp.

Storage fungi in barley

Storage fungi (also called storage moulds) are the fungi that invade grain or seeds during storage. Storage fungi are usually not present to any serious extent before harvest. Small quantities of spores of storage fungi may be present on grain going into storage or may be present on spilled grain present in harvesting, handling, and storage equipment or structures. Under improper storage conditions, this small amount of inoculum can increase rapidly, leading to significant problems. After harvest and before storage, barley is usually dried to a moisture content below 13%.

The number of fungi propagules increased in storage conditions and fluctuated from 8.3 up to – 15.2 cfu g^{-1}.

The number of fungi propagules was higher than in barley ears before the harvest, possibly because during the harvest the working elements of the combine harvester interacted with the crop and the dust rising from the soil was carried into the tank of the combine harvester together with grain. The following fungi were isolated from dried and stored barley grain:

Table. **Contamination of barley grain samples with fungi**

Barley grain sample	Contamination (cfu g^{-1})	Prevailing genera of fungi (IF > 40%)
Barley from field	6.2–9.0	*Alternaria* spp. *Drechslera* spp. *Fusarium* spp.
Barley from a storage silo	8.3–15.2	*Aspergillus* spp. *Scopulariopsis* spp.
Barley after malting	18.8–22.5	*Geotrichum* spp. and other yeasts *Mucor* spp. *Fusarium* spp.

Arthrobotrys oligospora, *Aspergillus niger*, *A. flavus*, *Aspergillus oryzae*, *Fusarium sporotrichioides*, *F. poae*, *Penicillium expansum*, *P. verrucosum*, *P. viridicatum*, and *Scopulariopsis brevicaulis*.

Fungi in barley malting

Analysis of the samples of malted barley grain revealed that malting conditions were favourable for fungal growth. During technological processes of malting, the changes in the abundance of fungi in grain were evident.

Technically, malting involves three steps: steeping, germination, and kilning. Steeping is the most critical step in malting with respect to both the microbial activity and microbial safety. Although some of the microbes are washed away along with the steeping water, the viable numbers increase substantially during steeping. The fungal microbiota is generally dominated by different yeasts and the most frequent is *Geotrichum candidum* (Figure), which prefers growth temperatures below 20°C.

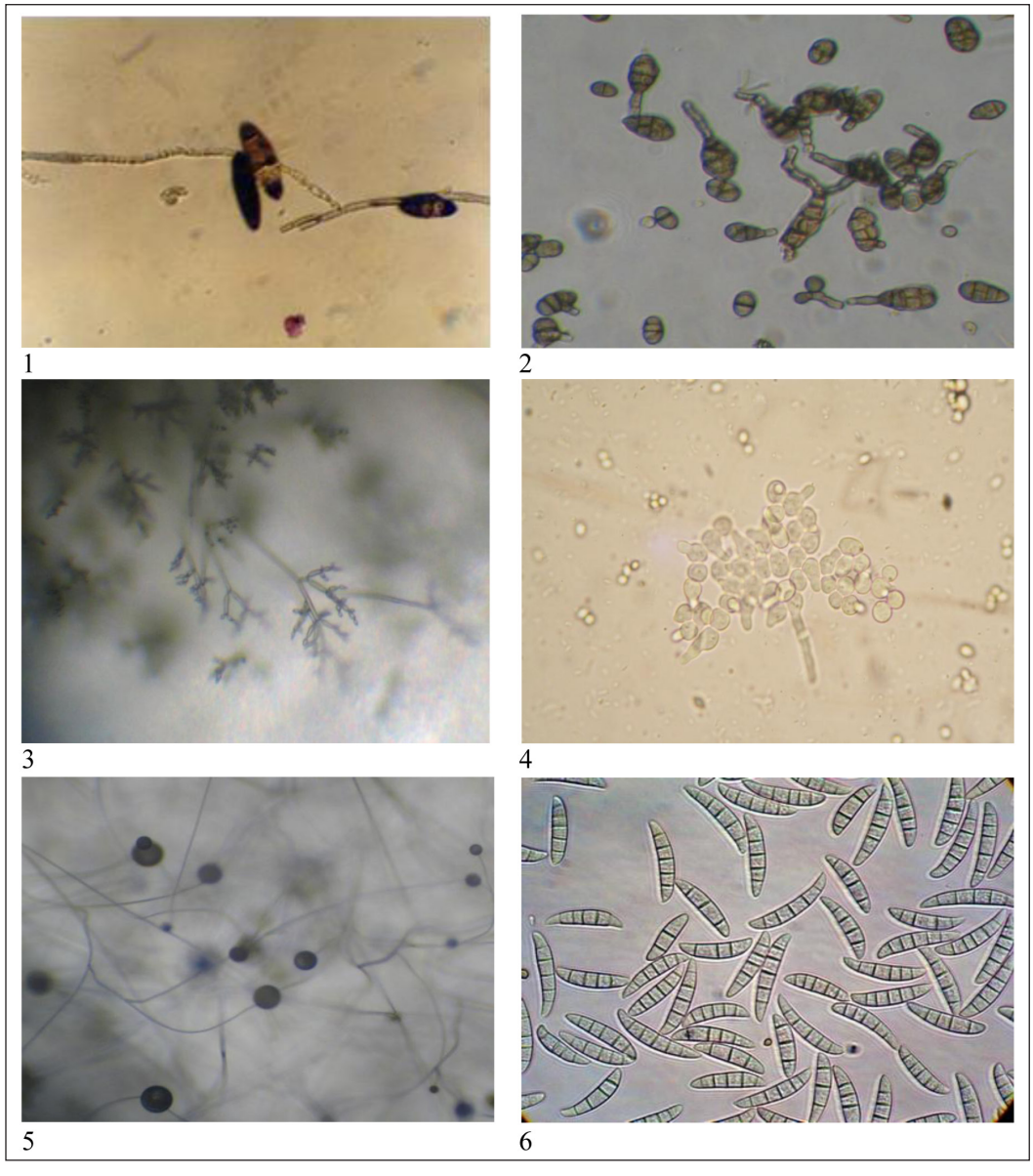

Figure. A microscopic view of the most frequent fungi in barley grain (×600 magnification): 1. *Drechslera sorokiniana*; 2. *Alternaria alternate*; 3. *Cladosporium* sp., 4. *Geotrichum candidum*; 5. *Mucor* sp.; 6. *Fusarium culmorum*

Moreover, genus *Fusarium* was quite frequent as the most important group. Intensive growth of *Fusarium* was observed during steeping, even when the barley from the storage silo had only a low level of *Fusarium* contamination.

Metabolic changes in the barley kernel, including conversion of residual carbohydrates to fermentable sugars, and increase in the fungi count (observed from 18.8 up to 22.5 cfu g^{-1}), took place during germination. The levels of some field or storage fungi such as *Alternaria, Cladosporium* and *Aspergillus* declined during germination.

CONCLUSIONS

1. Fungi of 26 kinds were isolated and identified from barley grain. These fungi belong to 16 genera. Of all isolated fungi, 15% consisted of sterile mycelium *Mycelia sterilia*, which on the standard used agarised mediums produced no reproductive organs. Fungi of the *Fusarium* genus were prevailing.

2. The quantity of fungi in grain conversion technology from the field to the malting process was increasing. Metabolic changes in barley and high water activity increase the fungi count from 6.2 cfu g^{-1} before harvest up to 22.5 cfu g^{-1} after steeping.

3. Some of the isolated and identified fungi species possessed the ability of active synthesis of toxins. The following species can be regarded as potential producers of toxins: *Alternaria alternata*, *Aspergillus flavus*, *Penicillium expansum*, *P. chrysogenum*, *P. verrucosum*, and others. Due to these toxins such grain loses its value and should not be used for food or feed.

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MIKROSKOPINIAI GRYBAI SALYKLINIUOSE MIEŽIUOSE IR SALYKLO GAMYBOJE

Santrauka

2012–2014 m. atlikti salyklinių miežių grūdų taršos mikroskopiniais grybais tyrimai derliaus nuėmimo ir skirtingais perdirbimo etapais. Subrendę miežių grūdai tyrimams buvo paimti iš lauko prieš derliaus nuėmimą, iš grūdų saugyklos, taip pat iš dviejų Lietuvos salyklo gamintojų paruoštų šviežio salyklo grūdų. Mikroskopiniai grybai buvo išskirti bei identifikuoti miežių grūduose prieš salyklo gamybą ir salyklo gamybos etapuose (po mirkymo ir daiginimo), nustatytas grybų pradų skaičius miežių grūdų mėginiuose visuose minėtuose etapuose. Daugiausia dėmesio miežių salyklo gamintojai ir naudotojai turėtų skirti salyklinių grūdų taršai tokiais plačiai paplitusiais mikroskopiniais grybais kaip *Aspergillus*, *Penicillium* ir *Fusarium*, kurie išskiria mikotoksinus. Kai kurių iš šių genčių grybų gaminami antriniai toksiški metabolitai sukelia potencialią toksikozės riziką žmonėms, vartojantiems mikotoksinais užterštus produktus.

Šio darbo tikslas buvo ištirti mikroskopinių grybų sudėties pokyčius ir jų bendrą skaičių salyklinių miežių grūduose – salyklo gamybos žaliavoje.

Raktažodžiai: salykliniai miežiai, salyklas, mikroskopiniai grybai, mikotoksinai