Mold and mycotoxin problems encountered during malting and brewing

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Abstract

Fusarium infections in grains can have severe effects on malt and beer. While some degree of Fusarium mycotoxins, such as deoxynivalenol, present in infected barley may be lost during steeping, the Fusarium mold is still capable of growth and mycotoxin production during steeping, germination and kilning. Therefore, detoxification of grain before malting may not be practical unless further growth of the mold is also prevented. Methods to reduce the amount of mold growth during malting are needed. Physical, chemical and biological methods are reviewed. Irradiation looks very promising as a means to prevent Fusarium growth during malting, but the effect on the surviving mold to produce mycotoxins and the effect on malt quality needs further study. Chemical treatments such as ozonation, which would not leave residual chemical in the beer also appear to be promising. Although biological control methods may be desirable, due to the use of “natural” inhibition, the effects of these inhibitors on malt and beer quality requires further investigation. It may also be possible to incorporate detoxifying genes into fermentation yeasts, which would result in detoxification of the wort when mold growth is no longer a problem. Development of these types of technological interventions should help improve the safety of products, such as beer, made from Fusarium infected grain.

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1. Introduction

Beer is consumed on a regular basis by many people around the world. Per capita consumption of beer in the United States for 2003 was 27 gal (Lakins et al., 2005), or about 289, 12 oz cans. This amount of beer consumption could possibly result in significant exposure to mycotoxins. The public health risk from mycotoxins in beer is uncertain, however risks to pets and livestock from mycotoxins in byproducts is a clear risk. Sources of mycotoxins in beer could include contaminated malt or adjunct grains used in malting and brewing.

Scott (1996) discussed the fate of several mycotoxins during the malting and brewing processes and reported results of surveys for mycotoxins in beer samples imported into Canada from various countries. Wolf-Hall and Schwarz (2002) reviewed the impact of molds and mycotoxins on beer quality and safety. Dupire (2003), Melotte (2004) and Boivin (2005) highlighted mycotoxins in the European malting and brewing industries. Odhav (2005), from South Africa, also reviewed the topic. Much of this previous material is also covered here, along with updated information about physical, chemical and biological control studies for prevention of mycotoxins in beer. Others (Castro, 2006) in these proceedings will overview ochratoxin A in beer. This review will focus on Fusarium mycotoxins.

2. Mycotoxins in barley

Malted barley is the most common ingredient used in the beer making process. Small grains, such as barley, have been greatly affected by the plant disease Fusarium head blight (FHB) also known as scab (McMullen, 1997). Trichothecene mycotoxins, including deoxynivalenol (also known as DON or vomitoxin), nivalenol, T-2 toxin, HT-2 toxin and diacetoxyscirpenol, as well as the estrogenic mycotoxin, zearalenone have been detected in FHB infected barley from the upper Midwest United States (Schwarz et al., 1995a). Of these, deoxynivalenol is the most frequently detected and the mycotoxin produced in greatest quantity in the FHB infected grain. Clear et al. (1996) also found deoxynivalenol to be the predominant mycotoxin in FHB infected barley in Manitoba during recent epidemics. In southwest Germany, the predominant mycotoxin found in barley crops from 1982 to 1992 was also deoxynivalenol (Müller et al., 1997). In a review of surveys
looking for mycotoxins in commercially available beers (Scott, 1996), it was shown that deoxynivalenol, nivalenol, T-2, HT-2, diacetoxyscirpenol, zearalenone, aflatoxins, ochratoxin A, and fumonisins have been detected in beers at trace (ppb) levels. Mycotoxins are also a concern in other byproducts of the malting and brewing processes such as various food ingredients and animal feeds (Flannigan et al., 1984).

3. Malting

Barley malt is produced by germinating the seed under regulated conditions of moisture and temperature. Malt is the source of amylase enzymes, which will break down starch in barley and adjunct grains to produce simpler sugars for yeast to metabolize during beer fermentation. To germinate the seed, the barley moisture content is raised to roughly 45% by steeping for 36–52 h at 12–20 °C and involves several stages of immersion in aerated water followed by air-drain cycles. The process rinses the barley during drain and fills cycles. After steeping, the seed is germinated at 15–20 °C in humidified air. The germinated barley (green malt), is then dried to roughly 4–5% moisture in a kilning step. Kilning temperatures and humidity are controlled to prevent inactivation of desired enzymes. Kilning temperatures rarely exceed 90 °C. Kilning causes chemical reactions to occur in the barley such as browning reactions. These chemical products affect the final beer qualities, such as flavor, color, odor and texture.

Mycotoxin production may occur with the growth of Fusarium during steeping, germination and possibly kilning in the malting process. Schwarz et al. (1995b) micro-malted several barley samples that were naturally infected with Fusarium graminearum and contaminated with deoxynivalenol (4.8–22.5 μg/g), zearalenone (1.5 μg/g) and 15-acetyldeoxynivalenol (2.1 μg/g). Steeping lowered deoxynivalenol concentrations to near or below the detection limit. Deoxynivalenol may have been lost due to solubilization or loss of dust with the overflow and fill-drain cycles of the steep. However, mold growth during germination increased deoxynivalenol concentrations by 18–114% on green malts compared to the original infected barley following five days of germination (Schwarz et al., 1995b). A significant increase in ergosterol (a molecular marker for fungal biomass) was observed between two and three days of germination, followed by deoxynivalenol production later in germination. This pattern of toxin production after growth of mycelia is typical for the production of secondary metabolites (Bu’Lock, 1980). A similar pattern was seen with samples contaminated with 15-acetyldeoxynivalenol and zearalenone (Schwarz et al., 1995b). No change in deoxynivalenol levels was observed during kilning. Deoxynivalenol is known to be stable up to 170 °C at neutral to acidic pH values (Wolf and Bullerman, 1998). Munar and Subree (1997) found deoxynivalenol produced during the early stages of kilning. Wolf-Hall and Bullerman (1998) were able to show increased production of deoxynivalenol and 15-acetyldeoxynivalenol by certain isolates of Fusarium graminearum at 35 °C versus 25 °C. The rise in temperature in the early stages of kilning may actually stimulate increased production of mycotoxins by certain strains of infecting mold. Many factors could be affecting the amount of toxin produced at various stages of malting, including strain of mold present, the viability of the infecting mold (injury, dormancy, or death), the amount of infecting mold present, the location of mycelia or spores within the seed structure, and competing and/or antagonistic organisms.

Other qualities affected by Fusarium mold growth during malting include reduced barley germination, gushing, change of color and off-flavors in beer (Gyllang et al., 1981). Barley used for malt must have germination rates above 95%. Below this rate problems develop such as low enzyme production and malt extract. The reduced rates of germination in FHB infected barley could be due to mycelium invading the embryo and/or presence of mycotoxins which may also inhibit germination (Haikara, 1983; Nummi et al., 1975; Schapira et al., 1989). Changes in color may be due to the production of pigments by the infecting mold (Sloey and Prentice, 1962), or due to increases in the levels of soluble amino nitrogen compounds in the wort due to proteolytic activity by the mold on barley proteins (Haikara, 1983; Prentice and Sloey, 1960; Zhou, 1998).

4. Brewing

After barley is malted, it is ground to a course particle size and extracted with hot water. This step is called mashing, where the ground malt is mixed with water and heated to about 70 °C. During this process, the barley’s proteolytic enzymes are most active and the result is roughly equal proportions of amino acids, peptides, and protein in the final malt extract. The soluble nitrogenous compounds contribute to the beer color, flavor and the yeasts’ metabolism during fermentation. Some of the proteins contribute to the foaming characteristics of the final beer. The amylases produced during malting break down the starch to simpler sugars; adjunct grain may be added as an additional source of starch. After mashing, the liquid portion or wort is separated from the solids or spent grains. The spent grains are usually used as animal feed. Water soluble mycotoxins tend to remain in the wort. The wort is boiled to kill spoilage organisms, then cooled, aerated and the yeast inoculation or pitch is added. The boiling process kills the contaminating fungi, but most mycotoxins will survive this process. The fermentation usually takes 5 to 7 days at 8 to 15 °C. The beer is then aged, further carbonated and packaged.

Some of the problems associated with using FHB infected barley malt are alterations in wort composition (soluble nitrogen compounds and carbohydrates), the presence of mycotoxins and other mold metabolites, and the propensity for beer gushing. The mold may produce proteases that will result in further digestion of proteins in the malt and wort beyond what the barley proteases would accomplish (Sloey and Prentice, 1962; Haikara, 1983). This will affect color, flavor, texture, and foaming characteristics in the final beer. Haikara (1983) also noted an effect on the degree of fermentation and beer pH which were both increased. This may be due to increased free amino nitrogen for yeast metabolism as well as to higher content of simple sugars. Yeast fermentation may also be affected by fungal metabolites in the wort. Lafont et al. (1981) noted a decrease in the rate of fermentation by Saccharomyces cerevisiae
by 58–80% in fermentation broths containing 10–50 μg/g T-2 toxin. This decrease was a temporary lag, and the yeast seemed to recover. It has been suggested that T-2 toxin inhibits mitochondrial function, causing slower oxygen utilization by the yeast and slowed log phase growth (Koshinsky et al., 1992). This slowed growth rate effect has also been shown with zearalenone at 5–50 μg/g (Flannigan et al., 1985), DAS at 5–10 μg/g (Whitehead and Flannigan, 1989), and deoxynivalenol at 50 μg/g (Ryman, 1994). However, there was no effect with deoxynivalenol at 20 μg/g (Whitehead and Flannigan, 1989).

Boeira et al. (1999) found no effect of nivalenol at concentrations below 50 μg/g on strains of both ale and lager yeasts. Boeira et al. (2000) studied the interactions of combinations of deoxynivalenol, zearalenone and fumonisin B1 on yeast. They found a synergistic interaction between deoxynivalenol and zearalenone, but only at very high concentrations. Even if FHB infected grain was utilized for brewing, it is unlikely that any of these mycotoxins would be found at concentrations this high.

Studies have shown increases in deoxynivalenol levels during yeast fermentation. Böhml-Schräm et al. (1997) showed an increase in deoxynivalenol within 20 h of fermentation, which progressively decreased afterward up to 100 h. The decrease in deoxynivalenol was theorized to be due to absorption by yeast cells or extracellular metabolism. In a baking study using wheat naturally contaminated with deoxynivalenol, Young et al. (1984) saw an increase in deoxynivalenol levels in a yeast fermented doughnut and attributed this to deoxynivalenol precursors in the flour which were further converted to deoxynivalenol by the yeast.

By increasing inoculum density, Whitehead and Flannigan (1989) were able to decrease effects of diacetoxyscirpenol on fermentation. They suggested that certain yeasts may detoxify mycotoxins such as T-2 toxin, and suggested that the development of mycotoxin resistant yeasts would be of great benefit to the fuel ethanol industry, as heavily mycotoxin contaminated grain could be better utilized as it would not be acceptable for malting or animal feed. There are microorganisms that detoxify certain trichothecenes (Shima et al., 1997; Westby et al., 1997), which may be sources of genetic material for modified ethanol producing microorganisms.

Deoxynivalenol appears to be very stable during the brewing process. Niessen (1993), found deoxynivalenol to be carried over into the final beer. This study also showed a four-fold increase in deoxynivalenol concentrations during mashing. It was suggested that deoxynivalenol may be released from protein conjugates during mashing. Wolf-Hall et al. (1999), found that deoxynivalenol recoveries from water extracts of spiked, extruded corn grits could be improved by 26% using an amylase enzyme in the extraction process. It may be that the action of proteases and amylases release additional deoxynivalenol from infected grains.

Schwarz et al. (1995b) pilot-brewed beers from naturally infected barley malt containing 1.8–17.3 μg/g deoxynivalenol and 1.6–4.8 μg/g zearalenone. The results showed 80–93% of the deoxynivalenol remained in the final beers and only trace amounts were present in the spent grains. No zearalenone was detected in the final beers and about 60% of the zearalenone was recovered from the spent grains. It has been suggested that zearalenone is converted by yeast metabolism to zearalenol (Scott, 1996), however no zearalenol was detected using a GC/MS method of detection (P. Schwarz, North Dakota State University, unpublished results).

Besides the effects of and presence of Fusarium mycotoxins in beer, there are other qualities of beer affected by using FHB infected grains. Gushing, or sudden over production of foam upon opening a container, is a defect that is closely associated with the use of FHB infected grain in beer making. Gushing can be a serious quality defect resulting in the permanent loss of consumers. Gushing appears to be caused by the formation and stabilization of large amounts of microbubbles in beer (Casey, 1996; Drager, 1996; Fischer et al., 1997). The nucleation centers for these microbubbles seem to be a product of mold growth in the grain, and not only Fusarium mold may cause it — others like Aspergillus and Penicillium may also cause gushing. However, Fusarium species seem to be the most problematic (Haikara, 1983). The topic of beer gushing has been reviewed by Casey (1996). Schwarz et al. (1997) proposed that the Fusarium gushing factor is a water soluble component produced by the mold, and were able to induce gushing in beer by adding cell-free extracts of Fusarium graminearum grown in peptone water. The gushing factor(s) has been correlated with the presence of deoxynivalenol in malt (Schwarz et al., 1996), and both the gushing factor(s) and deoxynivalenol are thought to be produced in parallel by the mold during growth in the germinating barley. It may be that the presence and amount of deoxynivalenol can be used to predict gushing propensity, but it is apparent that much more research is needed to develop analytical tests to determine gushing factor content in malt.

5. Control

The most effective current control for problems associated with FHB infected barley is to avoid it. Malting companies in the United States have adopted a standard of 0.5 ppm deoxynivalenol or less in purchased grain (McMullen et al., 1997). However, avoidance may not always be feasible. This is especially true in developing countries where food and feed supplies may be very limited, or during years of extreme epidemics of FHB in barley crops. The economic costs associated with avoidance can be devastating to barley producing regions. Growers can be severely discounted in price for FHB infected grain, and processors can have the additional costs of testing and importation of grain from other regions. With the current inability to control FHB in the field, it is important that technology be developed in order to be able to permit the utilization of at least a portion of the infected crop. Various techniques have been explored for the utilization of infected grain, and they generally fall into one of three categories: removal or separation of infected kernels, treatments designed to decontaminate or eliminate mycotoxins present in grain, and treatments intended to prevent or inhibit mold growth.

In wheat, deoxynivalenol can be significantly reduced by removing heavily FHB infected kernels known as tombstone kernels. These kernels are shriveled, smaller and less dense
problems with germination and clumping (Kunze, 1996). However, in barley, if infection occurs later in kernel development, shriveling of kernels may be minimal (Steffenson, 1996). Attempting to clean barley may not be an effective means to lower deoxynivalenol concentrations. Unlike wheat, the hull is usually left on barley and seems to be where the highest concentrations of both mold and deoxynivalenol occur (Clear et al., 1997). Dehulling damages the embryo, decreasing germination rates, left on barley and seems to be where the highest concentrations of deoxynivalenol concentrations. Unlike wheat, the hull is usually clean barley may not be an effective means to lower levels of deoxynivalenol. Some chemical treatments such as sodium bisulfite, chlorine gas, moist ozone, and ammonia have been shown to reduce deoxynivalenol concentrations. However, these harsh treatments can either be too severe on the grain quality, the reaction product too unstable, or undesired residues may be left on the grain. Young et al. (1987, 1986) used sodium bisulfite to decontaminate deoxynivalenol in infected corn and wheat. The reaction product was a deoxynivalenol sulfonate which could be hydrolyzed upon heating, as was evident in baking studies where deoxynivalenol concentrations increased 50–75% in the wheat samples when made into bread.

Chemical destruction of trichothecene toxicity can be achieved rapidly in alkaline solutions of sodium hypochlorite (Faifer et al., 1994; Young et al., 1986). Trenholm et al. (1992) saw reductions of 74 and 81% in deoxynivalenol and zearalenone respectively in FHB infected barley soaked in 1 M sodium carbonate for 30 min followed by two, 30 min distilled water rinses. Deoxynivalenol is unstable at alkaline pH values (Wolf and Bullerman, 1998). In fact, a traditional practice used by some maltsters is to include lime in the first steep. This, however, may have no effect on later growth and toxin production by Fusarium during germination. It may also be possible to develop genetically modified yeasts that degrade the toxicity of mycotoxins carried over into the wort. As stated previously, there are microorganisms that can detoxify certain trichotheccenes (Shima et al., 1997; Westby et al., 1997) which may be sources of genetic material to be incorporated into modified ethanol producing microorganisms.

Methods to prevent or inhibit mold growth include physical, chemical and biological based technologies. There has been very little research reported regarding physical treatments of barley, such as heat. There may be ways to heat pasteurize grain in a way to kill mold but still allow germination (Berjak et al., 1992). Kottapalli et al. (2003) used hot water treatments and irradiation to reduce Fusarium infection while maintaining adequate germination in barley. Kottapalli et al. (2006) further evaluated irradiation for control of Fusarium in malting barley and found dosages of 6–8 kGy to control deoxynivalenol levels in final malts with minimal impacts on malt quality.

There are references showing significant effects for chemical treatments affecting Fusarium in barley, including formaldehyde, hypochlorite, and mercuric chloride. These, however, would not be acceptable to the brewers who do not want the chemicals or potentially harmful reaction products to be retained in the beer. Fungicides applied in the field may prevent the initial Fusarium infection (McMullen, 1997), but treatments can be expensive and the fungicide needs to be applied within narrow periods to be effective. Sanitizing agents such as sodium hypochlorite have been shown to eliminate Fusarium culmorum from inoculated barley seeds (Ramakrishna et al., 1991), and may detoxify the grain; however, high levels of sanitizer would be needed due to the rapid depletion of active components by reactions with organic matter in the grain. Sanitizers may also affect desirable competitive microorganisms on the barley. High levels of residual sanitizing agents or byproducts may be detrimental to yeast performance during fermentation and may affect the final beer quality. A promising newer method is the use of ozone, which rapidly decomposes to oxygen. This method may be effective for sanitizing the barley as well as detoxification. Ozone has been shown to detoxify aflatoxins, cyclopiazonic acid, ochratoxin A, secalonic acid D, and zearalenone (McKenzie et al., 1997). Kottapalli et al. (2005) tested ozone and hydrogen peroxide for effects on Fusarium and germination in barley, and concluded these treatments may be effective chemical controls.

The development of FHB resistant barley cultivars is being explored, but may take many years of breeding to achieve results. Even if FHB resistance is achievable, these new cultivars will also need to have characteristics that will command malting quality. There is currently no truly resistant barley cultivar.

There are references to microbiological control methods using competing fungi, such as Geotrichum candidum (Bovin and Malanda, 1997), added as starter cultures to the steeping process. Bovin and Malanda (1997) showed a reduction in Fusarium infection rates from 86% to 0% in steeped barley. G. candidum is not typically found on barley, but can be very commonly found in malt (Flannigan et al., 1984). However, the starter cultures of G. candidum would need to be monitored for toxin production as well, since certain strains are capable of producing clavine alkaloiods (Flannigan et al., 1984).

Lactic acid bacteria have shown an ability to produce antifungal compounds. Lowe and Arendt (2004) reviewed the use and effects of lactic acid bacteria in malting and brewing. Vaughan et al. (2005) described some novel approaches for biological control to maintain malt and beer quality using lactic acid bacteria and modified yeasts. Although microbiological methods show promise, the brewing and malting industries are looking for controls they can do as soon as possible; ideally, a physical method that could be placed “online” in the process.

For any method to prevent mold growth, tests should be done on the affected microflora to determine if injuring the molds, such as Fusarium, that mycotoxin synthesis is not induced. Sub-lethal or inhibitory concentrations of chemicals may prevent fungal growth, but actually stimulate mycotoxin production. This would include chemicals such as organic acids and fungicides. It has been shown that application of fungicides increases the occurrence of mycotoxins (Miller, 1994). Sorbic acid has been found to stimulate the production of aflatoxin B1 and T-2 toxin (Bauer et al., 1984). Studies evaluating the effects of irradiation on Aspergillus flavus and A. parasiticus have shown surviving isolates with enhanced ability to produce aflatoxins (Moss and Frank, 1987),
however this pattern was not observed for *Fusarium* in barley (Kottapalli et al., 2006). Altering proportions of normal microflora in barley could potentially cause other problems, and Kottapalli et al. (2006) did observe significant changes in the dominant microflora in malts from irradiated barley. Fungi and bacteria have chemical interactions that are not well understood, and alteration in their proportions may result in increased mycotoxin production by *Fusarium* or increased mycotoxin production by other genera of fungi (Moss and Frank, 1987). The practicality of combining intervention methods to impose a hurdle effect to mycotoxin production should be investigated.

6. Conclusion

While some degree of mycotoxins, such as deoxynivalenol, in infected barley may be lost during steeping, the *Fusarium* mold is still capable of growth and mycotoxin production during steeping, germination and kilning. Detoxification of grain before malting may not be practical unless further growth of the mold is prevented. Irradiation holds promise as a means to prevent *Fusarium* growth during malting, but the ability of the surviving mold to produce mycotoxins and the effect on quality needs to be further studied. Chemical treatments like ozonation, which would not leave residual chemical in the beer also hold promise. Biological control methods may be desirable due to the use of “natural” inhibition, but the effects on quality will need to be evaluated. It may also be possible to incorporate detoxifying genes into yeasts through biotechnology, which would allow detoxification of the work when mold growth is no longer a problem. Development of these types of technological interventions should help improve the safety of products, such as beer, made from FHB infected grain.

References


