Journal of Food Protection, Vol. 68, No. 6, 2005, Pages 1236–1240

Research Note

Evaluation of Gaseous Ozone and Hydrogen Peroxide Treatments for Reducing *Fusarium* Survival in Malting Barley

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MS 04-537: Received 30 November 2004/Accepted 4 February 2005

ABSTRACT

The use of *Fusarium*-infected barley for malting can lead to mycotoxin production and decreased malt quality. Methods for treatment of *Fusarium*-infected barley might prevent these safety and quality defects and allow use of otherwise good-quality barley. Gaseous ozone and hydrogen peroxide (HP) were evaluated for effectiveness in reducing *Fusarium* survival while maintaining germinative energy (GE) in barley. Gaseous ozone treatments (GOT) included concentrations of 11 and 26 mg/g for 0, 15, 30, and 60 min. HP treatments included 0, 5, 10, and 15% concentrations with exposure times of 0, 5, 10, 15, 20, and 30 min. For GOT, in naturally *Fusarium*-infected barley, a statistically significant ($P < 0.05$) decrease (24 to 36%) of *Fusarium* survival occurred within 15 min of exposure at either concentration. GE was significantly ($P < 0.05$) affected by 30 min at both concentrations in naturally *Fusarium*-infected barley, but not in sound barley. GOT did not cause any significant ($P > 0.05$) effect on GE in sound barley at either concentration over the full 30-min exposure time. For HP, *Fusarium* survival was significantly decreased (50 to 98%) within 5 min of exposure. With the exception of two treatments (10 and 15% HP agitated for 20 min), GE was not statistically significantly different from the control in naturally *Fusarium*-infected barley. In sound barley, HP had no significant ($P > 0.05$) effect on GE. The results suggest that GOT and HP might have potential for treatment of *Fusarium*-infected malting barley.

Different microorganisms can contaminate barley from field through storage *(9)*. Fusarium head blight (FHB) of barley, incited by several species of *Fusarium*, has become an increasingly persistent problem in regions of North America and Europe *(17, 28)*. The primary concern is contamination of FHB-infected grain with tricothecene mycotoxins, which reduces its suitability for malt, feed, and food use *(25)*. The malting and brewing industries are reluctant to accept mycotoxin-contaminated grain because of concerns over public safety, public perception, and product quality *(20, 25, 26, 33)*. Studies have revealed that the growth of *Fusarium* during the malting process can result in mycotoxin production and can affect the germinative capacity and malting characteristics of the barley *(9, 27)*. *Fusarium graminearum* and *Fusarium poae*, commonly found in FHB-infected grain, have been reported to reduce kernel plumpness and increase wort-soluble nitrogen, free amino nitrogen, and wort color. *Fusarium* mycotoxins such as deoxynivalenol (DON) and diacetoxyscripenol have been reported to affect the malting process by reducing the alpha-amylase activity and alpha-amino nitrogen levels in wort, and could also have a negative effect on yeast growth and fermentation *(24, 31)*. Gushing of bottled beer is another problem that is associated with the use of FHB-infected grain *(8, 27)*. It is believed that gushing is induced by proteinaceous secondary metabolites produced by fungi, and not by mycotoxins. Hydrophobins have recently been identified as a potential causal agent *(12)*.

Because of the various negative effects associated with FHB-infected grain, maltsters generally do not accept infected grain, or they purchase it at a lower price *(17, 20)*. Screening and associated price discounts in North America have been based on the presence of DON, which is the predominate mycotoxin within the region. Grain with DON levels of >0.5 mg/kg has generally been highly discounted, and it is unlikely that significant amounts of grains with more than 2 mg/kg DON have been used for the production of malt. This situation has affected both price and the available barley stocks, resulting in negative effects on both the barley growers and malting and brewing industries in the United States *(10, 18, 19)*. As a result of FHB, it has become very difficult for the regional barley growers to profitably market even mildly FHB-infected grain for malting. Postharvest controls have focused on chemical, physical, and biological approaches with limited success *(25, 27, 33)*.

Two chemical agents of interest for reducing *Fusarium* in malting barley include ozone and hydrogen peroxide (HP). These chemicals are particularly interesting to maltsters because they would not leave chemical residues in finished malt. Both ozone and HP are allowed in direct contact or as an addition to certain foods by regulatory authorities in the United States *(5, 7)*. Both ozone and HP are listed by the National Organic Program (§205.605) as

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substances allowed as ingredients in or on processed products labeled as ''organic.''

The first and only study we are aware of to evaluate the use of ozone on barley was published by Allen et al. *(3),* who evaluated various doses (0.0 to 1.2 mg per g of barley per min) of gaseous ozone on the inactivation of mixtures of nonspecified spores and mycelium of fungi in barley adjusted to moistures of 19, 22, 25, and 30%. The barley with an initial moisture content of 11% was not included in the study treatments. Fungi were enumerated by a spread plate method. The percent survival of fungi was reported to decrease with increase in the dosages and exposure times used. At an ozone concentration of 0.1 mg per g of barley per min for 5 min, the fungi were inactivated by 96%. They concluded that ozonation could inactivate fungi, with mycelia being more susceptible than spores, while maintaining germination if the dosage was not too high in barley at these moisture contents. Further study on grain at moistures that would reflect average storage conditions and evaluates specific fungi of concern is needed.

The use of HP to treat malting barley has not been published; however, Schwarz *(25),* mentioned that HP (0.75%) was ineffective in reducing the growth of *F. graminearum* during malting of FHB-infected malting barley. Higher concentrations of HP have been shown in other studies to have significant fungal inactivation in different types of matrices. Aharoni et al. *(2)* reported that HP inhibited the mycelial growth of *Alternaria alternata* and *Fusarium solani* in galia melons and concluded that a concentration of 48% HP was effective in reducing postharvest decay in galia melons. Abdou and Galal *(1)* found that HP at 1×10^{-3} M concentration was effective in reducing sporulation, mycelial growth, and conidial germination in *Fusarium oxysporum, Gibberella fujikuroi,* and *F. solani.* Furthermore, Linfield *(14)* reported that 0.5% peratol, a chemical compound containing a combination of HP and peroxyacetic acid, eliminated the chlamydospores of *F. oxysporum* f. sp. *narcissi* when treated for 80 min.

The objective of this research was to evaluate gaseous ozone treatments (GOT) and HP treatments for reducing viable *Fusarium* in naturally FHB-infected barley grain. Because the malting process requires at least 95% of the grain to germinate, the effect of these treatments on germinative energy in barley was also evaluated. If effective, these types of treatments might allow the use of mildly FHB-infected barley for malting because reduction in viable *Fusarium* should reduce risks of mycotoxin production and quality defects.

MATERIALS AND METHODS

Experimental design and statistical analysis. This project included two sets of experiments. These experiments evaluated the effect of GOT and HP treatments on *Fusarium* survival (FS) and germinative energy (GE) in two barley samples (infected and sound). For all experiments, a randomized complete block design to test the treatment differences for FS and GE was used. The results of FS and GE were analyzed with analysis of variance and a general linear model procedure to compare differences between treatments. The level of significance used was 5% *(23).*

Barley. Two, six-rowed malting barley cultivars from the 2002 crop were used for this study. These included a naturally FHB-infected sample of *Robust* (infected) with 1.27 μg of DON per g of barley and a sample of *Lacey* showing no physical signs of FHB (sound) and containing no detectable DON (<0.10 μg of DON per g of barley) and were obtained from Farmers Grain Elevators (Alvarado, Minn.). All DON determinations were done by gas chromatography with electron capture detection *(30).* The moisture content for the samples was 14.9% for the FHB-infected barley and 11.7% for the sound barley. Moisture content was determined with an automatic grain moisture meter (Motomco Instruments, Paterson, N.J.).

GOT. Ozone gas was produced from a lab-scale OS-8C ozone generator (Ozone Solutions Inc., Sioux Center, Iowa) equipped with an ozone monitor (model 450-H, Advanced Pollution Instrumentation Inc., San Diego, Calif.). Teflon tubing (Nalgene Nunc International, Rochester, N.Y.) was used to pass the ozone produced from the ozone generator to the samples. The Teflon tubing was connected to a 500-ml side-arm conical flask equipped with a cork stopper.

Barley seeds (200 seeds per treatment) were randomly selected and treated at 0, 11, and 26 mg/g concentrations for time periods of 0, 15, 30, and 60 min. All treatments were done at room temperature (~25°C). Following treatment, the barley seeds were immediately analyzed for FS and GE. Each treatment was performed in triplicate.

HP treatments. Barley seeds (200 seeds per treatment) were randomly selected and added to 50 ml of HP solution in a 100-ml conical flask sealed with double-layered parafilm (Pechiney Plastic Packaging, Menasha, Wis.). The seeds were agitated during exposure in a shaker (model G25, New Brunswick Scientific Co. Inc., Edison, N.J.) at a speed of 200 rpm. The treatments included four different concentrations (0, 5, 10, and 15%) of HP dissolved in sterile distilled water for six different time periods (0, 5, 10, 15, 20, and 30 min). After the respective treatments, the seeds were rinsed three times with sterile distilled water for 1 min. The seeds were then immediately analyzed for FS and GE. All treatments were done at room temperature (~25°C). Each treatment was performed in triplicate.

***Fusarium* survival.** FS was determined as described by Kottapalli et al. *(13).* One hundred barley seeds (five per standard-sized petri dish) were aseptically transferred into petri dishes containing half-strength acidified (pH 3.5 with lactic acid) potato dextrose agar *(21).* The petri dishes were then incubated under ambient lighting for 5 days at room temperature. After 5 days, the molds that grew from the seeds were identified to the genus level *(22)* and counted, and the number of seeds colonized by *Fusarium* spp. was represented as a percentage.

GE. GE was determined according to American Society of Brewing Chemists method Barley-3C *(4).* One hundred barley kernels of each sample to be tested were placed into a sterile glass petri dish containing moistened filter paper (4 ml water). The petri dishes were placed into an environmental chamber (model 6030, Caron Products and Services Inc., Marietta, Ohio) maintained at 20°C and 100% relative humidity. Sprouted, or chitted kernels, were counted and removed after 24, 48, and 72 h. GE represents the percentage of kernels that germinate by 72 h.

RESULTS AND DISCUSSION

GOT. The results of GOT are summarized in Figure 1. Exposure to gaseous ozone for 15 min at either 11 or 26

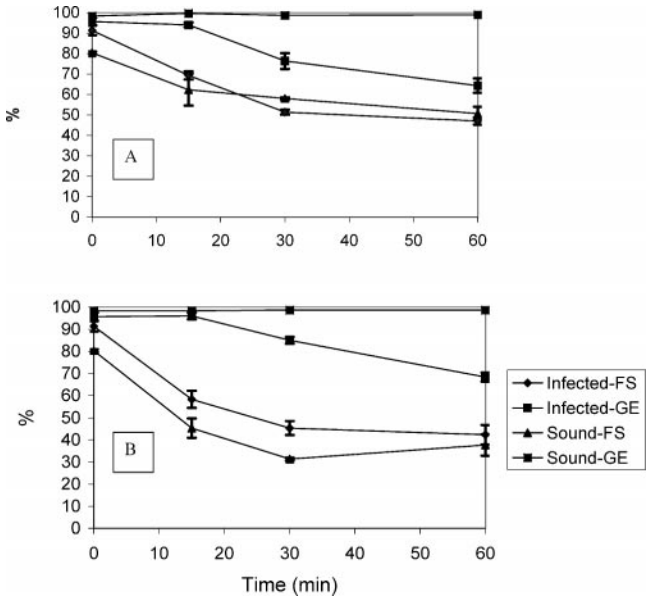

FIGURE 1. *Response of* Fusarium *survival (FS) and germinative energy (GE) in sound and FHB-infected barley treated with gaseous ozone at 11 mg/g (A) and 26 mg/g (B). Error bars represent standard deviations of three replications.*

mg/g significantly decreased ($P < 0.05$) FS but had no significant effect on GE. For FS, the largest decreases of 61 and 53% were observed at a concentration of 26 mg/g for sound and infected samples, respectively. GE in the sound barley was not significantly ($P > 0.05$) affected by any of the concentrations or exposure times used in this study; however, exposure of the infected sample to both 11 and 26 mg ozone per g for 30 min resulted in a significant ($P < 0.05$) reduction in GE. This is problematic because barley must generally exhibit a GE of ≥96% to be accepted for malting. Beattie et al. *(6)* previously reported poor germination in barley samples that were heavily infected with *F. graminearum*. It could be that an interaction of ozone and fungal damage was responsible for causing reduced GE in the infected sample. Less severely infected barley might retain germinative ability at ozone and time combinations that reduce FS. The study by Allen et al. *(3)* indicated that increased moisture content did result in less generic fungal survival; however, germination was affected at the highest moisture content (30%) after 15 min of exposure. The moisture content of the infected sample in this study was higher than that of the sound sample and could possibly have contributed to the effect on GE in the infected sample. The moisture content of the infected sample (14.9%) was higher than the 13.5% recommended for stored barley *(32)*.

Allen et al. *(3)* reported that an ozone concentration of 0.1 mg per g of barley per min for 5 min reduced the overall fungal population by 96% (initial count was 8.3×10^5 CFU/g), or a 1.4-log reduction. The GE of the barley at the corresponding concentration was reported not to be significantly different from the control (near 100%). In this study, *Fusarium* survival did not drop as drastically as fungal survival did for the Allen et al. *(3)* study. Although the quantification methods used were not comparable, this perceived difference between the studies could be because *Fusarium* might have more resistance to ozone than the genera that might have been present in the Allen et al. *(3)* study, or the *Fusarium* might have been more protected within the seed matrix than the genera affected in the other study. The quality of the barley used in the Allen et al. study *(3)* was not indicated, but the near 100% germination of the controls would indicate that the seeds were of good quality and might not have had significant internal infection compared with the FHB-infected kernels in this study.

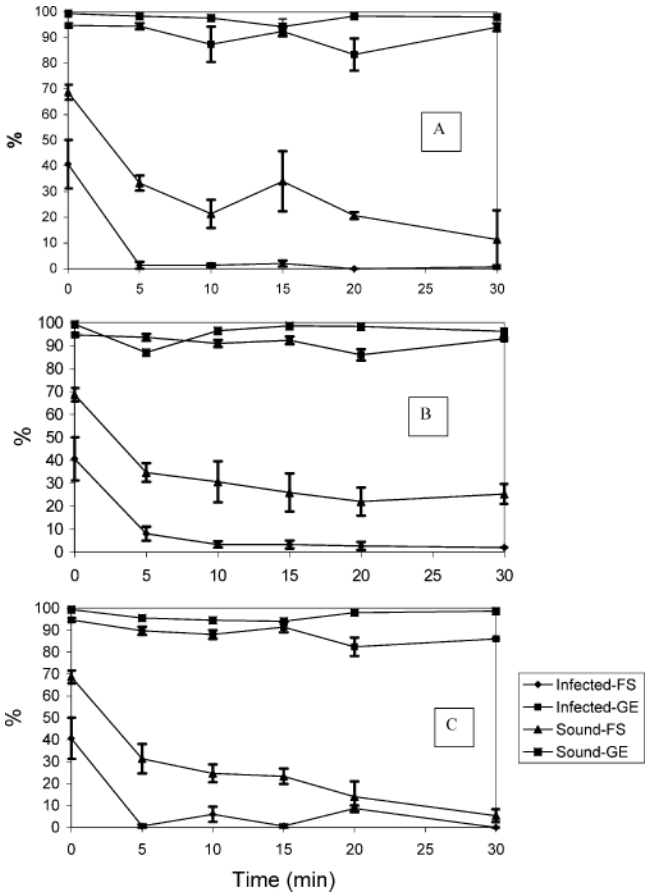

FIGURE 2. *Response of* Fusarium *survival (FS) and germinative energy (GE) in sound and FHB-infected barley treated with hydrogen peroxide at 5 (A), 10 (B), and 15% (C). Error bars represent standard deviations of three replications.*

HP treatments. The results of HP treatments are summarized in Figure 2. In both samples, FS significantly ($P < 0.05$) decreased with increase in HP concentration and treatment time. The largest reduction (50 to 98%) in FS was achieved within the first 5 min of exposure to HP. With the exception of two treatments (10 and 15% HP exposure for 20 min), GE was not significantly ($P > 0.05$) affected in the infected sample. As with ozone, an interaction between HP and fungal damage might explain the loss of GE in the infected sample at higher concentrations and exposures of HP. Germination in the sound sample was not significantly ($P > 0.05$) effected at any of the concentrations of HP used. The distilled water control had no significant effect on FS or GE in either infected or sound barley.

Both ozone and HP appear to have potential for treating mildly FHB-infected malting barley. HP treatments

achieved greater levels of *Fusarium* reduction (50 to 98%) with no effect on germination for most of the treatments. Ozone also caused a significant decrease in *Fusarium* levels (38 to 54%) without affecting the germination in sound barley. These chemical treatments could be more acceptable solutions than the use of other inhibitory chemicals. In general, other chemicals can leave residues or undesirable reaction products in the malt, which could affect the quality of the malt and the yeast fermentation during brewing.

An advantage with HP and ozone, in addition to their fungicidal activity, is that they are ''residue-free oxidants'' *(14).* An advantage of gaseous ozone would be that a process could be developed to treat grain during storage and could have the added benefits of insect control *(11, 15)* and potential to degrade mycotoxins produced preharvest *(16).* HP might also degrade mycotoxins produced preharvest as well *(29).*

This report of preliminary studies indicates that both ozone and HP have potential for reducing FS while maintaining GE in mildly FHB-infected malting barley. Further research is needed to optimize and scale up treatment conditions. Treating barley steep water with sparged ozone will also be considered as a potential means of reducing *Fusarium* survival during malting. Furthermore, research will also be done to evaluate safety, in terms of mycotoxin residue, in finished malt and quality of malt and beer prepared from the treated barley.

ACKNOWLEDGMENTS

We thank the North Dakota Barley Association, Rahr Malting, and North Dakota State University for partial funding of this project.

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