Effect of Ozone Treatment on the Safety and Quality of Malting Barley

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ABSTRACT

Molds and their mycotoxins are an expensive problem for the malting and brewing industries. Deoxynivalenol (DON) is a mycotoxin that is associated with Fusarium spp. These fungi frequently cause Fusarium head blight in wheat and barley in the midwestern region of the United States; Manitoba, Canada; Europe; and China. Barley growers and malt producers would benefit from a postharvest control method for mold growth and DON production. We evaluated the use of gaseous ozone (O3) for preventing Fusarium growth and mycotoxin production while maintaining malt quality characteristics. Micromalting was performed in three replications under standard conditions. Ozone treatment was applied to malting barley during steeping via a submerged gas sparger. Ozone treatment conditions were 26 mg/cm2 for 120 min after 2 and 6 h of steeping. The effects of gaseous ozone on DON, aerobic plate counts, Fusarium infection, and mold and yeast counts of barley throughout the malting process were measured. Various quality parameters of the malt were measured after kilning. Statistical tools were used to determine the significance of all results. Ozonation of malting barley during steeping did not lead to significant reductions in aerobic plate counts but did lead to a 1.5-log reduction in mold and yeast counts in the final malt. The influence of gaseous ozone on DON concentration was inconclusive because of the low initial concentrations of DON in the barley. Ozone significantly reduced Fusarium infection in germinated barley. Gaseous ozone did not negatively influence any aspect of malt quality and may have subtle beneficial effects on diastatic power and β-glucan concentrations.

Fusarium head blight (FHB or scab) is a fungal disease of cereal grains. This disease has occurred sporadically in the midwestern region of the United States throughout the past century, with major outbreaks occurring in 1993 through 2003 (18, 21). Crops of six-row barley (Hordeum vulgare (L.)) in the region can be devastated by FHB epidemics. Salas et al. (18) found FHB infection in 51.3% of midwestern barley samples from 1994. The most severe infection was found in the Red River Valley (border region of North Dakota and Minnesota), with much lower rates in the northwestern region of North Dakota. The FHB epidemics of the 1990s were so severe that much of the region’s barley acreage was shifted to other crops, and many malt companies transferred more of their barley dependence to the western states and Canada (11).

The two most pathogenic and aggressive fungal pathogens for wheat and barley are Fusarium graminearum (Schwabe) and Fusarium culmorum (W. G. Smith) Saccardo (3). F. graminearum was the species found most commonly in barley samples from the midwestern United States in the FHB epidemics of the 1990s; it accounted for 62 to 64% of all Fusarium-infected kernels from 1994 through 1996 (18). The toxicity of F. graminearum derives predominantly from the trichothecene mycotoxin deoxynivalenol (DON or vomitoxin). A variety of other toxins also are produced by these fungi (4).

DON is the most commonly detected mycotoxin in grain with FHB, and it is often found in unacceptable concentrations. The average DON concentrations in barley were 0.5 to 10.3 μg/g during the crop period of 1993 through 2003 (18, 21). The concentration of DON has become a standard of measure for malting barley quality, and barley with high concentrations of DON is sharply discounted on the market (11). Malting companies do not generally accept barley with DON concentrations above 1.0 μg/g because of concern for consumer health and because of malt quality problems associated with DON-contaminated barley (11, 21). Some companies do not accept any grain with detectable concentrations of DON to guard their company from any suggestion of toxicity in their products. Producers, brewers, and malting companies could benefit greatly from an effective postharvest barley decontamination process for otherwise good-quality barley.

Several postharvest grain treatment techniques for decontamination have been tested to determine whether they are effective enough to make blighted barley usable for malting. For a detoxification technique to be usable in an
industrial setting, it must meet certain criteria; it must be economical, relatively simple to perform, and capable of removing the toxin without leaving chemical residue and must not affect the nutritional or physical properties of the grain (23). Treatment of grain with ozone is one way of meeting all of these requirements. Ozone (O₃) is a strong oxidizing agent that has been successfully used to disinfect drinking water (17). Raila et al. (16) found a significant reduction in the number of micromycete species in wheat when an ozone-air mixture was used to ventilate the grain during storage. Kottapalli et al. (14) found that exposure of Fusarium-infected barley to gaseous ozone for 15 min at 11 or 26 mg/g significantly decreased Fusarium survival without affecting germinative energy (GE). Young (26) found that DON concentrations in contaminated corn could be significantly reduced by using an ozone treatment. Ozonated water also has been effective for inactivating microconidia of Fusarium oxysporum (Schlecht. emend. Snyder and Hansen) (7). These results suggest that application of ozone to Fusarium-infected barley during the steeping stage of malting could be a promising method for reducing microbial loads and DON.

The objective of this study was to evaluate the effect of ozonation at the steeping stage of barley malting on microbial loads, DON concentrations, and various malt quality parameters. The concentration of ozone selected was based on a results of a previous study by Kottapalli et al. (14). A discussion of other treatment methods previously studied (13, 15) also is included.

MATERIALS AND METHODS

Barley lots. The two barley lots used for these experiments were from the 2002 crop of the six-row malting barley cultivar ‘Robust’ and had been kept frozen to maintain viability in clear plastic bags at −80°C. These lots were described by Kottapalli and Wolf-Hall (13) as lot 1 and lot 2 and were obtained from ConAgra Malting Company (Quebec, Canada). The high-quality barley (lot 2) included in the study had no visible symptoms of FHB, and initial DON test results were 0.25 μg/g. The barley naturally infected with FHB (lot 1) initially had a DON concentration of 1.4 μg/g. The moisture contents were 11.9% for the FHB barley and 10.2% for the high-quality barley. Moisture content was determined with an automatic grain moisture meter (Motomco Instruments, Paterson, NJ).

Experimental design and statistics. The four treatment groups were high-quality (<0.5 μg/g DON when screened at the elevator and graded as high-quality barley) nonozonated barley, high-quality ozonated barley, naturally infected (1.0 μg/g DON) nonozonated barley, and naturally infected ozonated barley. Ozonation was done twice during the malting process: after 2 h of steeping and after 6 h of steeping. Grains were analyzed for DON concentration, aerobic plate count (APC), mold and yeast count (MYC), Fusarium infection (FI), GE, and moisture content. Malt quality parameters analyzed were diastatic power, alpha-amylase, fine grind extract, viscosity, soluble protein, color, free amino nitrogen (FAN), and β-glucan.

The treatments were set up in a randomized complete block design to test for differences. All treatments were repeated three times, replicated on different days. Results (means) were subjected to an analysis of variance using the general linear model procedure to compare differences between treatments through Duncan grouping. All analyses were conducted with Statistical Analytical Software (19). Treatment differences were considered significant at the 5% level.

Micromalting and ozonation. The malting procedure used in this study was on a pilot scale and hence referred as micromalting. Steeping was performed with distilled water at 16°C (400 g of barley in 800 ml of distilled water in a 2-liter Erlenmeyer flask). The steeping schedule was 8 h of steeping followed by 2 h of air rest; this cycle was repeated three times for a total steep time of 40 h (32 h of steeping and 8 h of air rest). Ozone treatment was applied via a submerged gas sparger attached to a lab-scale ozone generator (model OS-8C, Ozone Solutions, Inc., Sioux Center, IA). Ozone concentrations were maintained with an ozone monitor (model 450-H, Advanced Pollution Instrumentation, Inc., San Diego, CA). Two ozone treatments of 26 mg/cm³ for 120 min were used, one after 2 h of steeping and one after 6 h of steeping. Steep water was changed before and after each ozone treatment. The remainder of the malting procedure was performed in a pilot-scale malting unit (Joe White Malting Systems, Melbourne, Australia). Germination time was 84 h at 16°C and 95% relative humidity. Germinating barley was turned daily to prevent matting (clumping), and moisture was replaced to compensate for weight lost. The malt was then kilned by increasing temperatures, in a steepwise manner, to 85°C over a 22-h period (12). After kilning, the malt was derooted and ground, and malt quality parameters were analyzed.

Sample collection. Samples weighing approximately 50 g (wet basis) were collected for microbial and mycotoxin analyses at various points during barley malting: prior to steeping (0 h), after each ozone treatment (4 and 8 h), after completion of steeping (40 h), after 24 h of germination, upon completion of germination, and after kilning. Microbial analyses began within 6 h of sampling; each sample was sealed and refrigerated until analyses could begin. Samples for GE analysis were collected before and after each ozone treatment and after steeping. Final malt samples were collected for malt quality analyses.

Microbial analyses. APCs, MYCs, and FI assays were performed according to the methods used by Kottapalli and Wolf-Hall (13).

DON analysis. Barley and malt samples were analyzed for DON using gas chromatography with electron capture detection according to the method of Tacke and Casper (24). Samples were ground with a model 3600 laboratory mill (Perten, Huddinge, Switzerland). DON standards (Sigma Aldrich, St. Louis, MO) in acetonitrile were used to produce standard curves based on peak areas. Recovery rates were 95%, and the range of quantification was 0.2 to 120 μg/g for both barley and malt.

GE. GE was analyzed according to a modified version of the standard method, barley-3C of the American Society of Brewing Chemists (ASBC) (2). Glass petri dishes (100 by 15 mm) were prepared by placing two pieces of filter paper (no. 1, Whatman, Clifton, NJ) at the bottom of each dish for water absorption, and 100 kernels were placed evenly in each dish. Distilled water (4 ml) was added to each dish, and the dishes were covered and placed in a humidified incubation chamber maintained at 21°C. Sprouted kernels were counted and removed daily for 4 days, after which the percentage of germinated kernels were determined.

Malt quality analyses. Malt quality analyses were performed after kilning and grinding the malted barley. Quality parameters
FIGURE 1. Effect of ozonation on mean Fusarium infection (percentage) in (A) high-quality and (B) naturally infected barley samples taken at each stage of malting. Each data point represents the mean of three replications. Different letters on each bar indicate significant differences based on $\alpha = 0.05$.

FIGURE 2. Effect of ozonation on mean aerobic plate counts (APCs) in (A) high-quality and (B) naturally infected barley samples taken at each stage of malting. Each data point represents the mean of three replications. Different letters on each bar indicate significant differences based on $\alpha = 0.05$. 
measured were moisture content, fine grind extract, dynamic viscosity, soluble protein, ASBC color, FAN, and β-glucan concentration. All parameters were measured according to standard ASBC methods (2). Methods described by Karababa et al. (12) were used to determine the diastatic power and alpha-amylase in the malt.

RESULTS AND DISCUSSION

Effect of ozonation on FI. The FI results in high-quality and naturally infected barley samples at each malting step and after each treatment are summarized in Figure 1. Significantly lower levels of FI (α = 0.05) were found in samples of both high-quality and naturally infected barley after each ozone treatment (steeping: 4 and 8 h). An increase in water activity of barley, which initiates fungal spore germination (1, 5, 6, 9), was observed during the steeping process. Thus, an increase in FI was observed during germination as the Fusarium propagules that survived the steeping process grew. However, a significant decrease in FI was noted for ozonated compared with nonozonated barley samples (for both high-quality and naturally infected barley). Decreased FI in the ozone-treated grain before and during germination could result in lower levels of Fusarium mycotoxins such as DON in final malts. Although Fusarium is destroyed during kilning, DON is able to survive the high temperatures associated with kilning and wort boiling (20). In this study, a significant reduction in FI was observed in the finished malt because the high temperatures during kilning inhibited Fusarium growth.

Effect of ozonation on APCs. Figure 2 summarizes the effect that ozone applied during steeping had on APCs. Although there were no reductions in APCs immediately after completion of ozone treatment (steeping: 8 h), there were reductions in APCs for ozonated samples of both high-quality and naturally infected barley after 1 day of germination (germination: 24 h); however, only the difference for the naturally infected barley was significant. Significant differences in APCs for all samples were observed between the end of germination and the completion of kilning, which can be attributed to the high temperature (85°C) of the kilning process.

Effect of ozonation on MYCs. The MYC results throughout the malting process are summarized in Figure 3. There was no clear reduction in MYCs after completion of ozone treatment (steeping: 8 h). As with the APCs, most of the fluctuation in MYCs during malting was due to the different moisture and temperature conditions for each process. The counts steadily increased during germination (16°C, 95% relative humidity) followed by reductions in both MYCs and APCs after kilning (85°C, decreasing humidity). A significant decrease in MYCs (α = 0.05) was noted for ozonated naturally infected malt samples (kilning:
However, the practical significance may be minimal with a 1.5-log reduction in the MYC. Such reductions would need to be evaluated in an economic analysis to determine feasibility of this ozonation process for industrial use.

Effect of ozonation on DON concentration. The DON concentrations in ozonated and nonozonated high-quality and naturally infected barley samples throughout the malting process are presented in Figure 4. The initial DON concentration in the infected barley was high enough to be considered unacceptable for malting and brewing (11), although not as high as those found in barley crops from 1993 through 1996, when concentrations were as high as 17.2 to 60.0 μg/g (18, 21). Higher concentrations of DON may be associated with other quality defects in barley.

No significant differences in DON concentrations were found between ozone-treated and untreated malt. A decrease in DON concentration would be expected for more heavily contaminated grain because of the decrease in FI. Schwarz et al. (20) noted some fluctuations in DON concentration between malting steps. A dramatic decrease in DON concentration after steeping was due to solubilization in the steep water and removal with the water replacement cycles, and the subsequent increasing DON concentrations were due to mold growth during germination. However, in the present study the DON concentrations did not increase significantly (α = 0.05) even after germination and kilning.

The results from a number of studies indicate that DON from Fusarium-infected barley survives the processes of malting and brewing and can be found in the finished beer. Schwarz et al. (20) found that DON concentrations increased during germination because of continued mold growth, and final beers contained 80 to 93% of the DON from the malted barley. Garda et al. (8) evaluated the stability of DON through alcoholic fermentation and found that 47% of the DON in the wort remained in the beer after 120 h of fermentation. In another study, Scott (22) found that DON concentrations were stable throughout 9 days of fermentation. According to these reports, heavily infected barley used for malting will produce beer with some DON. However, decreasing FI by ozone treatment during steeping may be a promising method for reducing DON concentrations in beer because this treatment reduces the production of DON during germination.

Effect of ozonation on GE and malt quality. Figure 5 illustrates the GE of samples of barley taken before, during, and after steeping. The mean GE was high enough in all samples (>95%) to be acceptable for malting (25). There was no significant difference in GE between ozone-treated and untreated samples at any of the sampling points. This result agrees with those of Kottapalli et al. (14), who found...
that ozone concentrations of 11 and 26 mg/g did not affect
GE in sound barley. Our findings suggest that GE was not
an issue of concern associated with ozone treatment of
malting barley.

Malt quality parameters of each malt portion are
summarized in Tables 1 and 2. In general, there was very
little difference in malt quality between samples. Some of
the quality parameters, alpha-amylase, fine grind extract,
dynamic viscosity, and soluble protein, remained constant
for both ozone-treated and untreated samples of both high-
quality and naturally infected barley. Thus, ozone treatment
did not have any effect on these malt quality parameters.
This finding is important because antimicrobial treatments
should not have any influence on malt quality. For the
naturally infected barley, the ozone-treated samples had
higher diastatic power than did their untreated counterparts.
No significant differences were observed in diastatic power
for the high-quality samples. The increase in diastatic power
observed in the ozonated grain, although small, could be
beneficial to malting operations because it means that the
malt has higher enzymatic activity. Therefore, more of the
starch from the barley can be converted to fermentable
sugars, making it possible to increase the amount of adjunct
added to the mash. However, further studies are needed to
confirm that ozone has an effect on malt diastatic power.

Ozone treatment seemed to have mixed effects on both
color and FAN concentrations of malt. Ozone-treated
samples of high-quality barley had more color and FAN

![Effect of ozonation on germinative energy (GE%) in (A) high-quality and (B) naturally infected barley samples taken at each stage of malting. Each data point represents the mean of three replications. Different letters on each bar indicate significant differences based on α = 0.05.](http://meridian.allenpress.com/jfp/article-pdf/74/12/2134/1684844/0362-028x_jfp-11-193.pdf)

![Table 1. Effect of ozone on malt quality parameters: malt moisture, diastatic power, alpha-amylase, and fine grind extract](http://meridian.allenpress.com/jfp/article-pdf/74/12/2134/1684844/0362-028x_jfp-11-193.pdf)
TABLE 2. Effect of ozone on malt quality parameters: dynamic viscosity, soluble protein, ASBC color, free amino nitrogen (FAN), and β-glucan

<table>
<thead>
<tr>
<th>Barley sample</th>
<th>Dynamic viscosity (mP×s)</th>
<th>Soluble protein (% dry basis)</th>
<th>ASBC color (‘SRM)</th>
<th>FAN (mg/liter)</th>
<th>β-Glucan (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High quality, nonozonated</td>
<td>1.73 ± 0.03 A</td>
<td>5.37 ± 0.02 A</td>
<td>2.11 ± 0.02 BB</td>
<td>235 ± 15 BB</td>
<td>841 ± 106 AA</td>
</tr>
<tr>
<td>High quality, ozonated</td>
<td>1.69 ± 0.02 A</td>
<td>5.73 ± 0.10 A</td>
<td>2.20 ± 0.06 AB</td>
<td>251 ± 15 AB</td>
<td>670 ± 139 AB</td>
</tr>
<tr>
<td>Naturally infected, nonozonated</td>
<td>1.62 ± 0.05 A</td>
<td>5.91 ± 0.35 A</td>
<td>2.59 ± 0.17 AA</td>
<td>282 ± 8 AA</td>
<td>575 ± 82 AB</td>
</tr>
<tr>
<td>Naturally infected, ozonated</td>
<td>1.60 ± 0.05 A</td>
<td>5.95 ± 0.23 A</td>
<td>2.43 ± 0.16 AB</td>
<td>268 ± 10 AB</td>
<td>450 ± 28 BB</td>
</tr>
</tbody>
</table>

Values are the means ± standard errors for three replications. Within a column, means with different letters are significantly different at \( \alpha = 0.05 \).

Discussion of barley treatment methods. This experiment was the third in a series of experiments designed to evaluate three methods for reducing microbial loads in malting barley. Each of these three experiments was completed using similar methods and analytical techniques. The first study was conducted to investigate the effect of electron-beam irradiation on the safety and quality of malting barley with FHB (15). In that study, irradiated (8 to 10 kGy) barley and malt had significantly lower FI rates \((P = 0.05)\) than did untreated barley and malt. Electron-beam irradiation also significantly decreased \((P = 0.05)\) both APCs and MYCs in Fusarium-infected barley. The DON concentrations in malt samples were effectively reduced (54 to 100%) by electron-beam irradiation (4 to 10 kGy), with the greatest reduction (93 to 100%) observed at 10 kGy. The seed germination rate (GE) was more than 95% when electron-beam irradiation of up to 4 kGy was used. The results suggested that with minimal effects on malt quality, 6 to 8 kGy radiation was effective for reducing DON in malt and FI in barley.

The second study was conducted to determine the effect of hot water treatment on the safety and quality of malting barley (13). Hot water treatment resulted in significant reduction \((P = 0.05)\) in APCs, MYCs, and FI in Fusarium-infected barley. These reductions were greater at higher temperatures and after longer treatment times. However, the effect did not carry over to the final malt, probably because of the continued proliferation of heat-resistant organisms during germination. DON concentration decreased significantly \((P = 0.05)\) in malts treated with hot water (45 to 50°C). Similar to the present study, a decrease in DON contamination was attributed to the reduction in FI before germination. Hot water treatment did not significantly affect \((P = 0.05)\) malt quality parameters or GE, except after treatments at 50°C for 10 min or longer.

In the present study, ozone treatment was effective for reduction of the microbial load in malting barley without negatively impacting malt quality. The DON concentrations also were decreased in these samples after ozonation; however, the decrease was not significant. Both ozone and hot water are more promising antimicrobial treatments for malting barley than electron-beam irradiation because they do not interfere with malt quality or GE. Ozone treatment increased diastatic power and decreased β-glucans (but not significantly) in malt.

Ozone treatment was applied to high-quality and naturally infected barley during the steeping process of malting. This treatment was slightly effective for reducing APCs and MYCs of the malt. The effect of ozone on DON could not be determined because of the low concentrations of DON in the original grain. We assume that ozonation would significantly decrease DON concentrations in heavily infected grain because the treatment decreased the amount of Fusarium present in the grain. The reduction in FI should result in less DON being produced during germination. Ozonation is a promising treatment for malting barley because it did not have any negative effects on the quality parameters of the final malt. Additional studies are needed to test the effects of ozone treatment on barley heavily infected with Fusarium. The economic feasibility of such treatments also should be studied to establish the practicality of such processes at the industrial level.

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