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Reduction of Deoxynivalenol in Barley by Chemical Treatments and Malting

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Abstract *Fusarium* mycotoxin deoxynivalenol (DON) contents and its critical quality parameters were analyzed using two-rowed, six-rowed, and hulless barleys. DON content in the two-rowed Korean barleys was lower than that in the six-rowed barleys. The average DON contents of six-rowed, two-rowed, and hulless barleys were 1.35 ppm, 0.36 ppm, and 0.49 ppm, respectively. The DON content was reduced by 6.2% by sieving, by 6.0% by washing with water and by 18.1-69.8% by treatment with aqueous chemical solutions. Of the reagents investigated, aqueous sodium bicarbonate gave the greatest reduction in the barley DON level. The DON content was reduced to 62.5% of initial level after 3 days of steeping and to 23.1% after 3 days of germination. DON was not detected after steeping barley for 24 hr in 0.1M Na_2CO_3 solution with 0.1% activated carbon.

Key words: barley, deoxynivalenol, reduction, adsorption, malting

Introduction

Deoxynivalenol (DON) is known as type B trichothecene mycotoxin produced by *Fusarium graminearum* and *F. culmorum*. DON frequently co-occurs in various cereal crops (e.g., wheat, barley, maize, oats, rice, and rye) (1, 2) and processed foods worldwide (e.g., malt, beer and bread) (3-5). When processed foods, such as malt, beer and bread, are produced from the grains contaminated with DON, the products also contain a large quantity of DON which is a serious issue for consumers' food safety (3-6). Moreover, when people or animals consume grains contaminated with DON, they show symptoms including weight loss, emesis, feed refusal, and decreased feed intake (6, 7). For these reasons, many countries regulate DON by setting its maximum limits. For example, the maximum DON level allowed in wheat is 1 ppm in USA, 1 ppm in Russia, 1 ppm in China, and 0.75 ppm in Austria (8). DON can also contaminate various cereal grains, such as wheat (57% positive), maize (41% positive), oats (68% positive), barley (59% positive), rye (49% positive) and rice (27% positive) (8). In the European Union, 57% of the several thousands of samples showed positive contamination for DON (9).

A recent study has shown that 67% of the Korean barley samples had DON contamination (10). Other reports have shown that the Korean barleys are contaminated by mycotoxin of the *Fusarium* genus such as DON (11, 12). King *et al.*(13) have also analyzed fungi of the *Fusarium* genus that were isolated from agricultural products such as barley and showed a high potential for the DON production in Korean-produced barleys.

Grains contaminated by DON are usually discarded, which leads to a great loss in the production of grains. In order to minimize such loss, researchers attempted to remove or decompose mycotoxin in grains, stock feed and food (14). The methods studied for DON removal have included sieving (15), dehulling (16), extrusion (17) and active carbon adsorption (9). It is important to study methods of complete DON removal in the process of adsorption and in the manufacture of many kinds of barley foods including barley malt, barley tea and barley biscuit. Nevertheless, studies on DON removal during barley processing and on the quality after removal are rare.

This study investigates the variations in DON contents and the DON removal efficiencies during sieving, washing, steeping, germination and chemical treatments. This study also compares the important characteristics in samples that had undergone these removal treatments, and suggests important implications for establishing DON removing methods that are applicable to barley product processing.

Materials and Methods

Materials Korean barleys grown at Jeonnam and Gyeongnam provinces in southern areas of Korea were collected in early June 2003. Five samples of two-rowed barley, two samples of six-rowed barley, and four samples of hulless barley were collected from small-scale farmers who dried barley crops in sunlight after harvest. Barley samples were stored at 4°C for this study. The standard DON and florisil (60-100 mesh) used for cleaning DON were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents for this study were the highest grades available from commercial sources.

Grain quality of barley The barley samples were ground in a Udy cyclone mill (Udy Corporation, Fort Collins, CO, USA) using a 0.5 mm screen before analysis. The samples

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were analyzed with standard procedures for moisture and crude protein (18). Germination capacity was analyzed according to the standard methods of the American Society of Brewing Chemists (19), and determined from triplicate samples.

Pilot malting The modification of malting procedure described in Schwarz *et al.*(3) was used in this study. Individual samples (25 g, dry basis) were steeped at 16°C for three days, and then were germinated at 16°C and relative humidity of 90% for three days. During germination, the samples were stirred and sprayed with water. In order to measure the variations in DON concentrations during soaking and germination, DON was extracted from the samples and measured using HPLC on days 1, 2 and 3 of steeping and germination.

DON extraction and cleanup The barley samples were ground in a Udy cyclone mill (Udy Corporation, Fort Collins, CO, USA) using a 0.5 mm screen. Each sample (25 g) was placed in 100 mL extraction solution (acetonitrile: water=84:16, v/v) and shaken at 200 rpm for 30 min. The extract was filtered through Whatman No. 4 filter paper (20). A 4 mL aliquot of the filtrate was placed into a 10 mL culture tube and evaporated just to dryness under a stream of nitrogen. Solid phase extraction was adopted using a florisil column to purify the residue (12). The residue was dissolved in 1 mL of methanol and applied onto a florisil column containing 10 g of florisil. The column was washed with 100 mL of n-hexane and eluted with 100 mL chloroform:methanol (9:1, v/v). The eluate was concentrated by drying and the residue was redissolved in 0.4 mL of acetonitrile:water (8:92, v/v). DON was measured by HPLC.

Determination of deoxynivalenol by HPLC A Beckman 110B liquid chromatography system was used with an Ultrasphere® 5 μm Spherical 80Å pore, length 250 mm, ID 4.6 mm column (Alltech, Deerfield, IL, USA). The mobile phase for HPLC was acetonitrile:water (8:92, v/v) and the flow rate was 0.7 mL/min. The UV detector was set at 220 nm for DON analysis. Twenty microliters of the extract was injected into the HPLC column.

Reduction of DON by washing and sieving In order to measure the level of DON removed by washing with water, 25 g of each barley sample was added into 125 mL of water and washed 5 times at 980 rpm using a homogenizer (Type Z-1000, Rikakikai Co. Ltd., Tokyo). After being washed, the samples were dried at 50°C for 12 hours, and the DON residues in the samples were measured. In order to examine DON removal efficiency during sieving, kernels were separated according to size (2.0-2.36 mm, 2.36-2.8 mm and >2.8 mm) using testing sieves (Testing Sieve, Chung Gye Co., Seoul, Korea).

Effect of chemical treatments Twenty-five grams of barley samples were added to 16 mL of 0.1 M Na_2CO_3 solution, hydrogen peroxide solution (7.5%), ascorbic acid (4%), NH3 solution (7%), 0.1 M hydrochloric acid, 0.1 M NaOH, 5% $Ca(OH)_2$, 6% NaCl solution or distilled water. The sample was left overnight, dried and measured with HPLC.

Binding of DON by activated carbon and loess Stock solutions of DON were prepared by dissolving 500 ppm of toxin in methanol. Standard solutions were prepared at different concentrations in distilled water for in vitro tests, in methanol for spiking purposes, and in the mobile phase for HPLC detection. One or ten milligrams of adsorbent material was weighed separately in a 2 mL centrifuge tube to which 1 mL was added as a standard solution. After shaking for 1 hr at room temperature, the adsorbent materials were separated by centrifugation at 10,000 rpm for 10 minutes. The supernatants were then transferred to clean tubes and analyzed for DON by HPLC. The amount of bound DON was calculated for each material using the difference between the initial and the final DON concentrations in the testing solution, expressed as a percentage of the initial concentration.

Statistical analysis Data were analyzed with analysis of variance (ANOVA) in the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Least-square means procedures were used to separate mean values when the difference was statistically significant ($p \leq 0.05$). Measurements were made in triplicate.

Results and Discussion

Chemical composition Table 1 shows the moisture and protein contents, 1,000-kernel weight, germination capacity, floating kernels, and DON contamination of barley samples. Moisture content, ranging from 10.2% to 10.8%, showed no significant differences among the types of barley. The average protein content was 10.1% in two-rowed barley, 11.4% in hulless barley, and 13.2% in six-rowed husked barley. The average 1,000 kernel weight was 36.8 g for two-rowed barley, 29.9 g for six-rowed barley and 27.2 g for hulless barley. The average DON content was 1.35 ppm in six-rowed barley, 0.49 ppm in six-rowed hulless barley, and 0.36 ppm in two-rowed barley, showing the difference among the barley types. The average DON concentration in six-rowed barley was higher than that in two-rowed barley. In general, barley with over 2 ppm of DON is not purchased for malting, and some brewers do not accept barley with DON level higher than 0.5 ppm (21).

Reduction of DON by washing Table 2 shows the

Table 1. Quality characteristics of barley[1)]

Quality	2 Rowed (n=5)	6 Rowed (n=2)	Hulless (n=4)
Moisture (%)	10.2±0.4	10.2±0.2	10.8±0.7
Protein (%)	10.1±0.1	13.2±0.4	11.4±0.2
1000 K.W. (g)	36.8±0.8	29.9±4.9	27.2±1.5
Germination capacity (%)	85±2	73±24	76±21
Floating kernels (%)	7±1	23±27	3±2
DON content (ppm)	0.36±0.07	1.35±1.03	0.49±0.27

[1)]Means±standard deviation of triplicate determinations.

Table 2. DON contents of barley after 2 min washing with distilled water at 22°C[1)]

	2 Rowed barley		6 Rowed barley		Hulless barley	
	DON conc. (ppm)	Reduction (%)	DON conc. (ppm)	Reduction (%)	DON conc. (ppm)	Reduction (%)
Unwashed kernel D.W.	0.34±0.01	-	2.08±0.12	-	0.69±0.01	-
Washing 1st	0.33±0.05	3.0	2.04±0.05	2.0	0.62±0.06	10.1
Washing 2nd	0.28±0.06	17.6	1.96±0.04	5.8	0.50±0.02	27.5
Washing 3rd	0.27±0.03	20.6	1.97±0.10	5.3	0.52±0.06	24.6
Washing 4th	0.27±0.01	20.6	1.96±0.05	5.8	0.51±0.03	26.1
Washing 5th	0.26±0.03	23.5	1.95±0.10	6.0	0.50±0.05	27.5

[1)]Means±standard deviation of triplicate determinations.

Table 3. Distribution of DON after sieving[1)]

Sieving mesh	>2.8 mm	2.36-2.8 mm	<2.36 mm
Weight ratio (%)	55.3±4.5	42±3.8	2.5±0.3
DON content (ppm)	0.17±0.07	2.32±0.06	2.82±0.20
Increase or reduction of DON content (%)	-91.8±3.3	+11.5±2.7	+35.6±9.7
DON content of the composition (%)	4.5±1.8	46.8±1.1	3.4±0.2

[1)]DON content of untreated six-rowed barley was 2.08 ppm. Means±standard deviation of triplicate determinations.

DON contents of barley after washing for 2 min with distilled water at 22 °C. In the two-rowed, six-rowed and hulless barleys, the DON removal rates were 3%, 2% and 10%, respectively, by the first washing, confirming that DON cannot be removed much by washing just once. Furthermore, 6.0% DON was removed from six-rowed barley, 23.5% from two-rowed barley and 27.5% from hulless barley after the 5th washing, and these differences from the second washing were insignificant. In contrast to this result, Trenholm *et al.* (17) reported that 65-69% of DON was removed by washing three times for 30 minutes each time. In our experiments, the DON removal efficiency of washing was low and varied according to the variety of barley, degree of DON contamination, and washing times. It is thus necessary to examine washing conditions according to different sample types.

Effect of sieving The process of barley germination usually includes sieving for uniform steeping and germination. The DON contamination of barley was also analyzed by size. For this study, six-rowed barley with 2.08 ppm of DON was used due to its high DON content. The DON contents of the two-rowed and hulless barleys were too low to effectively analyze the removal rates. As shown in Table 3, the DON content was high in the small kernels. The DON contamination of 2.0-2.36 mm kernels increased 35.6% after separation. These results suggest that unripened kernels are severely contaminated with fungi, indicating that their DON contamination level is high. However, small-sized kernels were only 2.5% of the entire sample by weight. Sieving reduced the DON level by only 6.2%. Therefore, the separation of small-sized kernels is considered to be ineffective for DON removal. On the other hand, kernels bigger than 2.8 mm were 55.25% of the entire sample by weight and the DON contamination decreased 91.8% after separation. Kernels of this size seemed to be relatively safe from DON contamination compared to the small-sized barley kernels. Medium size (2.36-2.8 mm) kernels were 42% of the entire sample by weight and their DON contamination increased 11.5% after separation. Since kernels of this size were 42% of the entire sample, they are important in noxious contamination. Therefore, the removal of toxic materials should be focused on this size range.

Effect of chemical treatments As shown in Table 4, the removal efficiency of 0.1 M Na_2CO_3 solution was significantly higher than that for other chemical reagents. Removal efficiency was 46.5% with NaOH, 43.9% with hydrogen peroxide, 39.4% with NaCl, 34.7% with hydrochloric acid, 28.7% with NH_3 solution, 26.4% with ascorbic acid, and 18.1% with $Ca(OH)_2$. These results are similar to those by Young *et al.* (14) and Xie *et al.* (22). Although chemical treatments can remove toxic materials, they are disadvantaged by the requirement for large quantities of chemical reagents.

Binding of DON by activated carbon and loess When using 1.25, 2.5 and 5 ppm DON solutions, 1% Korean loess showed very poor adsorption (Table 5). For 0.65 ppm DON solution, loess adsorption of DON was 57%. In

Table 4. Effects of treatment with chemical reagents[1)]

Reagents	Concentration of aqueous reagents	Reagent vol. (ml/kg)	DON reduction (%)
Na_2CO_3	0.1 M	600	69.8±1.2
Sodium hydroxide	0.1 M	600	46.5±2.9
Hydrogen peroxide	7.5%	600	43.9±3.2
NaCl	6%	600	39.4±1.9
Hydrochloric acid	0.1 M	600	34.7±3.3
NH_3 Solution	7%	600	28.7±2.5
Ascorbic acid	4%	600	26.4±1.3
$Ca(OH)_2$	5%	600	18.1±0.6

[1)]DON content of untreated six-rowed barley was 2.08 ppm. Means± standard deviation of triplicate determinations.

Table 5. DON adsorption ability of activated carbon and loess

DON standard solution (ppm)	Percentage of DON [1]	
	Activated carbon (0.1%)	Korean loess (1%)
0.65	100±0.0	56.9±6.2
1.25	90.0±3.2	20.1±1.2
2.5	87.9±5.1	12.9±2.3
5.0	60.5±2.1	7.1±1.8

[1]Means±standard deviation of triplicate determinations.

comparison, the 0.1% activated carbon showed higher adsorption ability. About 60-100% of the available DON was adsorbed on the activated carbon at initial DON concentrations of 0.65, 1.25, 2.5 and 5 ppm.

Changes of DON contents during steeping and germination In order to examine the changes of DON contents during steeping and germination, barley samples were steeped for 1, 2 and 3 days, dried in a drier, and the DON level was measured with HPLC. As shown in Table 6, 98% of DON remained after one day of steeping, 79.3% after two days, and 62.5% after three days. The DON residue decreased significantly over time.

The DON residue was 23.1% in the barley sample after a day of germination, 22.6% after two days, and 23.1% after three days. A large quantity of DON was removed by various biochemical reactions in the barley during the process of germination. Nevertheless, a considerable quantity of DON remained after steeping and germination, so a new method of removing DON needs to be developed.

Composite treatments with active carbon and 0.1 M Na_2CO_3 in steeping Avantaggiato *et al.* (10) reported that active carbon adsorbs mycotoxins such as DON with an adsorbing capacity of 35.1 μmol/mg. Furthermore, Trenholm *et al.* (16) reported that 40-100% of DON was removed by soaking with 0.1 M Na_2CO_3 and Xie *et al.* (22) reported an average DON removal efficiency of 83.9% by 0.1 M Na_2CO_3. Our study found the DON removal efficiency of 0.1 M Na_2CO_3 to be approximately 70%. Based on these previously reported results (10, 16, 27), in order to further measure the DON removal efficiency of 0.1 M Na_2CO_3 solution and the adsorption of active carbon during steeping, active carbon was added to water and 0.1 M Na_2CO_3 solution to give a final concentration of active carbon of 0.1, 0.5 and 1%. Table 7 shows the results of extracting DON from the samples that were steeped for 1, 2 and 3 days and measured with HPLC. Substantial differences were observed between the samples with and without the addition of active carbon. The DON removal efficiencies of 0.1 M Na_2CO_3 solution for the active carbon concentrations from 0.1 to 1% were 67-100%, 87-100% and 100% in the process of steeping for 1, 2 and 3 days, respectively. The removal efficiencies using active carbon and water but without Na_2CO_3 solution were 27-50%, 56-78% and 75-84% in the process of steeping for 1, 2 and 3 days, respectively. The DON removal efficiency by 1, 2 and 3 days of steeping with 0.1 M Na_2CO_3 solution was 67%-100%, which was much higher than the 27-84% removal rate achieved without 0.1 M Na_2CO_3 solution.

On the other hand, DON removal efficiencies were 27-75%, 47-80% and 50-84%, when active carbon was added at a rate of 0.1, 0.5 and 1%, respectively. These results again showed significant differences according to the quantity of active carbon added. When 0.1 M Na_2CO_3 was used in steeping, the efficiencies were 67-100%, 68-100% and 100%, respectively. When 0.1 or 0.5% of active carbon was added, DON was removed below the level of detection after two days of soaking, and when 1% was added, DON was removed below the level of detection after a day of steeping. Significant differences were observed according to the quantity of active carbon added. Accordingly, the addition of 0.5-1% of active carbon and the use of 0.1 M Na_2CO_3 in steeping is an effective and practical method for DON removal in the process of barley germination.

Table 6. Changes of DON in barley during steeping and germination

Treatment	days	DON content[1] (ppm)	DON reduction rate (%)
Steeping	1	2.04±0.04	2[a2]
	2	1.65±0.02	20.7[b]
	3	1.30±0.05	37.5[c]
Germination	1	0.48±0.03	76.9[d]
	2	0.47±0.04	77.4[d]
	3	0.48±0.05	76.9[d]

[1]DON content of untreated six-rowed barley was 2.08 ppm.
[2]Values followed by the same letter in the same column are not significantly different ($p<0.05$).

Table 7. Reduction of DON during barley steeping with active carbon[1]

Steeping (days)	Activity carbon content (%)	DON remaining(ppm) (% reduction)	
		Distilled Water	0.1 M Na_2CO_3
1	0.1	1.53(27)	0.89(67)
	0.5	1.11(47)	0.87(68)
	1.0	1.04(50)	0.0(100)
2	0.1	0.92(56)	0.27(87)
	0.5	0.90(57)	0.0(100)
	1.0	0.45(78)	0.0(100)
3	0.1	0.52(75)	0.0(100)
	0.5	0.42(80)	0.0(100)
	1.0	0.33(84)	0.0(100)

[1]DON content of untreated six-rowed barley was 2.08 ppm.

Effect of chemical treatments on germination The effects of activated carbon and sodium carbonate treatments on germination are shown in Fig 1. The levels of germination were reduced by only an insignificant degree by sodium carbonate treatment compared to pure water treatment. If germination efficiency is assumed to be 100 % when using only water, germination efficiencies of 96 %, 82% and 81% were obtained when using only 1% activated carbon, a combination of 1% activated carbon and 0.1 M Na_2CO_3, and only 0.1 M Na_2CO_3, respectively. In conclusion, DON in barley could not be completely removed by sieving and washing alone. However, DON

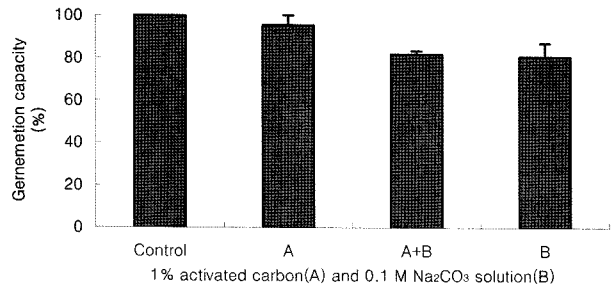

Fig. 1. Effect of 1% activated carbon and 0.1 M Na_2CO_3 on germination. Error indicates standard error of mean. The effects of activated carbon and Na_2CO_3 solution on germination were not significantly different ($p<0.05$).

was not detected after steeping barley for 24 hr in 0.1 M Na_2CO_3 solution with 1% activated carbon. In the future, the effect of Na_2CO_3 solution with activated carbon on enzyme activities and other malting qualities should be further studied for potential application in the malting process.

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