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보리 가공에 의한 Deoxynivalenol의 감소 효과

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Effects of Barely Processings on the Reduction of Deoxynivalenol

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ABSTRACT – This study examined the effects of pearling, simple heating, baking and extrusion in processing of barley contaminated with deoxynivalenol (DON) on the reduction or destruction of DON. The DON contamination level of barley used in the experiment was 2.08 ppm. The DON-forming strain isolated from the barley was *Fusarium graminearum*, and the DON formation potential of which was 6.3 ppm on the average in Czapek-Dox broth medium. The DON removal efficiency of pearling was 56.5%, simple heating 3.8-32.7%, extrusion 53-59%, and baking 10.4%. As a large quantity of DON still remains after processing, it is necessary to develop a new method of removing DON completely.

Key words: mycotoxin, barley, processing, reduction

Deoxynivalenol (DON) (3α,7α, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one) is known as type B trichothecene mycotoxin that causes vomiting to stocks like pigs[1]. It is detected frequently in wheat, barley, corn and stock feed produced from North America, Europe and Southeast Asia.[1,2] Park *et al.*,[3] Lee *et al.*[4] and Ryu *et al.*[5] reported the results of their investigation of DON contamination of home-produced barley. King *et al.*[6] isolated strains with DON formation potential from agricultural products such as barley, suggesting the possibility of DON contamination of home-produced agricultural products including barley. In addition, Pei *et al.*[7] and Shim *et al.*[8] found DON in barley processed foods and beer. These results raise questions on the safety of barley processed foods.

For the removal and disintegration of DON, Young *et al.*[9] examined the DON removal efficiency of water-soluble chemical reagents using a process that added water during wheat processing. In addition, Trenholm *et al.*[10] studied DON removal by sieving and dehulling and by washing.[11] Accerbi et al.[12] measured DON destruction by extrusion and Avantaggiato *et al.*[13] investigated DON removal by adsorption using active carbon. Scott *et al.*,[14] Abbas *et al.*,[15] Lee *et al.*[16] and Young *et al.*[17] reported the change of DON in processes such as cleaning, milling and baking. However, there have been few studies on the change and removal of DON in the processing of home-produced barley, so it is keenly necessary to accumulate basic experiment data. Thus the present study aimed to experiment in the reduction and destruction of DON in processing home-produced barley through pearling, simple heating, extraction and baking, to measure the effects, and to provide basic data in the applicability of DON-contaminated barley as food.

MATERIALS AND METHODS

Materials

Barley used in the experiment was a large quantity of sample contaminated with *Fusarium* species (red mold), which was collected from farms in Gyeongnam-province, southern area of Korea in June 2003, and wheat flour for baking (strong flour, Samyangsa), Hulless barley (Hongje Yutong), salt (Haepyo), sugar (Samyangsa),

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yeast (Ottogi), shortening (Heinz), etc. were obtained from the market. The DON standard and florisil used for cleanup of DON were from Sigma Chemical Co. (St. Louis, MO), and the organic solvents and reagents used in cleanup and detection DON were of HPLC grades (Merck Co.).

Methods

Quality characteristics of barley – This experiment analyzed the general properties of the barley sample such as moisture, crude protein and 1,000-kernel weight according to ASBC[18] method. To measure the degree of mold contamination, 100 kernels were randomly selected, and sterilized in 1% sodium hypochlorite solution by shaking a minute, washed with sterile water three times, and 20 kernels were placed on potato dextrose agar (PDA) plate medium, to which 200 ppm of streptomycin sulfate was added aseptically, and cultured at 28 for 5 days and calculated the percentage of kernels contaminated with mold.[7]

Isolation and identification of *Fusarium* species – In order to isolate *Fusarium* species, 1 g of finely powdered barley sample was mixed with 9 of sterile water in a test tube (18×200 mm), and made 10-fold serial dilutions of the mixture solution by sterile distilled water. Duplicates of 200 μL volumes were added to petri dishes containing 10-15 ml of rose bengal agar plate medium, to which 35 mg/L of rose bengal was added, and incubated at 28°C until a single colony was formed.[6] After cultivation, the colony that was assumed to be *Fusarium* species was isolated, and it was inoculated into a carnation leaf agar (CLA) plate medium, till forming a large quantity of spores, and identified *Fusarium* strains according to Nirenbergs[19] simplified method.

Production of DON by *Fusarium* species – In order to measure the DON producing ability of the isolated *Fusarium* strains, 9 ml of Czapek-Dox Broth (Becton Dickinson, USA) liquid medium was put into a test tube and autoclaved at 121°C for 15 minutes. Five ml of sterile distilled water was added to each slant of culture and the CLA agar surface was gently scraped to give a turbid suspension, corresponding to 1×10^6 spores/ml. The cultures were Incubated at 28°C for 14 days and again at 12°C for 14 days.[20] Culture liquid was filtered through a filter paper (Whatman No. 2). Exactly 4ml of culture solution was taken into the tube and 8ml of chloroform were applied to the tube, and stirred sufficiently with an agitator to extract DON. After separating the chloroform layer, the process was repeated by applying the same quantity of chloroform, and combined it with the previous one, and concentrated to dryness.

Determination of DON – The DON concentration of each sample was measured using modified methods of Trucksess *et al.*[21] and Ryu *et al.*[5] A 25 g of powdered sample was placed with 100 ml of extraction solvent (acetonitrile:water = 84:16), in a blender jar and blended for 3 minutes at high speed. The extract was filtered through Whatman No. 4 filter paper. A 4ml aliquot of the filtrate was placed into a 10 ml culture tube and evaporated to dryness on a steam bath of nitrogen. The residue was dissolved in 1ml of methanol, and applied onto a florisil column (10 g, 60-100 mesh, Sigma Chemical Co). The column was washed with 100 ml of n-hexane and eluted with 100 ml of elution solvent (chloroform:methanol = 9:1). The elute was concentrated to dryness and the residue was redissolved in HPLC mobile phase solvent and DON was measured by using HPLC. Beckman 110B liquid chromatography system was used with Ultrasphers® 5 μm Spherical 80Å Pore, Length 250 mm, ID 4.6 mm column (Alltech, Deerfield, IL, USA). The mobile phase for HPLC was acetonnitrile:water(8:92)(v/v) with the flow rate of 0.7 ml/min, which was quantified using UV detector (220 nm).

Reduction of DON by pearling process – After pearling each barley sample contaminated with DON using Satake Test Mill (Satake Engineering Co., Ltd., Tokyo, Japan), the DON contamination of the kernels as well as byproducts was determined and the quantity of DON removed by pearling was calculated.[22]

Reduction of DON in baking and heating processes – Bread was made by mixing DON-contaminated barley flour and wheat flour and kneading it using the straight-dough method (AACC 10-10A). The mixture ratio of basic materials used in baking is as in Table 1. Materials were kneaded using mixer (MVM-12-29, Dae Young Co. Seoul, Korea) and fermented at temperature of 30°C and humidity of 85% for 55 minutes. After punching, it was fermented again for 25 minutes. After the second fermentation, the dough was divided, rounded, rested for 10 minutes, sheeted, molded,

Table 1. Baking formula based on wheat flour weight

Material	Composition (g)	Composition (%)
Wheat flour	1040	100
Barely flour	260	25
Sugar	86	8.3
Salt	22	2.1
Shortening	52	5
Yeast	26	2.5
Non fat dried milk	40	3.8
Water	832	80

panned, proofed for 38 minutes, and baked at 200°C for 20 minutes using a oven (OFP-202, Dae Young Co., Seoul, Korea).

In order to see the change of DON by simple heating, twenty-five g of barley was placed into a Stir Mantle Set (EXT-F-25, PRECISON & INDUSTRY Co. LTD., USA) and heated at each of 150, 200 and 250°C for 7 minutes, and DON was extracted and measured using the same method above.

Reduction of DON by extrusion process – In order to measure the reduction of DON in barley sample by extrusion, the finely ground barley sample contaminated with DON was extruded using a co-rotating twin screw extruder (Model THK 31T, Baeksang Machine, Korea) and the condition of extrusion was as follows. The diameter of the screw was 31 mm, L/D ratio was 22 and the diameter of the die (circular type) was 3.7 mm. In addition, a high shear screw was used. The rotating speeds of the screw were 200 and 300 rpm, and the temperatures were 110, 115 and 120°C. The extruded samples were dried so that their water contents became 10%. DON was extracted using the method explained above and measured using HPLC.

Statistical analysis – In order to test significance among mean values obtained from experiments, we performed ANOVA at significance level of α=0.05 using Statistical Analysis System[23] (SAS Institute Inc., Cary, N.C.).

RESULTS AND DISCUSSION

Quality characteristics of barley samples

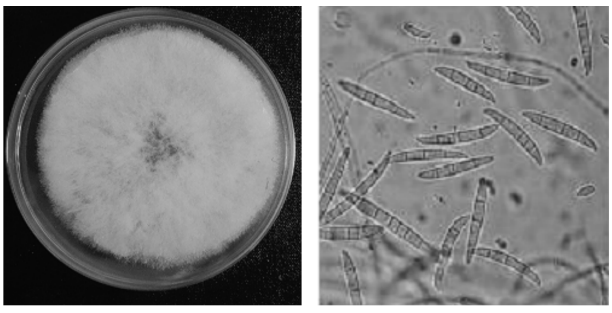
The general qualitative characteristics of home-produced barley sample used in the experiment are as in Table 2. The moisture content was 11.3%, the crude protein content was 11.0%, and the 1000-kernel weight was 26.4 g, which was relatively low. The low 1000-kernel weight is probably because of the mold contamination (100%) and DON contamination (2.08 ppm) of the barley sample. In order to examine the causes of DON contaminating the barley, molds were isolated from the barley sample and measured the DON formation potential of each strain isolated.

Table 2. Quality characteristics of barley

Component	Quality characteristics
Moisture (%)	11.3±0.8
Protein (%)	11.0±1.2
1,000 Kernel Weight (g)	26.4±0.7
Volume weight (g/L)	490±2.0
Contamination of Molds (%)	100±0.0
DON producing ability of identified strains* (ppm)	6.3±5.5
Concentration of DON (ppm)	2.08±0.05

*Eleven isolates of *Fusarium graminearum* were detected for DON producing ability.

The DON formation potential of 11 mold strains with DON formation potential was 6.33 ppm on the average in Czapek-Dox Broth liquid medium, and significant differences were observed in DON formation potential among the strains. King *et al.*[6] also isolated DON forming molds from agricultural products such as barley using the immunoanalytical method. They cultivated them in a solid medium, and reported DON formation potential of 0-100 ppm, which suggested that DON formation potential was different among molds. On the other hand, according to the result of identifying DON forming molds using Nirenberg's[19] simplified method as shown in Fig. 1, after 10 days cultivation in a PDA plate



Aerial mycelium Macroconidia (x400)

Fig. 1. Cultural and morphological characteristics of *Fusarium.* sp.

medium, the colony was morphologically over 7cm in diameter, and at its center was formed a yellow orange conidia mass and on its back was secreted red pigment. When macrospores were formed using the slide cultural method[19] and observed with a microscope, their length was over 50 μm, the number of septa was 3~7, and DON formation potential was reconfirmed through the re-cultivation of the spores. Based on these characteristics, the isolated strain was identified as *Fusarium graminearum*. In previous research, Lee *et al*.[24] identified *Fusarium graminearum* and *Fusarium culmonum* as major species that contaminated home-produced barley. Similarly, this study also found that *Fusarium graminearum* was a major species contaminating home-produced barley.

Effect of pearling on DON content

Table 3 shows changes in the DON content in each composition after pearling DON-contaminated barley sample. DON was contained more in the bran of the contaminated sample than in the kernel, which is similar to reports by other researchers.[14,16,17] The DON content in bran appeared to increase by 71.1% compared to that in barley sample before pearling and the DON content in kernel after pearling appeared to be decreased by 39% compared to that before pearling. With regard to the distribution of DON in bran and kernel after pearling, on the other hand, 56.5% of DON in barley sample before pearling remained in bran after pearling and 40.9% of DON in kernel. This shows that 56.5% of DON can be removed by pearling barley.

Effect of baking on DON content

Baking has been shown to cause little or no effect on DON levels in flour and dough. A 35% reduction in DON levels was observed in cookies and doughnuts baked from flours containing 0.5 ppm DON.[17] In contrast, Niera *et al*.[25] observed a 29% difference in DON levels between fermented doughs and baked products. Little or no reduction in DON concentration was observed when dough containing flour contamination with DON levels of 1-7 ppm was baked into bread.[14] Similarly, EI Banna *et al*.[26] reported no reduction in DON levels when wheat flour (2-3.5 ppm) was baked into Egyptian bread at 350°C, for 2 min. In addition, Tanaka *et al*.[27] reported no reduction in DON concentration when flour contamination with 0.38 ppm DON was baked into a sponge cake at 350°C for 30 minutes.

In this experiment, when DON-contaminated barley flour was mixed with wheat flour in the composition as in Table 1, the DON content in the mixed flour was 1139.7 μg. As shown in Table 4, when bread was made of the mixed flour, the DON content changed to 1017.9 μg after the first fermentation, 1012.4 μg after the second fermentation and 1020.5 μg after baking. Although no significant difference was observed in DON removal efficiency among processes from the first fermentation to baking, the DON contents decreased significantly by around 10.4-10.7% compared to that in the flour. However, the result of this study shows that DON remains in bread and the DON removal efficiency of baking is quite low. This result is similar to reports by EI Banna *et al*.[26] and Tanaka *et al*..[27]

Effect of heat treatments on DON content

Barley foods such as barley tea processed through simple heating are produced widely throughout the Korea, but there have been few researches on the reduction and destruction of the toxicity of harmful molds such as DON by parching heat. Thus, this study was carried out on the destruction of DON at different tem-

Table 3. Distribution of DON after pearling process

Composition	Bran	Kernel
Weight rate (%)	33	67
DON content (ppm)	3.56	1.27
Increase of reduction of DON content (%)	+71.1	-39.0
DON content of the composition (%)	56.5	40.9

[1] DON content of untreated barley was 2.08 ppm.
[2] Data in the table are the average values of triplicate determinations.

Table 4. Variation of DON in baking process

Samples	DON remaining rate[1] (μg)	DON reduction (%)
Flour	1139.7^{a}±23.6	-
1st fermented dough	1017.9^{b}±153.2	10.7
2nd fermented dough	1012.4^{b}±157.5	11.1
Baking	1020.5^{b}±105.2	10.4

[1] Meanstandard deviation of triplicate determinations
[2] Values followed by same letter in the same column are not significantly different ($p<0.05$)

Table 5. Reduction of DON by heat treatments[1)]

Heating temperature (°C)	DON content[2)] (ppm)	Reduction rate (%)
150	2.0[a]0±.1	3.8
200	1.8[b]±0.2	13.5
250	1.4[c]±0.32	32.7

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

peratures of simple heating. According to the result as shown in Table 5, the level of DON was reduced by 3.8% at 150°C, 13.5% at 200°C and 32.7% at 250°C. This shows that the reduction of DON is significantly different according to heating temperature. Charlene *et al.*[28)] reported that DON in decreased by 12% as a result of autoclaving DON-contaminated corn, indicating that DON is relatively resistant to heat. In the present experiment, heating at temperature below 200°C destroyed only 13.5% of DON. This suggests that it is difficult to remove DON completely just by simple heating.

Effect of extrusion process on DON content

In order to prepare a large quantity of sample for extrusion, hulless barley was obtained from the market and the concentration of DON in the mixed sample was measured using HPLC. The initial DON contamination of the sample was 0.32 ppm. Table 6 shows the DON content in extruded barley at different extruder barrel temperatures and screw speeds. DON reduction rates at 110°C, 115°C and 120°C were 53, 56 and 56-59% respectively, showing the tendency of increasing with the rise of temperature, but no significant difference was observed between 110-120°C. At each temperature, the DON reduction rate increased with the rise of screw speed but the difference was not significant. Accerbi *et al.*[12)] reported that 57.1% of DON was removed at 134-170°C and that the teratogenic contamination decreased

Table 6. Reduction of DON by extrusion process[1)]

Temperature (°C)	Extrusion screw speed (rpm)			
	200		300	
	Remaining (ppm)	Reduction (%)	Remaining (ppm)	Reduction (%)
110	0.15[a2]±[)]0.01	53	0.15[a]±0.01	53
115	0.14[a]±0.05	56	0.14[a]±0.05	56
120	0.14[a]±0.04	56	0.13[a]±0.04	59

[1)] DON contents of untreated barley grains are 0.32 ppm.
[2)] Values followed by same letter in the same column are not significantly different ($p<0.05$)

from 7.3 to 0.3 ppm after wheat was treated with 5% SO_2 solution and extruded. This suggests that extrusion is effective in removing DON not only for wheat processing but also for barley processing. In this experiment, the DON removal efficiency of extrusion was 53-59%, which was quite high despite low processing temperature. Thus, extrusion is considered applicable to the removal of DON from contaminated barley.

In processing barley, physical processing methods such as heating may contribute to the destruction or reduction of DON to a certain degree but it was found that the effect is as low as 3.8-32.7% in simple heating and 10.4% in baking. When high pressure is applied together with heating as in extrusion, the DON removal efficiency increases up to 53-59%. Thus, in the future research, it is necessary to optimize DON removal through composite processing including physical and chemical treatments in order to remove DON completely in barley processing.

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국문요약

Deoxynivalenol (DON)에 오염된 보리의 가공과정에서 도정, 단순가열, 제빵 및 압출성형 등 공정에 의한 DON의 감소 및 파괴에 미치는 영향을 시험하였다. 실험에 사용된 보리의 DON 오염도는 2.08 ppm이었으며 보리로부터 분리한 DON 생성균은 *Fusarium graminearum*으로 생성능은 Czapek-Dox Broth 액체배지에서 평균 6.33 ppm이었다.

도정에 의한 DON의 제거 정도는 56.5%였으며 단순가열에 의한 감소는 3.8-32.7%로 나타났다. Extrusion에 의한 제거 정도는 53-59%였으며 제빵과정에서 최종 10.4%의 DON이 제거 된 것으로 나타나 가공 후 상당한 DON이 잔류되어있어 DON의 완전제거 할 수 있는 방법을 새로 개발할 필요가 있다고 사료된다.

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