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Reduction of aflatoxin B_1 contamination in wheat by various cooking treatments

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Abstract

To investigate the effects of various cooking treatments such as washing, heating and steaming on the reduction of aflatoxin toxicity, a simultaneous analytical method for aflatoxin B_1, B_2, G_1, G_2 was established using high performance liquid chromatography (HPLC) with a fluorescence detector. The levels of aflatoxin B_1 (AFB_1) spiked in wheat—three varieties of United States (US) wheat and two varieties of Korean wheat—were analyzed according to washing time and heating temperature. Reduction of AFB_1 toxicity was directly proportional to washing time in both Korean and US wheat. The concentration of AFB_1 was reduced more by heating than washing treatment. The level of AFB_1 in dried wheat was decreased to 50% and 90% by heating at 150 and 200 °C, respectively. However, the reduction of AFB_1 in wet wheat in which water (10%) was intentionally added was higher by heating than in dried wheat. The reduction of AFB_1 was increased by 8% and 23% in 10% water-added US wheat (soft red white wheat) and Korean wheat (Anbaekmil) compared to dried US and Korean wheat, respectively, through heat treatment. Traditional processing used in Korean foods such as Sujebi (a soup with wheat flakes) and steamed bread caused 71% and 43% decrease in aflatoxin B_1 content.

Keywords: Aflatoxin B_1; Toxicity reduction; Cooking; Wheat; High performance liquid chromatography (HPLC)

1. Introduction

Aflatoxins (AFs) are secondary metabolites produced by various fungal species and have the highest toxicity, among mycotoxins. Due to their toxicity including carcinogenic activity, AFs affect not only the health of humans and animals but also the economics of agriculture and food (Hwang, Chun, & Lee, 2004). Recently, contamination of mycotoxins in food including imported ones has received much attention because of the increase in international food trade, due to new trade treaties. Therefore, it is urgently necessary to investigate toxicity-reduction of mycotoxin contamination in raw material as well as foods themselves.

AFs have 17 isomers including B_1, B_2, G_1, G_2, M_1, and M_2. Among them aflatoxin B_1 is considered the strongest hepatocarcinogen agent so far. AFB_1, AFG_1, and AFM_1 have higher carcinogenic activity than AFB_2, AFG_2, and AFM_2, respectively. AFs are produced under optimum temperature and moisture conditions from *Aspergillus* genus contaminated in carbohydrate-rich grains such as peanut, corn, cotton, and wheat (Jaimez et al., 2000). Many countries regulate aflatoxin levels in their foods, to lower than 20 ppb in USA and EU (Europe Union) and 10 ppb in Korea and Japan (Chiavaro et al., 2001).

It is very difficult to remove AFs since they are heat-resistant and soluble in intermediate polar solvents. Many experimental approaches have been attempted to detoxify AFs. So far, detoxification methods are classified as physical, chemical and biological ones. Ammoniation, one of the chemical methods, was reported to detoxify AFs in various raw materials with

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high efficiency (Buser & Abbas, 2002). The level of AFs was reduced to over 40% by roasting and heating peanuts (Rustom, 1997). Buser and Abbas reported that an extrusion process is able to decrease the level of AFs to 33%. As a biological treatment, antagonistic microorganisms were used to reduce toxicity of AFB1 (Cho & Kang, 2000). Studies on the reduction of aflatoxin by treatment with naturally occurring compounds such as antioxidants have been tried world widely. These studies are usually carried out through the elucidation of metabolic pathways in aflatoxin-forming microorganisms (Huwig, Freimund, Kappeli, & Dutler, 2001). It was reported that a mushroom extract inhibited AFB_1-8,9-epoxide formation (Lee, Jeong, Kim, & Choi, 2003).

In present study, the reduction of aflatoxin B_1 contaminated in Korean and US wheat was monitored according to physical treatments usually carried out in home cooking. In addition, the effect of Korean traditional food processes such as Sujebi (a soup with wheat flakes) and steamed bread on the level of aflatoxin in wheat was carried out. This study could be used to provide basic and valuable information about reduction of aflatoxin in wheat.

2. Material and methods

2.1. Materials

Korean wheats (Anbaekmil and Keumkangmil) were kindly donated by Dr. Choon-Ki Lee of Rural Development Administration and US wheat such as HRW (hard red winter wheat), DNS (Dark north spring wheat), SRW (soft red winter wheat) were donated by Ms. Hyo-Sook Kim of Daesun Mills Co. Aflatoxins were purchased from Sigma Co. (USA) and various organic solvents used in HPLC analysis were purchased as HPLC grade.

2.2. Contamination of aflatoxin B_1 to wheat

Wheat (100 g) was aged in various concentrations of aflatoxin B_1 (AFB_1) dissolved in 50 ml distilled water for 24 hours. The contaminated wheat was used for the aflatoxin reduction study.

2.3. Washing and heat treatment

Wheat (20 g) contaminated with various concentrations of AFB_1 was put in 200 ml water and agitated to 155 rpm for 10, 20, 30 min using stirrer. Then, wheat was homogenized and derivatized with TFA (trifluoroacetic acid) for AFB_1 analysis according to the method previously reported (Jaimez et al., 2000). The concentration of AFB_1 in washed water was also analyzed.

For heating wheat, contaminated wheat was heated in an oven at various temperatures for 30, 60 and 90 min. The AFB_1 level of heated wheat was analyzed by HPLC as described above. Wet wheat samples were prepared by soaking wheat in water up to 10% moisture levels. Wet samples were heated at 100 °C for 30 min. The analysis of AFB_1 in wet wheat samples was performed in the same way as for the dried ones.

2.4. Cooking Sujebi (a soup with wheat flakes) and steamed bread

For Sujebi, wheat flakes (30 g) was put into boiling water (500 ml) and cooked for 15 min. For steamed bread, 30 g dough was put in a steamer and heated at 100 °C for 15 min. The foods were dried and AFB_1 in the foods was extracted with 150 ml of methanol.

2.5. Sample preparation for AFB_1 analysis

For extraction of AFB_1 from wheat, wheat samples were milled and extracted with 10 ml of methanol in a sonicator for 10 min. The extract was filtered using Whatmann No. 2 filter paper and analyzed by TFA derivatization method. For TFA derivartization, 2 ml of extract was dried by nitrogen gas at 40 °C in a test tube. Then, 100 μl of TFA was added to the sample and mixed using a vortex for 1 min. After adding 4 ml of acetonitrile, the sample was filtered with a 0.45 μm syringe filter followed by HPLC analysis.

For analysis of AFB_1 in water, AFB_1 was extracted by solid phase extraction (SPE). SPE was activated by adding 5 ml of methanol and distilled water consecutively in an Oasis HLB cartridge and 20 ml of sample was loaded. Then, AFB_1 was eluted by adding 10 ml of distilled water, 10 ml of 5% methanol and 4 ml of methanol consecutively.

2.6. AFB_1 analysis using HPLC

Analysis of aflatoxin was performed using HPLC (model 600, Waters, USA)-fluorescence detector (model 474, Waters, USA). A extracted sample (20 μl) was injected by auto-sampler (Waters, USA) and eluted through a Capcell-Pak C18 UG 80 column (4.6 mm I.D. × 250 mm length, 5 μm particles, Shiseido, Japan). Fluorescence detector was set at wavelength of excitation 474 nm and emission 484 nm. Mobile phase (acetonitrile:water = 25:75 (v/v)) was eluted at a flow rate of 1.0 ml/min with samples. Each peak area was integrated by an integrator (Ds Chrom 2000, Donam Instruments Inc., Korea).

2.7. Statistical analysis

All experiments were independently performed a minimum of three times and data was expressed as

mean ± standard deviation (SD). The statistical significance of the data was analyzed using Student's *t*-test.

3. Results and discussion

Standard curves of AFs (AFB_1, AFB_2, AFG_1, and AFG_2) are shown in Fig. 1. Each AF peak was well separated by the method described in the materials and methods.The retention time of AFB_1 was 8.9 min and mobile phase was 25% acetonitrile. The detector response to AFs was found to be linear over a range of 1–100 μg/l injected, with an R^2 value of 0.9996. The detection and quantification limits for AFB_1 were 0.14 and 0.79 μg/l, respectively. The effect of washing on the reduction of AFB_1 in US and Korean wheat is shown in Tables 1 and 2. All values are averages of three replications (mean ± standard deviation). Initial concentrations of AFB_1 in US and Korean wheat were 45.8 and 43.3 μg/l, respectively. The reduction of AFB_1 in all wheat samples was proportional to washing time. The level of AFB_1 in US wheat samples such as SRW, DNS, and HRW decreased by 62%, 56%, and 58%, respectively, after 30 min washing. About 60% and 56% reductions of AFB_1 were obtained when Korean wheat—Anbaekmil and Keumkangmil—was washed for 30 min, respectively. There was no difference between US and Korean wheat in terms of the reduction degree (%) of AFB_1 by washing. However, the degree did vary according to wheat variety. Due to the low solubility of AFs in water it is generally hard to remove

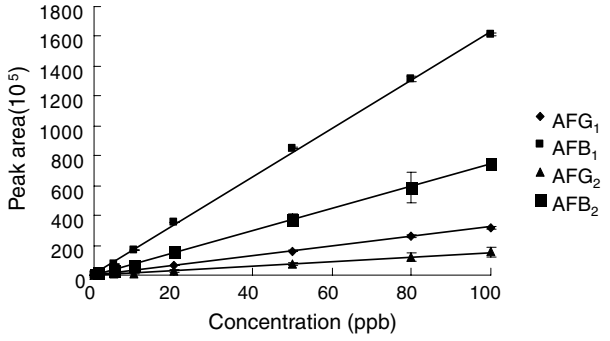

Fig. 1. Standard curves of AFB_1, AFB_2, AFG_1 and AFG_2.

Table 2
Reduction of AFB_1 in Korean wheat (Anbak, Kumkang) by washing

Washing time (min)	Anbak		Kumkang	
	μg/l	Reduction[a] (%)	μg/l	Reduction (%)
10	21.3 ± 0.7	50 ± 1	25.2 ± 2.5	41 ± 6
20	21.2 ± 0.1	50 ± 0.3	19.4 ± 1.6	55 ± 4
30	17.2 ± 2.6	60 ± 6	18.9 ± 1.1	56 ± 3

The values are mean ± standard deviation ($n = 3$).
[a] Initial concentration of AFB1: 43.3 μg/l.

AFs by washing. However, in this study about 40% of AFB_1, usually attached on surface of wheat, could be removed by washing. The reduction degree of 10 and 30 min-washing did not show a significant difference, which means it is very difficult to remove AFB_1 bonded or attached strongly to wheat. Only 20 min washing of SRW showed a significant difference ($P < 0.05$) compared to 10 min washing. This result was in agreement with a previous report (Yeo & Kim, 2002).

To investigate the effect of thermal treatment on the destruction of AFB_1, contaminated wheat samples such as SRW and Anbaekmil were heated in oven at various temperatures (50, 100, 150, 200 °C) and heating periods (30, 60, 90 min). The effect of heating on the level of AFB_1 is shown in Table 3. Dried SRW and Anbaekmil were selected for testing heat treatment since their reduction of AFB_1 was higher than the others by washing. Heating at 100 °C or lower did not lead to any marked reduction in AFB_1 levels, whereas at over 150 °C the reduction of AFB_1 was much higher. Heating SRW at 150 °C caused AFB_1 destruction of about 60%, 75%, and 82% for 30 min, 60 min, and 90 min, respectively. In case of 200 °C heating, over 90% of AFB_1 were destroyed. Compared to heating SRW at 50 °C, heating at 100 °C for 60 min displayed a significant difference of AFB_1 reduction ($P < 0.05$). However, over 150 °C heating in SRW showed a significant difference versus 50 °C heating regardless of heating time ($P < 0.01$ or $P < 0.05$). In Korean wheat, Anbaekmil, the effect of heating was almost similar to that of US wheat, SRW. At over 150 °C, greater decreases of AFB_1 were apparent in Anbaekmil. However, it is impossible to heat foods at over 100 °C to reduce AFs level. An other treatment is necessary to destroy AFs in cooking besides

Table 1
Reduction of AFB_1 in US wheat (S/W, DNS, HRW) by washing

Washing time (min)	SRW		DNS		HRW	
	μg/l	Reduction[a] (%)	μg/l	Reduction (%)	μg/l	Reduction (%)
10	22.8 ± 1.2	50 ± 3	25.0 ± 3.1	45 ± 7	24.4 ± 3.0	46 ± 6
20	18.2 ± 1.5*	60 ± 3	23.6 ± 7.4	48 ± 18	21.9 ± 4.4	52 ± 9
30	17.1 ± 3.0	62 ± 7	20.0 ± 8.9	56 ± 21	18.8 ± 3.0	58 ± 6

The values are mean ± standard deviation ($n = 3$).
[a] Initial concentration of AFB1: 45.8 μg/l.
* $P < 0.05$.
** $P < 0.01$, versus 10 min washing.

Table 3
Effect of heat treatment on reduction of AFB_1 in dried wheat

Temperature (°C)	30 min		60 min		90 min	
	AFB_1 (μg/l)	Reduction (%)	AFB_1 (μg/l)	Reduction (%)	AFB_1 (μg/l)	Reduction (%)
SRW[a]						
50	98.8 ± 15.0	1 ± 15	94.8 ± 10.3	12 ± 10	94.8 ± 15.5	18 ± 15
100	61.6 ± 6.9	38 ± 7	69.2 ± 2.8*	31 ± 3	57.5 ± 4.7*	42 ± 5
150	39.6 ± 13.5**	60 ± 13	25.0 ± 6.2**	75 ± 6	17.6 ± 0.7*	82 ± 1
200	2.2 ± 1.3**	97 ± 1	11.0 ± 2.3**	89 ± 2	7.0 ± 4.9**	93 ± 5
Anbak[b]						
50	51.6 ± 3.6	6 ± 7	52.7 ± 2.0	4 ± 4	49.3 ± 0.4	10 ± 1
100	45.6 ± 3.2	17 ± 6	49.9 ± 1.6*	9 ± 3	42.9 ± 2.8*	22 ± 5
150	25.9 ± 2.3**	53 ± 4	17.8 ± 1.8**	67 ± 3	12.2 ± 2.9**	77 ± 5
200	8.4 ± 0.7**	84 ± 1	3.4 ± 0.3**	93 ± 1	3.7 ± 0.8**	93 ± 1

The values are mean ± standard deviation ($n = 3$).
[a] Initial level of SRW (μg/l): 100.2.
[b] Initial level of Anbak (μg/l): 55.3.
* $P < 0.05$.
** $P < 0.01$, versus 50 °C treatment of each heating period.

heating. Regarding the type of heating used to destroy AFs, it was reported that oven heating was more effective to reduce AFs than other heating types such as microwave (Soliman, 2002; Soliman, El-Faramawy, Zakaria, & Mekkawy, 2001).

To investigate the effect of the moisture content on the reduction of AFB_1 level in wheat, SRW and Anbaekmil (10% moisture content) were heated for 30 min at 100 °C (Table 4). The initial levels of AFB_1 in SRW and Anbaekmil were 28.6 and 14.8 μg/l, respectively. The reduction of AFB_1 in wet SRW and Anbaekmil were 40% and 47%, respectively. Compared with those of dried wheat samples, reductions of AFB_1 in wet SRW and Anbaekmil were higher by 2% and 20%, respectively. Heating wet Anbaekmil showed a significant difference compared to heating dried Anbaekmil under the same conditions ($P < 0.05$).The relationship between moisture content in foods and destruction of AFs has been already reported several times (Mendez-Albores et al., 2002; Torres, Guzman-Ortiz, & Ramirez-Wong, 2001). According to these reports, the increased moisture content enhances the destruction of AFs during cooking or baking. In addition, AFs are stable up to their melting points when heated without moisture (Mann, Codifer, & Dolear, 1967). Samarajewa, Sen, Cohen and Wei reported that moisture is required to hydrolyze the lactone ring of the AFs with heating at home cooking temperature (85–95 °C). So far, for detoxifying AFs it is the most important step to open the lactone ring of the AFs (Buser & Abbas, 2002).

To elucidate the reduction of AFB_1 during real cooking processes, two Korean traditional foods such as Sujebi (soup with wheat flakes) and steamed bread were selected (Table 5). Wheat in Sujebi and steamed bread is heated by hot water and steam, respectively. Cooking Sujebi (wheat flakes) and steamed bread caused 71% and 43% decrease in AFB_1 content, respectively ($P < 0.01$). There was no AFB_1 found in soup of Sujebi. Though the temperature of cooking for Sugebi and steamed bread was about 100 °C, the reduction of AFB_1 was quite higher than in the case when wheat itself was heated. Through this experiment, it was confirmed that moisture is an important factor to reduce AFs by heating. In Sujebi, opening of the lactone ring of AFB_1 takes

Table 5
Reduction of AFB_1 during the cooking process

	AFB_1 level (μg/l)	Reduction[a] (%)
Sujebi flakes	4.9 ± 0.6**	71 ± 3
Sujebi soup	ND[b]	ND
Steamed bread	9.9 ± 0.7**	43 ± 3

The values are mean ± standard deviation ($n = 4$).
[a] Initial level of AFB_1 (μg/l): 17.55.
[b] ND: not detection.
* $P < 0.05$.
** $P < 0.01$, versus control.

Table 4
Effect of heat treatment (100 °C) for 30 min on reduction AFB_1 in wet wheat

Moisture content (%)	SRW			Anbak		
	Initial level (μg/l)	μg/l	Reduction (%)	Initial level (μg/l)	μg/l	Reduction (%)
10	28.6	17.0 ± 3.0	40 ± 10	14.8	7.8 ± 2.35	47 ± 16*

The values are mean ± standard deviation ($n = 4$).
* $P < 0.05$.
** $P < 0.01$, versus the reduction (%) of dry wheat treated at the same condition.

place easily since wheat dough contacts directly with hot water. The reason why reduction of AFB_1 in steamed bread is lower than that of Sujebi flakes is due to low heat transfer in steamed bread. Steamed bread has a lower surface to volume than Sujebi.

4. Conclusion of the study

In present study, the effects of various cooking treatments such as washing, heating and steaming on the reduction of aflatoxin toxicity were carried out. A simultaneous analytical method for aflatoxin B_1, B_2, G_1, G_2 was established using HPLC with a fluorescence detector. Reduction of AFB_1 toxicity was directly proportional to washing time in both Korean and US wheat. The level of AFB_1 was reduced more by heating than washing treatment. The reduction of aflatoxin in wet wheat was higher than in dried wheat by heating. Processing of traditional Korean foods such as Sujebi (a soup with wheat flakes) and steamed bread caused 71% and 43% decrease in aflatoxin B_1 content. This study would provide valuable data for the aflatoxin-reduction research in wheat by cooking.

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