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Reduction of aflatoxins (B_1, B_2, G_1, and G_2) in soybean-based model systems

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ABSTRACT

The effects of chemical, physical, and cooking treatments on the reduction of aflatoxin B_1 (AFB_1), B_2, G_1, and G_2 in soybean matrix were investigated. A HPLC-FLD with a Kobra cell system was used for the quantitative analysis of aflatoxins (AFs). To decrease the level of AFs during the soaking process, the contaminated soybeans were submerged in organic acid solutions. The reduction rates of AFB_1 in 1.0 N citric acid, lactic acid, succinic acid, and tartaric acid for 18 h were 94.1%, 92.7%, 62.0%, and 95.1%, respectively. In the case of pH and autoclave treatment, the level of AFB_1 was significantly decreased during autoclaving process at pH 7.4, 9.0, and 11.1, compared with the non-autoclaved samples ($p < 0.05$). In the case of physical treatment, the heating process at 100 and 150 °C for 90 min significantly decreased the level of AFB_1 by 41.9% and 81.2%, respectively ($p < 0.05$). The reduction rate of AFB_1 after cooking was 97.9% for soybean milk and 33.6% for steamed soybeans.

1. Introduction

Aflatoxins (AFs) are known to be secondary metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (Gourama & Bullerman, 1995). The source of these fungi has been traced to a toxic contaminant in a groundnut meal used in feed (Lancaster, Jenkins, & Philp, 1961). Since, AFs have virulent, carcinogenic, mutagenic, and teratogenic characteristic, they are considered highly harmful substances for both human and animal health (Hall, Wild, Eaton, & Groopman, 1993; Massey, Stewart, Daniels, & Liu, 1995). Among the 20 kinds of AFs, the aflatoxins B_1, B_2, G_1, and G_2 are commonly found in food materials (MFDS, 2010). Aflatoxin B_1 (AFB_1) is the most toxic substance, as classified by the International Agency for Research on Cancer (IARC) under Group 1 as carcinogenic to humans (IARC, 1993). AFB_1 in the liver is activated by cytochrome p450 enzymes and it then converts to AFB_1-8,9-epoxide, which produces a carcinogenic effect by creating peroxidase in kidney (Massey et al., 1995). In Korea the legal limit (MRL: Maximum residue level) of AFB_1 and total AFs are 10 and 15 ppb, respectively (MFDS, 2010).

Due to the toxicity of AFs post-harvest food, various studies for the reduction of AFs have been carried out across the world. Generally, the removal of AFs is very difficult, since they are stable and heat-resistant in dried materials (MFDS, 2010). Many researchers have tried to reduce AFs in foods and their materials through various methods. In general, the detoxification methods of removing AFs from contaminated foods and feeds are classified as physical, chemical, or biological treatments. For physical treatments, Hwang and Lee (2006) reported that washing and heating processes were able to decrease the level of AFs in contaminated wheat by 50% and 90%, respectively. In the case of pressure-cooking in rice, it was able to reduce AFs by 80% (Park & Kim, 2006). In food additives, organic acids were studied for their ability to reduce the level of AFs. Méndez-Albores, Martínez-Bustos, Gaytán-Martínez, and Moreno-Martínez (2008) reported that the level of AFs in maize was reduced by organic acids such as citric acid and lactic acid. In particular, Fan et al. (2013) reported that alkaline-electrolyzed water (AIEW) influences the substantial degradation of AFs, depending on pH, the volume of AIEW, and the oil matrix in edible plant oils. In addition, *Rhodococcus erythropolis* decreased the level of AFs with high efficiency in lipid cultures (Alberts, Engelbrecht, Steyn, Holzapfel, & Van Zyl, 2006). Food irradiation as a technique to bring down the AFs contamination in foods was reported (Herzallah, Alshawabkeh, & Fataftah, 2008). With gamma radiation of 25 KGy, reduction rates of AFs were up to 40%.

The soybean is one of the most common food ingredients in East Asia. Various soybean foods such as soybean milk, *Tofu* (soybean curd), *Kongbab* (rice with soybeans), *Kongjaban* (soybeans cooked in soy sauce) and *Kongguksu* (cold soybean noodles) are made of soybeans. In addition, soybeans are used in fermented foods such as *Doeanjang* (soybean paste) and *Ganjang* (soy sauce). In many

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previous studies, AFs have been detected in raw material and fermented soybean products such as *Doenjang*, *Meju*, and *Miso* (Bae, Kwak, Park, Kim, & Shon, 2003; Kim et al., 2001; Tanaka, Goto, Manabe, & Matsuura, 2002). Most soybean-based food involves the soaking, heating, and steaming of soybeans to reduce the cooking time. The soaking and heating process are necessary to reduce cooking times. The steaming process is essential for fermentation, particularly in fermented soybean foods such as *Doeanjang* and *Ganjang*. Although soybeans are a commonly consumed food, the detoxification methods of AFB_1 in the soybean matrix have not yet been applied to the soaking, heating, and steaming processes.

In this study, the effects of chemical (soaking in various organic acids) and physical (heating with pH or moisture content control) on the reduction of AFB_1, AFB_2, AFG_1, and AFG_2 in soybean matrix were investigated. In addition, to evaluate the effect of cooking methods, soybean milk, soybean curd (which require dehulling and boiling processes) and steamed soybeans (which require steaming and pressure processing) were processed on a laboratory scale.

2. Materials and methods

2.1. Chemicals and reagents

The mixture of aflatoxins B_1, B_2, G_1, and G_2 was bought from Trilogy Analytical Laboratory (Washington, MO, USA), and its standard solution was prepared in methanol (J.T. Baker, Philipsburg, NJ, USA). The solution was stored at −18 °C until use. HPLC-grade water (J.T. Baker, Philipsburg, NJ, USA), acetonitrile (J.T. Baker, Philipsburg, NJ, USA), and methanol were used in HPLC analysis solvents. Analytical reagents such as a sodium chloride, potassium bromide, and nitric acid in aqueous solution were obtained from Samchun Pure Chemicals (Pyeongtaek, Korea). Anhydrous citric acid, succinic acid, tartaric acid, and aqueous DL-lactic acid were purchased from Sigma Aldrich (Tokyo, Japan).

2.2. Validation of the analytical method for AFs

Linearity (R^2), limit of detection (LOD), limit of quantitation (LOQ), and recoveries and precision (RSD, %) tests were conducted for the validation of AFs analysis in the soybean matrix. Linearity and R^2 were calculated by each calibration curve of the AFs as six points (1, 5, 10, 20, 50, and 100 μg/L). LOD and LOQ were estimated by the 3.3 × sigma (σ)/slope factor, and the 10 × sigma (σ)/slope factor of the calibration curve. Sigma was obtained by the standard deviation of the *y*-intercept of seven specific calibration curves, each of which consisted of three points of the AF concentrations (Harris, 2010). Recoveries were obtained by spiking 0.1 mL and 1 mL of the AFs mixture (10 μg/L) in 10 g of soybean matrix with 6 repetitions about each experiment. In case of blank test, AFs-free soybean was analyzed as the blank sample. The precision of the AFs was determined by inter-day (RSD, %) for 3 days and intra-day (RSD, %) for triplicate.

2.3. Sampling

Soybeans (*Glycine max* (L.) Merr.) were purchased from a local market in Seoul, South Korea. All soybean samples were pulverized equably by a grinder (HR-2011, Philips, Netherlands) for 2 min, and then the samples were passed through a 40-mesh sieve to get a fine powder. The soybean samples were stored in clean plastic bags at −18 °C until use.

To prepare the soybeans contaminated by AFs, a solution of AFs mixture (10 μg/L) was prepared for each experiment: 10 mL of the AFs mixture (10 μg/L) was diluted with methanol and then added to each 10 g of ground soybean material. The contaminated soybeans were stored overnight at room temperature (25 °C) to evaporate the solvent. Analysis of the contaminated samples was carried out before treatments for AFs degradation to calculate the initial concentration of AFs in the samples.

2.4. Effect of organic acids during soaking process on the reduction of AFs

Each sample contaminated by AFs was submerged in the same volume of solutions such as distilled water (control), 1.0 N citric acid, 1.0 N lactic acid, 1.0 N succinic acid, and 1.0 N tartaric acid for 6 h and 18 h at room temperature (3.0 mL per g of contaminated soybean). The steep liquor was removed, and then the treated samples were washed with running water three times to neutralize them. The washed samples were then dried at room temperature (25 °C) in a fume hood for 72 h.

2.5. Effect of autoclaving with different pH treatment on the reduction of AFs

To evaluate the effect of pH on the reduction of AFs, the pH of distilled water was adjusted to 5.1, 7.4, 9.0, or 11.1 by 1 M HCl and 1 M NaOH. They were measured by pH-meter (InLab Export Pro, Mettler-Toledo, Greifensee, Switzerland), and then 30 mL of adjusted HPLC water was added to 10 g of contaminated soybeans. Each sample was treated at a different pH level (5.1, 7.4, 9.0, and 11.1) and was autoclaved (AC-60, HYSC, Seoul, Korea) at 121 °C and 110 kPa for 15 min. Non-autoclaved samples were treated under same conditions (pH ranges, temperature and time).

2.6. Effect of heating time and moisture content during the heating process on the reduction of AFs

To investigate the reduction effect of heating time and temperature, soybeans that have been artificially contaminated with AFs were heated in a dry oven (OF-22 GW, Jeio Tech, Seoul, Korea) at different temperatures (50 °C, 100 °C, and 150 °C) for different times (30, 60, and 90 min). To evaluate the effect of moisture content during the heating process on the reduction of AFB_1, the moisture content of the samples from the soaking soybeans were adjusted to 10%, 20%, or 30%. Then the samples were heated at 100 °C for 90 min. The moisture content of the soybeans was calculated by moisture analyzer (MB45, OHAUS, NJ, USA). After the heating process, they were cooled rapidly with ice flakes to room temperature.

2.7. Effect of cooking methods on the reduction of AFs

Ten gram of soybeans contaminated by 10 mL of the AFs mixture (50 μg/L) was prepared for soybean milk and as steamed soybeans according to common Korean recipes (Lee & Hwang, 1994). To prepare the soybean milk, contaminated soybeans were soaked in distilled water for 6 h. They were washed roughly to peel their husks. The peeled soybeans and 5 g of NaCl were put into a pot, and then boiled at 100 °C with 100 mL of distilled water for 15 min. After that, the soybeans were washed with running water and mixed with 200 mL of water by blender (HR 1372, PHILIPS, China). To separate the soybean milk and soybean curd, the blended soybeans were squeezed out by cotton cloth.

To prepare the steamed soybeans, contaminated soybeans were submerged in distilled water for 30 min. The soybeans were cooked in two different ways. One was the steam method at 100 °C for 15 min with a commercial cooking pot (Fissler, Germany) with 100 mL of distilled water. The other cooking method was carried out in a commercial pressure cooker (Fissler, Germany) at 116 °C and 150 kPa for 15 min with 100 mL of distilled water. After

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