Evaluation of reduction of aflatoxins by near infrared spectrometric sorting in the highly aflatoxin-contaminated peanut lots

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Abstract

Peanuts are one of the most susceptible crops to aflatoxigenic fungi, such as Aspergillus flavus and Aspergillus parasiticus, and is often contaminated with aflatoxins (AFs). AF contamination of peanuts is a major human and animal health hazard that has an economic impact worldwide. Numerous control and management strategies have been developed to reduce the levels of AFs in peanuts and sorting is the final stage in the process targeted at decreasing AF levels. Previous studies have shown that handpicking and electric color sorting are not entirely effective in reducing AF levels because of the occurrence of apparently healthy but internally moldy kernels with high AF levels. A previous study (Hirano, et al. 1998) showed that near infrared (NIR), the ratio of transmittance energies at two wavelengths of 700 to 1100, was applicable for efficiently removing internally moldy kernels. However the use of this procedure was limited to a sample that contained artificially inoculated kernels and low levels of AFs. This present study evaluated the performance of spectrometric sorting in naturally and highly AF-contaminated peanut lots on a commercial scale. The NIR spectrometric sorting clearly showed that AFs could be effectively decreased to levels of < 10 µg/kg even when the lot was highly contaminated with > 700 µg/kg of AFs by rejecting deteriorated kernels, including those that were internally moldy.

Introduction

Aflatoxin (AF) is a potent carcinogenic mycotoxin produced by fungi such as Aspergillus flavus (Link) and Aspergillus parasiticus (Speare). Mycotoxin contamination of agricultural products is a serious global problem with human health and economic impacts¹. Over 100 nations have set regulatory limits on allowable AF levels in human foods and animal feeds⁵. The European Union has a maximum level of 2 µg/kg for AFB1 and 4 µg/kg for total AFs, and the Japanese government set regulatory limits of 10 µg/kg for total AFs. Peanut (Arachis hypogaea) is an extremely nutritious crop that is widely grown worldwide and is one of the host crops that is most susceptible to invasion by toxigenic molds and subsequent AF contamination³⁴.

Numerous control and management strategies have been used to reduce the presence of AFs in food and feed chains from the pre-harvest and post-harvest stages, including biocontrol using atoxigenic strains of A. flavus⁵⁶⁷. Studies have found that AF contamination in peanuts is not uniform, and a few highly contaminated kernels are distributed among a large number of uncontaminated kernels⁸⁹. Therefore, sorting is useful in the final stage of processing to reduce the levels of AFs if the contaminated kernels are removed efficiently.

Common sorting methods, including handpicking and electric color sorting, can effectively mitigate the levels of AFs¹⁰¹¹. However, a study reported that these sorting types, which are principally dependent on appearance (such as color sorting and handpicking), are effective but not complete, and the removal efficiency is highly variable¹¹. Studies have also demonstrated that a few apparently sound and mature kernels contain high levels of AFs⁶. In addition, a positive relationship was observed between AF content and the presence of kernels with a specific “lumen” or a hollow space observed inside when the kernels were examined internally⁶. These results indicate
that sorting methods that depend on appearance and applied reflected light are often unable to completely eliminate contaminated kernels because apparently healthy but internally moldy kernels with AF-producing fungi are often present in AF-contaminated lots.

Spectrometric methods have been developed as rapid and non-destructive tools to identify AF-contaminated kernels and detect AFs in certain crops\textsuperscript{12,13,14}. A previous study\textsuperscript{15} showed that the ratios of transmittance energies at 700 –1100 nm, near infrared (NIR), were different between normal and moldy kernels even when the mold was internal. NIR spectrometry detected changes caused by fungal digestion of cell components such as triglycerides and carbohydrates, whereas it did not detect AFs present inside contaminated kernels. Furthermore, the study also demonstrated that the spectrometric principle had been adapted to the preliminary apparatus and could remove kernels artificially inoculated with \textit{A. flavus} from the toxigenic peanut lots that contained 1.6 and 4.5 µg/kg of AF.

In this study, we evaluated the performance of a developed Q-sorter in separating naturally AF-contaminated peanut lots. The results showed that NIR spectrometric sorting effectively decreased AFs to levels < 10 µg/kg even when the sample was highly contaminated. This observation indicated that removing the peanut kernels with \textit{A. flavus}-derived mold effectively reducing the levels of AFs even when the sample was highly contaminated.

\section*{Materials and Methods}

\textbf{Peanut Sample} Two samples of Chinese large shelled nuts were examined in the separation experiment. One sample from the 2014 crop year, which was used after size screening and handpicking, contained AFs at an average of 212.2 (range, 181–270.1) µg/kg and was designated as sample C. The other sample from the 2018 crop year, which was termed sample B, had been stored for nearly 1 year after color sorting and was extremely contaminated with AFs at an average of 706.6 (range, 257.4–1146.2) µg/kg.

\textbf{Sorter and sorting} The two types of sorters used were manufactured by Anzai Manufacturing Co., Ltd., Chiba, Japan. One, which was the chute type MK-06, had a higher performance processing capacity than the MK-06 and is illustrated in \textit{Supplementary Fig. 1}. The other sorter was the Leo-300 MK belt carrier system, which had a higher performance processing capacity than the MK-06 and is illustrated in \textit{Supplementary Fig. 2}. The chute type sorter was used to sort 278 kg of sample C, which contained an average of 706.6 µg/kg of AFs, three times at a removal ratio (no. of rejected kernels/no. of tested kernels) of 4.2%, 3.9%, and 1.1%. Sorting was conducted at a processing speed of 600 kg/h and the sorting flow of sample C is shown in \textit{Fig. 1}. The belt type sorter was used to separate 470 kg of sample B containing 706.6 µg/kg of AFs three times with removal ratios of 8.3, 3.8, and 16.4%.

\textbf{AF analysis} For the sorted samples, AFs in the original unsorted and accepted subsamples after sorting were determined according to the official method for AF analysis recommended by the Ministry of Health, Labor, and Welfare of Japan\textsuperscript{15}. Briefly, 5 kg of the sample was prepared for the analysis after mixing four 5 kg subsamples, for a total of 20 kg. Furthermore, 5 kg of the sample rejected after sorting was determined when the weight of the sample exceeded 5.0 kg and the entire sample was analyzed when it was < 5.0 kg. After milling the subsample using a Dickens Mill (mesh 2 mm)\textsuperscript{16} it was mixed using the SKH-40 (Misugi Ltd., Osaka, Japan) for 30 min. Then, 50 g of the powdered peanut sample was extracted with 200 mL acetonitrile-water (9:1, v/v) by blending the mixture for 5 min at a speed of 7,000–10,000 rpm. Five milliliters of the filtrate was loaded onto the multifunctional MultiSep™ #228 column (Romer Labs. Inc., USA) and passed at a flow rate of 1.0 mL/min. Then, 2 mL of the eluate was dried under a gentle nitrogen stream at 40°C. Trifluoroacetic acid (0.1 mL) was mixed with the residue, allowed to stand for 15 min in the dark, and then 0.9 mL acetonitrile-water (9:1, v/v) was added to the mixture, which was shaken vigorously.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Flow diagram of the sorting in the highly aflatoxin-contaminated Chinese shelled nuts by the NIR spectrometric sorter (Chute type, MK-06).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Photographs of the internally moldy kernels after opening to two halves of cotyledons. The kernels were taken as the rejected category sorted by the high performance NIR spectrometric sorter (Belt type, Leo-300 MK) in the extremely highly aflatoxin-contaminated sample (Table 5). Left: kernel No. 7: Mycelial growth (→) and almost entirely discolored to brown and contained 6.0 µg/kg of aflatoxins in the kernel. Right: kernel No. 4: Mycelial growth (→) and partly discolored to brown but aflatoxin was not detected.}
\end{figure}
Then, the AF content of the sample was determined using high-performance liquid chromatography (HPLC) using a Hitachi-2000 Elite system (Hitachi High-Technologies Corporation, Tokyo, Japan), which was conducted under the following conditions: column, Atlantis-T3 (Waters, USA); column temperature, 40°C; mobile phase, acetonitrile-methanol-water (1:3:6, v/v/v); flow rate, 0.4 mL/min; detection wavelength, 365 and 450 nm for excitation and emission, respectively; and injection volume, 20 μL. The limits of detection and quantification for the method were 0.2 and 0.5 μg/kg, respectively.

For individual kernels, 10 mL 80% ethanol was added to one peanut kernel except for those from the apparently healthy group and the mixture was coarsely ground, followed by homogenization using a polytron (PT10-35GT, Kinematica, Switzerland) for 3 min at 800 rpm. Then, 1 mL of the filtrate was determined for the AF contents according to the Japanese Official Methods as previously mentioned. Water was added to the kernels collected from the apparently healthy group at a 5:4 ratio (w/w) and AFs in the filtrate were determined as described above after finely grinding the mixture. In the spike and recovery test, a sample of each of the four AFs was added to the filtrate prepared from non-toxigenic kernels to obtain a final concentration of 5 ng/mL. More than 90% of the added AFs were recovered.

**Visualization of peanut kernel interior and group classifications**  Peanut kernels from the accepted or rejected categories collected after sorting were manually split into the two halves of the cotyledon to investigate the interior of the kernel. The kernels were visually observed using a microscope at low magnification and then they were classified into groups depending on their appearance, including the presence or absence of fungal growth, discoloration, or both.

**Detection of aflatoxigenic fungi and AF production by isolate**  Approximately 25 g of the peanut kernels was homogenized with 225 mL sterile 0.1% peptone water for 1 min using a stomacher and then 10 mL of the homogenate was diluted with 90 mL sterile water. Then, 0.5 mL of the diluted sample was spread on two Dichloran Rose-Bengal Chloramphenicol (DRBC) agar plates and they were incubated at 25°C for 7 days.

Colonies that developed morphologically similar to A. flavus on the plate were counted as colony forming units (CFU, no. colonies per gram of sample). Some colonies were transferred to a potato dextrose agar (PDA) slant culture for isolation and the isolates were identified macroscopically and microscopically. The colonies were then cultured in 15 mL 15% sucrose yeast extract sucrose (YES) liquid culture and the culture filtrate of the isolates was assayed for AF production using thin-layer chromatography, as previously reported

### Results

#### Sorting

**Chute Type Sorter**  Sample C (278 kg) was partitioned into the accepted category (CA-1) consisting of 266.8 kg (yield: 95.8%) and the rejected category (CR-1), which was 11.7 kg (removal ratio, 4.2%). The initial average AF level (212.2 μg/kg) decreased to 45.1 (range, 15.1-103.9) μg/kg after the sorting. After the first sorting process the AF levels decreased by 78.7%. In contrast, 3737.3 μg/kg of AF was detected in the CR-1 group. Similarly, 246.3 kg of the CA-1 sample was divided into the CA-2 and CR-2 samples, which were 236.7 kg (yield: 96.1%) and 9.6 kg (removal ratio: 3.9%), respectively. The average AF content of the CA-1 sample was reduced to 12.9 (range, from 4.8-24.9) μg/kg after the second sorting. The decrease in AF levels after sorting was 71.4%. Then, 46 kg of the CA-2 sample was separated into 45.5 kg of the CA-3 (yield: 98.9%) and 0.5 kg of the CR-3 (removal ratio: 1.1%) sub-samples. The AF content of the CA-2 sample decreased to 4 μg/kg and the overall decrease in AF level calculated from the initial and final (4.0 μg/kg, CA-3). AF concentrations obtained for the three sorting processes was 98.1%. The cumulative overall percentage of the rejected category was 8.9%. These results clearly showed that the AF content could be reduced to < 10 μg/kg and a removal of approximately 10% was achieved with the three NIR spectrometric sorting processes, even though the AF level of the sample was > 200 μg/kg.

**Belt Type Sorter**  The shelled kernels of sample B (470 kg), which contained extremely high levels of AFs were examined three times (Table 2) using a process similar to the previously described method (Table 1). The starting material was separated into samples consisting of 431 kg of the accepted category (BA-1, yield: 91.7%) and 39 kg of the rejected category (BR-1, removal ratio: 3.9%). Furthermore, the initial average concentration of total AFs was decreased to 319.8 μg/kg after the first sorting. The AF level was decreased by 54.7% and then, 400 kg of the BA-1 sample was separated into 385 kg of the CA-3 (yield: 96.3%) and 15 kg of BR-2 (removal ratio: 3.8%) by subsequent sorting. The average AF content of the accepted category decreased to 182.1 μg/kg, whereas the decrease following the second sorting was 43.1%, which was slightly lower than that of the first sorting. A 297 kg sample of BA-2 was separated into 251 kg of BA-3 (yield: 84.5%) and 46 kg (removal ratio: 15.5%) of BR-3. The decrease in AF following the last sorting increased to 97.4%, and the overall decrease following the three sorting processes was 99.3%. The overall removal ratio of the rejected category calculated from the accumulation was 26.3%. These results showed that when < 30% of the deteriorated peanut kernels were included in the rejected category, the contamination of 70% of the residual kernels could be mitigated to < 10 μg/kg using high-performance spectrometric sorting. This result was also achieved even when the sample was extremely contaminated with an AF content > 706 μg/kg.
Table 1 Reduction of AFs in the highly AF-contaminated sample (C) by the NIR spectrometric sorter (Chute type, MK-06).

| Sorting | Sample examined | Segregated category after sorting | Removed category |
|---------|-----------------|-----------------------------------|-----------------|
|         | AFs conc. (µg/kg) | Accepted category | AFs conc. (µg/kg) | AF decrement (%) | Weight (kg) Ratio (%) | AFs conc. (µg/kg, avg.) |
|         | Range<sup>a</sup> | Avg.<sup>c</sup> | Weight (kg) Yield (%) | Range | Avg. | CR - 1 | (4.2) |
| 1st     | 278.5           | 181 – 270.1          | CA-1 266.8 (95.8) | 15.1 – 103.9 | 45.1 | 78.7 | 11.7 |
|         | 212.2           |                       |                   |               |               |               | 3737.3 |
| 2nd     | 246.3           | 15.1 – 103.9         | CA-2 236.7 (96.1) | 4.8 – 24.9 | 12.9 | 71.4 | 9.6 |
|         | 45.1            |                       |                   |               |               |               | 982.2 |
| 3rd     | 46              | 4.8 – 24.9           | CA-3 45.5 (98.9) | 2.2 – 5.6 | 4 | 69.0 | 0.5 |
|         | 12.9            |                       |                   |               |               |               | 134.6 |

<sup>a</sup> AFs concentration. <sup>b</sup>N=4. <sup>c</sup>In average. <sup>d</sup>AF decrement = (1-accepted category of AFs conc. /AFs conc. before the sorting) × 100.

Table 2 Reduction of AFs in the extremely highly AF-contaminated sample (B) the high performance NIR spectrometric sorter (Belt type, Leo-300 MK).

| Sorting | Sample examined | Segregated category after sorting | Removed category |
|---------|-----------------|-----------------------------------|-----------------|
|         | AFs conc. (µg/kg) | Accepted category | AFs conc. (µg/kg) | AF decrement (%) | Weight (kg) Ratio (%) | AFs conc. (µg/kg, avg.) |
|         | Range<sup>a</sup> | Avg.<sup>c</sup> | Category | Weight (kg) Yield (%) | Range | Avg. | BR - 1 | (8.3) |
| 1st     | 470             | 257.4 – 1146.2      | BA - 1 431 (91.7) | 24.0 – 700.3 | 319.8 | 54.7 | 39.0 |
|         | 706.6           |                       |                   |               |               |               | 6478.8 |
| 2nd     | 400             | 24.0 – 700.3        | BA - 2 385 (96.3) | 11.4 – 285.3 | 182.1 | 43.1 | 15.0 |
|         | 319.8           |                       |                   |               |               |               | 3031.2 |
| 3rd     | 297             | 11.4 – 285.3        | BA - 3 251 (84.5) | 1.2 – 7.8 | 4.7 | 97.4 | 46.0 |
|         | 182.1           |                       |                   |               |               |               | 70.8 |

<sup>a</sup>N=4.

Visual inspection of interior of split kernels

**Kernel using chute type sorting** Peanut kernels randomly collected from each of the rejected or accepted categories CR-1, CA-2, and CR-2 were investigated visually after they were split open (Table 3). The kernels were classified into the following five groups depending on their appearance: 1) frequently discolored moldy kernels, 2) discolored kernels without visible mold growth, 3) insect-damaged kernels, 4) kernels with darkened plumule, and 5) apparently healthy kernels (Table 3).

In group 1, mycelial growth was frequently observed and a few kernels exhibited abundant sporulation. Fungal species other than *Aspergillus* section *Flavi* (formerly *Aspergillus flavus* group), such as *Eurotium* and *Penicillium*, were occasionally found in the kernels. Moldy kernels are commonly discolored brown. After the first sorting, among the groups in the CR-1 category, the moldy group had the highest weight percentage (8.8%) except for that of the apparently healthy group (80.7%). After the second sorting, the moldy group constituted 0.8% of the CA-2 category, whereas it was 5.6% of the CR-2 category, suggesting that moldy kernels were eliminated from the rejected category.

Similarly, discolored kernels were found in the rejected group of the CR-1 (3.4%) and CR-2 (3.6%) categories after the first and subsequent second sorting. The percentage of kernels with darkened plumules was decreased by sorting them multiple times. The percentage of insect-damaged kernels was almost constant with each sorting examination.

**Kernel using beelt type sorter** The peanut kernels (300, 294.9 g) were sampled randomly from the rejected category (BR-3) after the third sorting of the extremely AF-contaminated sample (Table 2). They were inspected and classified as described above (Table 4). The percentage of the internally moldy group was the highest at 3.7% (11 kernels), followed by that of the discolored group without visible mold (3.0%). The insect-damaged kernels exhibited commonly low or non-detectable levels of AF, which was similar to what was observed with the previous chute sorting process (Table 3). Images of the opened kernels showed abundant mycelial growth and brown discoloration in the cavity between the cotyledons of kernel No. 7, whereas kernel No. 4 showed little mycelial growth and partial discoloration (Fig. 2).
Table 3  Visual inspection and classification of inside of the kernels to the groups after opening in each of the category after NIR spectrometric sorting by MK-06 (Chute type) in highly AF-contaminated sample.

| Category after sorting | Weight of kernels inspected (g) | Classified group | Moldy | Discolored | Insect-damaged | Dark plumule | Apparently healthy |
|------------------------|--------------------------------|-----------------|-------|-----------|---------------|--------------|-------------------|
|                        |                                 | Weight (g) (%)  | Weight (g) (%) | Weight (g) (%) | Weight (g) (%) | Weight (g) (%) |
| CR-1                   | 2971.0                          | 262.4 8.8      | 101.5 3.4     | 3.1 0.1          | 206.2 6.9       | 2397.8 80.7       |
| CA-2                   | 2924.2                          | 24.8 0.8     | 44.1 1.5      | 2.9 0.1          | 28.1 1.0         | 2824.3 96.6       |
| CR-2                   | 2947.4                          | 165.4 5.6     | 106.6 3.6     | 0 0              | 187.2 6.4        | 2488.2 84.4       |
|                        | N. D.                            | N. D.         | N. D.         | N. D.            | N. D.            | N. D.            |

**See Table 1. Sample kernels were randomly taken from each of the category. **

Table 4  Visual observation and classification of inside of the kernels to the groups after opening in 300 kernels of the removed category (BR-3) after by NIR spectrometric sorting (Leo-300MK, belt type) in extremely high AF-contaminated sample.

| Category tested* | No. of kernels inspected (Weight) | No. of kernels in the classified group after observation (Weight % of kernels) |
|------------------|----------------------------------|--------------------------------------------------------------------------------|
|                  |                                  | Moldy  | Discolored  | Insect damaged | Dark plumule  | Apparently healthy |
| BR - 3           | 300 (294.9 g)                   | 11    | 3          | 0            | 22           | 258              |

**See Table 2. Sample kernels were randomly taken from the removed category (BR-3).**

AF contents of individual kernels in rejected category  The AF contents of 300 peanut kernels in the BR-3 sample (Table 4) were assayed after visual inspection. Furthermore, the 11, 3, and 22 moldy, discolored, and dark plumule kernels were also determined individually. As shown in Table 5, AFs were only found in kernels of the moldy group (7/11), especially in those with abundant mycelial growth and obvious discoloration. Kernels Nos. 7 and 4 (Fig.2) contained 6.0 µg/g and non-detectable amounts of AF, respectively, whereas the levels were undetectable in the other groups of kernels examined, including those in the dark plumule and apparently healthy groups (data not shown). These results demonstrate that spectrometric sorting separated AF-contaminated kernels effectively into the rejected categories and reduced AF to levels < 10 µg/kg, even in highly contaminated batches.

Detection of aflatoxicogenic fungi and AF production by isolate  Fungal units of section Flavi (CFU) in the original and accepted samples after sorting (CA-1 and CA-2, Table 1) were counted. The result showed that 5.0 \times 10^4 CFU were detected in the original sample before sorting and this value decreased to 1.8 \times 10^4 and 1.5 \times 10^4 CFU after the first and second sorting processes, respectively (Table 6). Forty strains each were isolated from the original samples, CA-1, and CA-2, for a total of 120 strains. The morphological examination identified all strains as A. flavus and A. parasiticus was not found when the G group of AFs was detected in the sample examined. Regarding AF production, only the B group of AFs was found in the strains tested in this study. The positive ratio of AF-producing strains to total strains examined decreased from 47.5% to 27.5% in CA-1 and was 15.0% in CA-2.

AFs concentration in individual peanut kernel of the moldy group after the visual inspection in the rejected category sorted by the NIR spectrometry*

| Kernel No. | AFs concentration (µg/kg) | Total in kernel (µg/kg) | Degree of discoloration in the kernel* |
|------------|---------------------------|------------------------|---------------------------------------|
| 1          | N. D.*                    | N. D.                  | ++                                    |
| 2          | N. D.                     | N. D.                  | -                                     |
| 3          | N. D.                     | N. D.                  | -                                     |
| 4          | N. D.                     | N. D.                  | ++                                    |
| 5          | 74.3                      | 326.4                  | +++                                   |
| 6          | 310.1                     | 99.5                   | ++                                    |
| 7          | 495.3                     | 6.0                    | +++                                  |
| 8          | 18.1                      | 0.2                    | +                                     |
| 9          | 0.4                       | N. D.                  | ++                                    |
| 10         | 0.2                       | N. D.                  | +++                                   |
| 11         | 0.2                       | N. D.                  | -                                     |

**See Table 4. *Not detected. **Degree of discoloration was symbolized as; -, scarcely or none; +, a little; ++, partly; +++; almost entirely.

Table 6  Fungal counts of section Flavi*a in the peanut samples and aflatoxin production of the isolates from the sample before and after the sorting by the NIR spectrometry.

| Sample*  | CFU* and positive ratio* of AF producing strain among the strains examined |
|----------|--------------------------------------------------------------------------|
| Original (before sorting) | 5.0 \times 10^4 | 19/40 (47.5) |
| CA-1     | 1.8 \times 10^4 | 11/40 (27.5) |
| CA-2     | 1.5 \times 10^4 | 6/40 (15.0) |

**Formerly Aspergillus flavus group.  *See Table 1.  *Colonies Forming Unit. **No. of AF-producing strains / No. of strains examined, (%).
Discussion

Our study clearly showed that the chute (MK-06) and belt (Leo-300MK) NIR sorters eliminated > 90% of the AF content in the original sample. Approximately 10%–30% of the peanuts were separated into the rejected category in three rounds of sorting, even though the samples were naturally and highly AF-contaminated, as shown in Table 1 and 2. The decrease in AF content changed considerably with the removal ratio, and selecting suitable operating conditions was the key to achieving an adequate reduction in AF contamination. Information on the product batch such as AF content and records of the sample before and after harvest is very important for determining the condition.

Visual inspection of the kernels after splitting (Table 3 and 4) indicated that the sorting process was likely to also eliminate discolored kernels along with moldy kernels to the rejected category. The additional weight percent of the moldy group to the discolored group reached more than 12% in CR-1. They also showed that internally moldy kernels, which were commonly discolored, were eliminated to the rejected category after sorting. Discoloration of the peanut kernel might be caused by fungal digestion of cell components, especially lipids, which are higher in peanuts and comprise nearly 50% of the kernel (USDA National Nutrient database, 2018).

The main components in the lipids are triglycerides, which are stored as small droplets called oil bodies. The oil bodies are distributed throughout the whole seed, which is surrounded by ultrathin (2–3.5 nm) biological membranes[18,19]. Therefore, the oil bodies were likely to be decomposed by phospholipase secreted by the invading fungal mycelium. The enzymes were reported to be highly potent in Aspergillus spp., especially A. flavus and related species[20]. The oil bodies are also likely to be broken by physical damage, including insect bites, which would enable the fungi to easily invade.

The released fatty acids exuded out of the cells of the peanut tissue, which resulted in the brown discoloration of the kernels. A previous report[17] demonstrated a linear relationship between the transmittance ratio (700/1100 nm) and the degree of triglyceride hydrolysis. Therefore, moldy kernels are likely to be placed in the rejected category by the NIR spectrometric sorting. Moreover, kernels in the discolored group without visible fungal growth might have been invaded by fungal mycelia and the lipids were likely decomposed; however, the kernels were probably not inspected for fungal growth.

The AF assay of the individual kernels in the last rejected category of the highly contaminated batch showed that AF–contaminated kernels were concentrated in the internally moldy group but not in any other groups in this study (Table 5). Numerous studies have shown that kernels that are discolored, insect–damaged, and shriveled are also commonly AF-contaminated[21,24]. In this study, the apparently discolored kernels with high levels of AFs might have been eliminated to the rejected group before the last sorting because both the severely deteriorated and insect-damaged kernels were likely efficiently eliminated by the preceding sorting depending on their appearance. Then, the severely deteriorated kernels that contained AFs might remain only in the groups of internally molded kernels before the last sorting in this study. These results of the individual examinations showed that the spectrometric sorting separated the internally moldy kernels, and there was a relationship between their removal and the reduction of AF levels of the accepted category.

These results also prove that eliminating AF-contaminated kernels based on strategies using their appearance, including electric color sorting, is useful. However, this strategy provides incomplete results because of the potential presence of internally moldy kernels with AF–contamination, as reported previously[12]. These results suggest that AF contamination from toxicogenic fungi might recur if the storage conditions are changed to those that support fungal growth. The combination of size screening, handpicking, and electric color sorting is recommended to efficiently reduce AFs before spectrometric sorting. Multiple sorting processes are also useful, especially for reducing AF levels of highly contaminated samples. Finally, NIR spectrometric sorting could contribute to mitigating health risk and economic impact associated with the global peanut trade. Furthermore, this strategy could enhance the efficiency of crop utilization through the reduction of AF contamination levels.

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Supplementary Fig. 1  Mechanical principles of chute type sorter: MK-06
Peanut kernel is feeding through 6 channels of chutes from a hopper. Light beam is generated from halogen lamp and irradiated to the dropping kernel. Transmitted light was separated by a dichroic mirror and bandpass filter. The ratio of the transmittance energies at two wave lengths of 700 to 1100 nm, are captured by a sensor and then amplified. The signals of judgement, acceptance or rejection, was transmitted to the air-ejector. (https://anzai-mfg.com/en/publics/indecs/56/)
Supplementary Fig. 2  Sorting system of belt type sorter: Leo-300MK
Peanut kernels are feeding on the belt of 300 mm in width. The light beam is generated from two LED lights depending on the wavelengths of visible (700 nm) and NIR (1100 nm). After the transmittance, the ratio of the energies at two wave lengths can be divided into two ranges, visible light and near infrared light, by a dichroic mirror as the previous type, and then captured by a CCD (700 nm) and an InGaAs cameras (1100 nm) depending on the wavelength. Judgement is transmitted to the ejector. (https://anzai-mfg.com/en/pages/42/#block)