Manual Sorting To Eliminate Aflatoxin from Peanuts

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ABSTRACT

A manual sorting procedure was developed to eliminate aflatoxin contamination from peanuts. The efficiency of the sorting process in eliminating aflatoxin-contaminated kernels from lots of raw peanuts was verified. The blanching of 20 kg of peanuts at 140°C for 25 min in preheated roasters facilitated the manual sorting of aflatoxin-contaminated kernels after deskinning. The manual sorting of raw materials with initially high aflatoxin contents (300 ppb) resulted in aflatoxin-free peanuts (i.e., peanuts in which no aflatoxin was detected). Verification procedures showed that the sorted sound peanuts contained no aflatoxin or contained low levels (<15 ppb) of aflatoxin. The results obtained confirmed that the sorting process was effective in separating contaminated peanuts whether or not contamination was extensive. At the commercial level, when roasters were not preheated, the dry blanching of 50 kg of peanuts for 45 to 55 min facilitated the proper deskinning and subsequent manual sorting of aflatoxin-contaminated peanut kernels from sound kernels.

The peanut (Arachis hypogaea L.) is a popular food because of its pleasing aroma and flavor and its dry crunchy texture (16). The peanut’s high protein and energy contents make it a suitable ingredient in other food products. Peanuts are a major component of several food products manufactured in the Philippines and throughout the world. Molds grow on peanuts and may lead to the production of mycotoxins, specifically aflatoxins, produced by particular strains of Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius. In the United States and the United Kingdom, maximum levels of aflatoxin in peanuts have been set by regulatory agencies at 20 ppb (8) and 4 ppb (9), respectively.

Studies to investigate methods for the elimination of aflatoxin from contaminated peanuts and other agricultural commodities are continuously being conducted. Heat treatment is not a useful method for the reduction of aflatoxin because the temperature required to destroy aflatoxin is ca. 270°C (3). Chemical degradation of aflatoxins may be brought about by the addition of chlorinating agents (sodium hypochlorite, gaseous chlorine), oxidizing agents (hydrogen peroxide, ozone, sodium bisulfite), or hydrolytic agents (acids, alkalis, ammonia). Some bacteria, yeasts, molds, actinomycetes, and algae are known to remove aflatoxin from or degrade aflatoxin in foods and feeds (12). The ability of Flavobacterium auranticum to remove aflatoxin B1 from milk, corn oil, peanut butter, corn, soybeans, peanuts (5), and peanut milk (10) was demonstrated.

At present, electronic color sorting and handpicking are widely used to separate aflatoxin-contaminated kernels from sound kernels. Electronic color sorting, however, is only 72% efficient (7), while handpicking, although more selective, is deemed impractical in the United States. Density-based separation schemes are theoretically feasible, but the loss of peanuts associated with such methods is high (9) and the efficiency of separation achieved with them is highly variable. A water flotation method based on the observation that contaminated kernels are usually less dense than sound kernels has been patented (11). This procedure has not gained wide commercial acceptance because of a requirement for an additional drying step after the flotation treatment.

A major problem for the peanut industry in the Philippines is aflatoxin contamination of raw kernels due to difficulty in controlling the temperature and relative humidity during storage. High levels of aflatoxin in peanut products exported from the Philippines have resulted in detention problems at the ports of entry of countries to which these products have been shipped. There is no other cost-effective method that peanut product manufacturers in the Philippines can use to reduce aflatoxin levels in peanuts except for the manual sorting of raw peanuts to separate kernels that are not fit for processing.

This study was undertaken to develop a technology for the manual sorting of peanut kernels to eliminate aflatoxin contamination. Specific objectives were (i) to determine methods for the manual sorting of peanuts, (ii) to evaluate and verify the effects of manual sorting at laboratory- and pilot-scale levels, and (iii) to scale up the manual sorting technology to commercial applications.

MATERIALS AND METHODS

Development of the sorting process. The sorting process was developed in the laboratory. A prototype roaster (Kosuge Takkosho, Japan) was used to test the applicability of the blanching procedure suggested by Woodroof (16). The roaster was preheated to 140°C. Five kilograms of shelled peanuts (large-seed variety,
Vietnam) was weighed and placed in a roaster to dry blanch at 140°C for 25 min. This step was carried out in two replications. After dry blanching, the peanuts were removed from the roaster, cooled with an electric fan (14 in.; Standard Electric Co., Manila, Philippines), and sorted manually by visually examining the kernels and separating discolored and damaged kernels. Two hundred grams of representative samples of both dry-blanched unsorted peanuts and sound sorted peanuts and all kernels sorted out as discolored and damaged were analyzed for aflatoxin by AOAC International (2) official method 49.2.08, 968.22-CB. The aflatoxin value for the total weight (5 kg) of raw peanuts was derived by the following formula: total aflatoxin value (ppb) = (aflatoxin content [ppb] × weight of discolored and damaged peanuts [g])/weight of starting raw peanuts (g).

Aflatoxin analysis. Aflatoxin analyses were conducted by thin-layer chromatography with the use of AOAC International (2) official method 49.2.08, 968.22-CB with a detection limit of 1 ppb. The sample was coarse ground and mixed. Approximately 10 g of the ground peanuts was reground to a finer consistency and reduced to paste with a hammer mill (Cemotec Sample Mill 1090, Hamburg, Germany). Fifty grams of the prepared sample was weighed into a 500-ml Erlenmeyer flask fitted with a glass stopper, along with 25 ml of water, 25 g of diatomaceous earth (Fisher Scientific, Fair Lawn, N.J.), and 250 ml of chloroform (Merck, Darmstadt, Germany). The mixture was secured in the flask with its stopper and placed on a wrist action shaker (Model 75, Burell Corporation, Pittsburg, Pa.) for 30 min. The first 50-ml portion of the chloroform filtrate was collected and passed through a silica gel (Merck, Darmstadt, Germany) cleanup column (22 by 300 mm). The eluate was evaporated nearly to dryness in a steam bath and transferred to a vial with chloroform, and the chloroform was evaporated with a stream of nitrogen (Merck, Darmstadt, Germany). The extract was dissolved in 200 μl of benzene/acetonitrile (98:2, vol/vol) solution. Aflatoxin standards (Sigma Chemical Co., St. Louis, Mo.) were dissolved in benzene/acetonitrile solution to a concentration of 8 to 10 μg/ml. Preliminary tests were conducted to estimate the amount of aflatoxin in the sample extract by determining the amount of the sample extract that would produce approximately the same fluorescence intensity as aflatoxin standards of known concentrations.

Portions of sample eluate (one 2-μl portion, one 5-μl portion, and two 10-μl portions, corresponding to 0.1, 0.25, and 0.5 g of sample, respectively) were spotted on silica gel-coated glass plates (20 by 20 cm). On the same plates, the same amounts (2, 5, and 10 μl) of aflatoxin standards were spotted. Five microliters of standard was spotted on top of one of the two 10-μl sample spots as an internal standard. At least one 5-μl resolution reference standard (a combination of all aflatoxin standards with the same concentration of 8 to 10 μg/ml) was spotted to determine whether adequate resolution was attained. The plates were developed in 50 ml of acetone/chloroform (1:9, vol/vol) for 40 min at 23 to 25°C or until aflatoxins reached an Rf (the ratio of the distance traveled by the sample spot and the mobile phase [acetone/chloroform solution]) of 0.4 to 0.7 within 90 min. The plates were removed from the tank, the solvent was evaporated at room temperature, and the plates were illuminated from below by a long-wave 15-W UV lamp in a dark room. Each sample spot was compared with the aflatoxin standards simultaneously spotted on the plates.

Samples that needed to be concentrated in order to be quantified were evaporated to dryness in a steam bath and redissolved in a calculated amount of benzene/acetonitrile solution to provide the needed concentration. These extracts were spotted on silica gel plates with aflatoxin standards and developed as described above. In samples, fluorescent spots thought to represent aflatoxins were found to have Rf values identical to those for aflatoxin standard spots and colors similar to those of aflatoxin standard spots when the unidentified spots and the internal standards were superimposed.

The fluorescence intensities of aflatoxin B1 spots in samples were compared with those of standard spots and matched with the intensities of the aflatoxin standards used. Aflatoxin B1, G1, and G2 spots were compared by the same procedure. The concentration of aflatoxin B1 was calculated as micrograms of aflatoxin/kilograms of sample = (S × Y × V)/(X × W), where S is the amount (μl) of the aflatoxin B1 standard required to match the unknown spot, Y is the concentration of the aflatoxin B1 standard (μg/ml), V is the amount (μl) of the final dilution of sample extract, X is the amount (μl) of the spotted sample extract required to match the fluorescence intensity of S (the B1 standard), and W is the weight (g) of the sample applied to the column (10 g if 50 ml chloroform extract is used). The same equation was used to determine the amounts of other aflatoxins, such as B2, G1, and G2. The reported value represents the total of all aflatoxins found in the sample.

Pilot-scale trials. Twenty kilograms of peanuts (large-seed variety, Vietnam) was weighed, dry blanched in a preheated roaster at 140°C for 25 min, and sorted by the sorting process developed previously. Two trials were conducted. Representative samples (1,000 g each) of dry-blanched unsorted peanuts, sound sorted peanuts, and all kernels sorted out from raw materials as discolored and damaged were analyzed for aflatoxin. The aflatoxin content of the starting raw material (20 kg) was computed by the equation used above.

Verification of the efficiency of the sorting process. Eleven 20-kg samples of peanuts of the same variety (large-seed, Vietnam) were obtained from various commercial sources to represent the wide range of aflatoxin levels found in peanuts on the market. Peanut samples were weighed, fed into a preheated roaster, dry blanched at 140°C for 25 min, and sorted by the process developed here. Two trials were conducted. Representative samples (1,000 g each) of dry-blanched unsorted peanuts, sound sorted peanuts, and kernels sorted out from raw materials as discolored and damaged were analyzed for aflatoxin. The aflatoxin content of the starting raw material (20 kg) was calculated.

Commercial-scale trials. Three trials were conducted at a commercial food-manufacturing plant with the use of the procedure employed by the company during normal manufacturing operations, which involved the use of the roaster’s flame setting with no preheating of the roaster. The roasted used (fabricated, Marigold Commodities Corporation, Philippines) had no temperature control devices. Fifty kilograms of raw peanuts was dry blanched at 140°C for 45 min, which facilitated the removal of peanut skins with a deskinner (fabricated, Marigold Commodities Corporation). Skins removed from the peanuts were blown off with electric fans (14 in.; Standard Electric Co.). The manual sorting of aflatoxin-contaminated kernels was carried out, and samples were analyzed as described above. The aflatoxin content of the 50 kg of raw material was derived by the equation given above.

Two successive trials were conducted to determine the level of ease with which peanuts could be deskinned after blanching when peanuts from different sources were used. Blanching time was extended to 55 min when there was difficulty in deskinning peanuts blanched for 45 min owing to differences in the maturity levels of peanut kernels.
RESULTS AND DISCUSSION

Development of the sorting process. The flow diagram of the process developed for sorting is presented in Figure 1. Dry blanching of raw peanuts resulted in increased visibility of damaged peanuts, which appeared more moldy, shriveled, and discolored than sound blanched peanuts.

The manual sorting of 5 kg of raw peanuts resulted in an average yield of 4.36 kg (87.2%) of dry-blanched peanuts (Table 1). Skins, peanut kernels not recovered from the roaster, and waste during the transfer of peanuts from blanching to sorting accounted for the 12.8% loss. Of the dry-blanched unsorted peanuts, on average, 98.7% were sorted as sound kernels and 1.3% were sorted as discolored and damaged peanuts.

The results of aflatoxin content analyses of samples collected during the development of the sorting process are shown in Table 1. Although aflatoxin was not detected in either the dry-blanched unsorted peanuts or the sound sorted peanuts, the aflatoxin levels of the discolored and damaged kernels ranged from 95 to 114 ppb, which is equivalent to 1.25 ppb in starting raw materials. The calculated aflatoxin content of raw materials (1.25 ppb) was low compared with the 15-ppb limit set by Philippine regulatory agencies. This value is also low compared with those found by the Food and Agricultural Organization for aflatoxin contents of market samples of peanuts in other Asian countries, such as Indonesia (7 to 8,000 ppb), Malaysia (>16 ppb), and Thailand (20 to 30 ppb) (8). These results imply

FIGURE 1. Flow diagram for the development of the manual sorting procedure for raw peanuts and the sources of the samples used for aflatoxin analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Trial</th>
<th>Total wt of peanut samples (kg)</th>
<th>Wt of samples submitted for analysis (g)</th>
<th>Aflatoxin content of sample (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of the sorting process (5 kg of starting raw material)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry-blanched unsorted peanuts</td>
<td>1</td>
<td>4.34</td>
<td>200.00</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.37</td>
<td>200.00</td>
<td>ND</td>
</tr>
<tr>
<td>Dry-blanched, discolored, and damaged sorted peanuts</td>
<td>1</td>
<td>0.04</td>
<td>44.10</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.07</td>
<td>72.98</td>
<td>114</td>
</tr>
<tr>
<td>Dry-blanched, sound sorted peanuts</td>
<td>1</td>
<td>4.30</td>
<td>200.00</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.30</td>
<td>200.00</td>
<td>ND</td>
</tr>
<tr>
<td>Pilot-scale trials (20 kg of starting raw material)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry-blanched unsorted peanuts</td>
<td>1</td>
<td>18.18</td>
<td>1,000</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17.80</td>
<td>1,000</td>
<td>300</td>
</tr>
<tr>
<td>Dry-blanched, discolored, and damaged sorted peanuts</td>
<td>1</td>
<td>0.28</td>
<td>280</td>
<td>16,000</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.50</td>
<td>500</td>
<td>611</td>
</tr>
<tr>
<td>Dry-blanched, sound sorted peanuts</td>
<td>1</td>
<td>17.90</td>
<td>500</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17.30</td>
<td>500</td>
<td>ND</td>
</tr>
<tr>
<td>Commercial-scale trials (50 kg of starting raw material)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry-blanched (45–55 min) unsorted peanuts</td>
<td>1</td>
<td>47.10</td>
<td>1,000</td>
<td>ND</td>
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<tr>
<td></td>
<td>2</td>
<td>46.90</td>
<td>1,000</td>
<td>ND</td>
</tr>
<tr>
<td>Dry-blanched, discolored, and damaged sorted peanuts</td>
<td>1</td>
<td>1.10</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.90</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>Dry-blanched, sound sorted peanuts</td>
<td>1</td>
<td>46.00</td>
<td>500</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>46.00</td>
<td>500</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND, none detected.*
that although the analysis failed to detect aflatoxin in the unsorted peanuts, this starting raw material was in fact contaminated with 1.25 ppb of aflatoxin. This finding indicates that if raw peanuts were not sorted, the end product would be contaminated with aflatoxin. This discrepancy in the aflatoxin values for the peanut samples may be accounted for by differences in testing procedures (methods of sampling, subsampling, and analysis), especially when large quantities of raw materials are tested (14, 15). Moreover, according to Whitaker and Wiser (15), sampling errors are large because although aflatoxin is found in only a small percentage (<0.5%) of the kernels in a lot, the concentration in a single kernel may be high. In a study of “wrinkled” peanuts by Cucullu et al. (6), aflatoxin contents varied not only in individual kernels but also in different parts of individual kernels, such as the skin, the heart, and the surface. In the study by Cucullu et al. (6), aflatoxin values for individual kernels range from no detectable aflatoxin to 19,800 ppb. Furthermore, different parts of the individual kernels contained $1 \times 10^4$ to $4 \times 10^6$ ppb of aflatoxin. The values found by Cucullu et al. (6) were much higher than the aflatoxin contents (95 to 114 ppb) of the samples collected during the development of the sorting process in this study. When a few kernels containing large amounts of aflatoxin are not sorted out, they will likely be homogeneously distributed when they are used in any product that requires a grinding step during processing, such as peanut butter, pastes, and spreads.

Pilot-scale trials. The manual sorting of 20 kg of raw peanuts resulted in a mean weight of 18 kg (90%) of dry-blanched peanuts (Table 1). Skins, peanut kernels not recovered from the roaster, and waste during the transfer of peanuts from blanching to sorting accounted for the 10% loss. Of the dry-blanched unsorted peanuts, on average, 97.8% were sorted as sound kernels and 2.2% were sorted as discolored and damaged peanuts.

The results of aflatoxin analysis of samples obtained during the pilot-scale trials are shown in Table 1. Dry-blanched unsorted peanuts were found to contain 300 ppb of aflatoxin, which is a high level compared with the maximum regulatory limit of 15 ppb in the Philippines. After sorting, the separated discolored and damaged peanuts contained high levels of aflatoxin (611 to 16,000 ppb, equivalent to 15 to 224 ppb of aflatoxin in the starting raw materials). The remaining sound sorted peanuts tested negative for aflatoxin. These results demonstrate the necessity for the implementation of an effective sorting process for raw peanuts. Raw peanuts, even those lots testing negative for aflatoxin, need to be sorted prior to their use in food products. Otherwise, damaged kernels would remain in the lots, and the final product could be contaminated with aflatoxin.

**Verification of the efficiency of the sorting process.**

The manual sorting of 20 kg of raw peanuts resulted in an average yield of 17.2 kg (86%) of dry-blanched peanuts (Table 2). Skins, peanut kernels not recovered from the roaster, and waste during the transfer of peanuts from blanching to sorting accounted for the 14% loss. Of the dry-blanched unsorted peanuts, on average, 3.6% were sorted as discolored and damaged kernels and 96.4% were sorted as sound peanuts.

For the 11 peanut lots, aflatoxin was detected only in two of the dry-blanched unsorted peanut samples (Table 2). The analysis failed to detect any aflatoxin in the nine remaining samples. However, the aflatoxin contents of the discolored and damaged kernels separated from the same lots of dry-blanched peanuts ranged from 9 to 2791 ppb. These values are equivalent to 0.045 to 104.70 ppb of aflatoxin in the 20 kg of starting raw material, demonstrating
the difficulty in sampling for aflatoxin analysis. One of 11 sorted batches was not devoid of aflatoxin but contained a level of 5 ppb after sorting had been carried out. However, this level was low compared with the regulatory limit of 15 ppb set by Philippines regulatory agencies. In a study by Cucullu et al. (6), it was shown that peanut kernels appearing to be sound may contain aflatoxin at levels ranging from $3 \times 10^2$ to $1.1 \times 10^6$ ppb.

The results of the verification trials demonstrate the importance of sorting peanuts whether or not the raw material shows an aflatoxin content. The results of the verification tests confirm that raw-peanut lots testing negative for aflatoxin are likely to contain kernels contaminated with aflatoxin. This likelihood was demonstrated by aflatoxin values of 9 to 2,791 ppb that were obtained for discolored and damaged kernels sorted from the same lots of raw peanuts. These results also indicate that the sorting process was effective in separating the aflatoxin-contaminated kernels from raw materials with high (104.7 ppb) and low (0.045 ppb) aflatoxin contents.

**Commercial-scale trials.** The manual sorting of 50 kg of raw peanuts resulted in an average yield of 47 kg (94%) of dry-blanced peanuts (Table 1). Skins, peanut kernels not recovered from the roaster, and waste during the transfer of peanuts from blanching to sorting accounted for the 6% loss. Of the dry-blanced unsorted peanuts, on average, 97.9% were sorted as sound peanut kernels and 2.1% were sorted as discolored and damaged peanuts.

Table 1 shows the results of the aflatoxin analysis carried out for samples collected during the commercial-scale trials at a manufacturing plant. Although samples of dry-blanced unsorted peanuts and sound sorted peanuts did not exhibit aflatoxin, the mean aflatoxin value of 200 ppb for the discolored and damaged peanuts indicates that the starting raw material was contaminated with aflatoxin at a level of 2 ppb. The separation of contaminated kernels with the use of a commercial procedure involving light roasting, deskinning, and electronic color sorting for 30 lots of peanuts (200 kg per lot) was also observed in a study by Chiu et al. (4). The procedure resulted in no aflatoxin in sorted sound peanuts, as did the manual sorting procedure described in the present study. However, of the 30 lots of discolored and damaged sorted peanuts Chiu et al. (4) collected, only 17 contained aflatoxin (at a mean level of 402 ppb), while 13 did not exhibit aflatoxin contamination. The electronic color sorting process resulted in false positives and the potential for reduced yields. In the present study, with the use of manual sorting at the commercial level, all kernels separated as discolored and damaged were contaminated with aflatoxin, as demonstrated in the two trials. Further trials are warranted to demonstrate the efficiency of the sorting process at the commercial level.

Aflatoxin contamination (at 2 ppb) of raw peanuts used in the commercial-scale trials of this study was not detected in the analysis except when sorting was carried out. This finding demonstrates a potential problem for peanut-manufacturing industries in the Philippines. Most peanut product manufacturers in the Philippines do not have access to electronic technology available in the United States to sort raw materials properly. The sampling procedures used for raw peanuts provide peanut processors with a false sense of security with regard to the aflatoxin contents of their products. Ali et al. (1) reported that most of the peanut products commercially sold in Malaysia and the Philippines were contaminated with aflatoxin. In the Philippines, roasted shelled peanuts and fried peanuts were found to have aflatoxin contents of 177 and 375 ppb, respectively. In Malaysia, 11 of 17 peanut product samples were contaminated with aflatoxin at a mean concentration of 50 ppb. This value is high compared with the aflatoxin values obtained for the raw peanuts used in this study and the maximum regulatory limits of 15 and 35 ppb in the Philippines and Malaysia, respectively.

Results obtained in the laboratory- and pilot-scale tests and in the verification trials show that the manual sorting of raw peanuts was efficient in separating contaminated kernels whether or not contamination in the raw materials was extensive. Thus, all peanuts should be sorted whether or not they test negative for aflatoxin in order to ensure that aflatoxin-contaminated kernels are removed. The implementation of the sorting procedure is critical for companies that use peanuts that have not been sorted with an electronic sorter, and this procedure should be used as a final step after peanuts have been electronically sorted. The results obtained in the present study provide support for the use of this sorting procedure by peanut product manufacturers and companies that use peanuts as an ingredient, especially where environmental conditions enhance mold growth. If this sorting procedure is implemented by the food industry, it will protect consumers from potential public health threats posed by aflatoxin.

**ACKNOWLEDGMENTS**

The authors acknowledge the Peanut–Collaborative Research Support Program (P-CRSP) of the U.S. Agency for International Development (USAID) for providing research funds through grant no. LAG-G-00-96-00013-00 and Mr. Kim Lapuz of Marigold Commodities for serving as the industry collaborator on this project.

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