Color Sorting of Lightly Roasted and Deskinned Peanut Kernels To Diminish Aflatoxin Contamination in Commercial Lots

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Moistened peanut kernels were inoculated with conidia of *Aspergillus parasiticus*, and moistened but uninoculated kernels were incubated at ambient temperature for 35 days. Glucose, free fatty acid, free threonine, proline, glycine, alanine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine contents increased in both types of peanuts, whereas sucrose, free glutamic acid, aspartic acid, valine, and serine contents decreased. When the incubated kernels were roasted at 160 °C for 15 min, discoloration of infected, manually deskinned kernels was detected by color sorting. The average aflatoxin contents in the discolored sublots of kernels inoculated with *A. parasiticus* and uninoculated lots were 18 200 and 12.5 ppb, respectively. When this process was applied to a commercial line, the aflatoxin content in the discolored sublots of 30 200-kg lots ranged from undetected to 976 ppb. Aflatoxin was not detected in kernels of unblemished sublots.

Keywords: *Peanut; aflatoxin; sorting; light roasting*

INTRODUCTION

Peanut kernels are a good substrate for mold growth when the moisture content, temperature, and time permit. The growth of aflatoxigenic molds and subsequent aflatoxin production in peanut attract a great deal of public concern. In many cases, mold growth results in discoloration of kernels. Removal of the discolored kernels in lots of raw and unblanched peanut is the most common means to diminish aflatoxin contamination in peanut products. Dickens and Whitaker (1975) reported that an average 72% of the aflatoxin in peanuts was removed by electronic sorting and subsequent hand picking of the discolored kernels. However, the efficacy of aflatoxin removal with electronic sorting was highly variable among peanut kernel lots.

Since mold growth on peanut kernels does not always result in significant discoloration, unblemished kernels may be contaminated with aflatoxin at a low level. Mold mycelium and spores on kernel surfaces may be removed by routine handling, rendering kernels acceptable by color sorting. Wilson and Flowers (1978) stated that a low level of aflatoxin contamination in peanuts may be endemic and current sorting procedures may not be effective in removing unblemished and contaminated peanuts.

In addition to color sorting of peanuts by hand or by an electronic sorting machine, other sorting systems have been developed. Pelletier and Reizner (1992) reported that fluorescence sorting of raw and unblanched peanut kernels was not as effective as an aflatoxin control method. Although electronic color sorting has been shown to be efficient in removing aflatoxin-contaminated peanuts from sound peanuts, hand sorting is more effective. Flotation separation and density characterization of aflatoxin-contaminated and sound peanuts have been extensively investigated (Henderson et al., 1989; Gnanasekharan et al., 1992). Separation of aflatoxin-contaminated kernels from sound kernels by hydrogen peroxide treatment has also been attempted (Clavero et al., 1993). However, when sorting was conducted with unblanched raw peanuts, complete removal of the aflatoxin-contaminated kernels was not achieved.

Growth of molds on peanuts causes a reduction of dry matter and oil content, an increase in free fatty acids, and deterioration of seed quality and nutritive value (Diener, 1973). Hydrolysis of the macromolecules, e.g., proteins (Cherry et al., 1975, 1978), lipids (Pattee and Sessoms, 1967), and polysaccharides, occurs during mold infection, resulting in the release of free amino acids, free fatty acids, and simple sugars. These breakdown products contribute to color development in peanut kernels during roasting. More rapid color formation is likely to occur in infected kernels compared to that in sound kernels. Color sorting is comparatively more efficient for deskinned kernels than for unblanched kernels, regardless of the degree of infection.

In this study, moistened peanut kernels inoculated with *Aspergillus parasiticus* were incubated at ambient temperature to promote mold growth. Infected peanuts were roasted for various times followed by analysis for color. Mold populations and compositional changes in infected peanut kernels were determined. Light roasting and deskinning of peanut kernels were done on a commercial processing line prior to sorting. Aflatoxin content in unblemished and discolored sublots of kernels was determined.

MATERIALS AND METHODS

Peanuts. Freshly harvested, sun-dried, shelled, and hand-sorted peanut kernels (Tainan No. 9, a Spanish cultivar, 7.5% moisture, dry basis) were used for mold inoculation studies. Prior to use, kernels were packaged in polypropylene bags and stored at −25 °C. Sealed bags of kernels were removed from the freezer and tempered at ambient temperature (25−30 °C) overnight before opening. A collaborative study was conducted in a peanut-processing company using commercial lots of

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Table 1. Free Fatty Acid Content, Conjugated Diene Hydroperoxide Content, and Fatty Acid Composition of Peanut Oils and Mold Populations in Peanut Kernels after Mold Infection

item	treatments and determinations[a]			
	control I[b]	control II[c]	natural mold infected	*A. parasiticus* infected
FFA, mg/g of oil	0.42 ± 0.01	0.62 ± 0.01	36.47 ± 5.82	56.79 ± 23.63
CDHP, OD_{234nm}/mg of oil	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.13 ± 0.01
fatty acid, %				
14:0	0.03 ± 0	0.03 ± 0	0.03 ± 0	0.03 ± 0
16:0	12.80 ± 0.04	12.75 ± 0.03	12.84 ± 0.01	12.97 ± 0.02
18:0	3.58 ± 0.06	3.57 ± 0.01	3.62 ± 0	3.58 ± 0.01
18:1	38.51 ± 0.05	38.23 ± 0.02	38.11 ± 0.39	38.17 ± 0.32
18:2	38.40 ± 0.01	38.57 ± 0.05	38.87 ± 0.17	38.79 ± 0.22
20:0	1.60 ± 0	1.60 ± 0.01	1.56 ± 1.73	1.55 ± 0.03
20:1	0.81 ± 0	0.83 ± 0	0.83 ± 0.01	0.83 ± 0.01
22:0	3.03 ± 0.01	3.10 ± 0.01	2.93 ± 0	2.90 ± 0
24:0	1.14 ± 0.03	1.14 ± 0.02	1.07 ± 0.02	1.06 ± 0.01
mold count, CFU/g of peanut	$<10^2$	$<10^2$	6.8×10^5	2.3×10^6

[a] Mean of values with standard deviation. [b] Control I, raw kernels stored at −25 °C. [c] Control II, nonmoistened kernels stored at ambient temperature.

peanuts destined for honey-roast processing. Commercially obtained kernels were size graded, electronically sorted, and hand picked prior to roasting and deskinning.

***A. parasiticus* and Natural Mold Infection of Peanuts and Determination of Total Mold Count.** For each experiment, 200 g of peanut kernels in polypropylene bags was evenly sprayed with 15 g of water to increase the moisture content to approximately 15% (dry basis). Twenty-five kernels were gently rolled on the surface of a 7-day-old culture of *A. parasiticus* NRRL 2999 grown on yeast−malt agar, added to each 200-g quantity of peanut kernels, and thoroughly mixed. The procedure for inoculation resulted in 9.8×10^4 conidia/g of peanut kernels as determined by plating appropriately diluted suspensions of washed kernels on oxytetracycline glucose yeast extract (OGYE) agar (Mossel et al., 1970) and incubating for 3 days at ambient temperature. Suspensions were prepared by combining 45 mL of phosphate buffer (pH 7.0, 0.05 M, containing 0.01% Tween 20) with inoculated kernels, vigorously shaking for 2 min, and subjecting to subsequent dilutions.

To promote the growth of molds naturally present, peanut kernels were moistened but not inoculated with *A. parasiticus*. Bags containing inoculated or uninoculated peanuts were punctured with a pin to facilitate air exchange and incubated at ambient temperature (25−30 °C) for 35 days. After incubation, total mold populations on kernels were determined as described before. The remaining peanuts were heated at 45 °C in an oven for 48 h to reduce moisture content to an original level. These peanuts were used in the following experiments.

Compositional Analyses of Mold-Infected Peanuts. Inoculated and naturally infected peanut kernels were freeze-dried and deskinned. Kernels were then hydraulically pressed to prepare oils and partially defatted press cake. Free fatty acid (FFA) contents, conjugated diene hydroperoxide (CDHP) contents, and fatty acid composition of the oils were determined (Yoon et al., 1985; Chiou et al., 1993). Partially defatted flour was prepared by grinding the press cake in a cyclone mill and defatting with *n*-hexane (−20 °C). Sucrose, glucose, and free amino acid contents were determined, and electrophoretic patterns of proteins were analyzed by SDS−PAGE (Chiou et al., 1991).

Color Determination of Peanuts during Roasting. Mold-infected and sound (uninfected) peanut kernels were deskinned, placed on a hot aluminum block, and roasted in an oven at 160 °C. The block was removed from the oven at 5-min intervals for the purpose of photographing the kernels. Less than 15 s was required for each photograph. The color of the deskinned peanuts in photographs, expressed as *L*, *a*, and *b* values, was measured with a color difference meter (Nippon Denshoku Ltd., Osaka, Japan). At each roasting time, color differences between *A. parasiticus* infected and sound peanuts and between natural mold infected and sound peanuts were determined by subtracting *L*, *a*, and *b* values measured for each treatment.

Peanut Roasting and Color Sorting. Preliminary results indicated that mold-infected peanut kernels discolored more rapidly than did uninfected kernels during roasting at 160 °C for 15 min. *A. parasiticus* infected and natural mold infected peanut kernels, as well as raw kernels, were roasted in an oven at 160 °C for 15 min, manually deskinned, hand sorted, and divided into unblemished (accepted) and discolored (rejected) sublots. On a commercial scale, 200-kg batches of peanut kernels were roasted at 160 °C for 15 min in a roller roaster. After roasting, peanuts were deskinned and hand sorted. Weight percentages of unblemished and discolored kernels in sublots were determined, and samples from sublots were subjected to aflatoxin analysis.

Aflatoxin Analysis. Peanut kernels were ground into meal with a cyclone mill. The procedure reported by Tarter et al. (1984) was followed for aflatoxin extraction with minor modification. Meal (10 g) was mixed with 40 mL of methanol and 10 mL of 0.1 N HCl, homogenized for 1 min with a homogenizer (Polytron PT Mr-3000, Kinematica AG, Littau, Switzerland), and then filtered using Advantec No. 2 filter paper (Toyo, Japan). Twenty milliliters of the filtrate was mixed with 20 mL of hexane and 20 mL of NaCl (10%) in a 250-mL flask with a silicone stopper and shaken at 250 rpm with an orbital shaker for 10 min. Then, 25 mL of the aqueous solution was mixed with 5 mL dichloromethane and shaken as described above. After the dichloromethane was withdrawn, the remaining solution was extracted twice with 5 mL of dichloromethane. The pooled dichloromethane extract solution was placed in a desiccator and evaporated to dryness with an aspirator. Methanol (0.5 mL) was added to dissolve extracted substances; 5 μL of the solution was applied to a TLC plate (DC-Alufolien Kieselgel 60F254, E. Merck, Darmstadt, Germany), which was then developed with chloroform−acetone (9/1 v/v). The quantity of aflatoxins separated on TLC plates was determined using a fluorescence densitometer (Scanner II, Camag, Switzerland) and an aflatoxin standard containing known quantities of aflatoxins B_1, B_2, G_1, and G_2 spread on the TLC plate concurrently.

Statistics. At least duplicate experiments were conducted. Mean values with standard deviations are presented.

RESULTS AND DISCUSSION

Free fatty acid contents, conjugated diene hydroperoxide (CDHP) contents, and fatty acid composition of peanut oils prepared from mold-infected peanut kernels are shown in Table 1. The free fatty acid content in infected peanuts was significantly higher than that of the control peanuts. This is in agreement with the observations described by Pattee and Sessoms (1967) and Diener (1973). Lipolysis of peanut oils resulted from lypase action of molds. However, extensive lipid

Table 2. Sucrose, Glucose, and Free Amino Acid Contents in Peanut Kernels after Mold Infection

item	treatments and determinations[a]			
	control I[b]	control II[c]	natural mold infected	*A. parasiticus* infected
sucrose, mg/g of defatted meal	85.40 ± 0.43	85.41 ± 0.25	63.79 ± 1.35	37.04 ± 7.96
glucose, mg/g of deffated meal	1.56 ± 0.02	1.62 ± 0.09	2.23 ± 0.02	1.72 ± 0.27
free amino acid, mg/g of protein				
Asp	0.20 ± 0	0.22 ± 0.01	0.11 ± 0	0.12 ± 0
Thr			0.17 ± 0.01	0.19 ± 0.01
Ser	0.54 ± 0.01	0.45 ± 0.02	0.45 ± 0.02	0.38 ± 0.02
Glu	2.69 ± 0.05	2.68 ± 0.09	0.75 ± 0.01	0.64 ± 0
Pro	0.23 ± 0.01	0.24 ± 0.02	0.32 ± 0.01	0.31 ± 0
Gly	0.11 ± 0	0.11 ± 0.01	0.34 ± 0.01	0.41 ± 0.01
Ala	0.80 ± 0.01	0.74 ± 0.03	0.93 ± 0.30	1.22 ± 0.06
Val	0.16 ± 0	0.26 ± 0.01	0.21 ± 0.02	0.25 ± 0
Met			0.05 ± 0.02	
Ile	0.11 ± 0	0.11 ± 0.02	0.28 ± 0.05	0.11 ± 0.01
Leu	0.06 ± 0	0.06 ± 0.01	0.21 ± 0.05	0.14 ± 0.01
Tyr	0.15 ± 0	0.13 ± 0	0.59 ± 0.18	0.36 ± 0.01
Phe	0.76 ± 0.06	0.78 ± 0.09	0.92 ± 0.11	0.95 ± 0.05
His	0.11 ± 0.01	0.08 ± 0	0.10 ± 0.01	0.22 ± 0
Lys	0.02 ± 0	0.03 ± 0	0.11 ± 0.02	0.12 ± 0.01
Arg	0.13 ± 0	0.13 ± 0	0.21 ± 0.01	0.29 ± 0.06
total	6.07	6.02	5.75	5.71

[a] Mean of values with standard deviation. [b] Control I, raw kernels stored at −25 °C. [c] Control II, nonmoistened kernels stored at ambient temperature.

peroxidation of oil did not occur. The CDHP content in mold-infected peanuts was not significantly higher than that in control peanuts. This was further supported by the fact that change in fatty acid composition resulting from mold infection was not significant (Table 1). In other words, the increase in free fatty acid content in oils of mold-infected peanuts did not necessarily influence the overall fatty acid composition.

The highest mold population was observed in peanuts infected with *A. parasiticus*, followed by populations in naturally infected peanuts. Mold populations were very low in peanuts that were not moistened, being similar to populations in peanuts stored frozen during the incubated period.

Sucrose, glucose, and free amino acid contents in infected peanut kernels are presented in Table 2. The difference between two control peanuts was limited. The minor difference might be mainly due to physiological change that occurred during storage at ambient temperature on the basis of the fact that no obvious mold growth was observed for unmoistened peanuts stored concurrently (control II) (Table 1). The sucrose content was much lower in infected peanuts than in control peanuts, while the glucose content was significantly higher in infected kernels. The total free amino acid content was highest in control peanuts, followed by peanuts infected by natural mold contaminants and *A. parasiticus*. In a comparison of mold-infected peanuts and control II peanuts, because most of the glutamic acid in peanuts disappeared after mold infection, contents of most amino acids except glutamic acid, aspartic acid, serine, and valine increased. Increases in threonine, proline, glycine, alanine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine contents in the infected peanuts were pronounced. Increased amounts of some amino acids undoubtedly affect color and flavor formation during roasting (Newell et al., 1967).

Analysis of peanut proteins extracted from mold-infected and control peanuts using SDS–PAGE (Figure 1) revealed that there was no extensive qualitative alternation in patterns. Quantitatively, slightly lower amounts of proteins with molecular weights higher than 43 000 were detected in mold-infected peanuts compared with control peanuts.

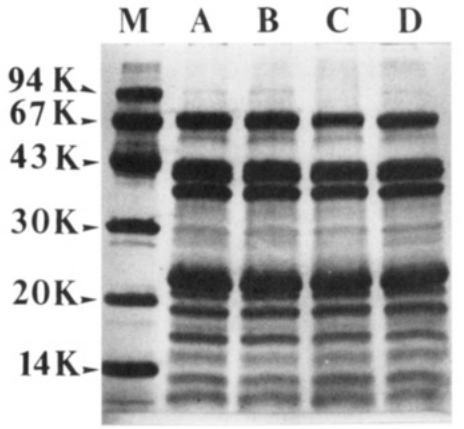

Figure 1. SDS–PAGE patterns of proteins extracted from peanut kernels before and after mold infection; M, protein marker; A, control I; B, control II; C, natural mold infected; D, *A. parasiticus* infected.

Color changes occurred in deskinned kernels during roasting at 160 °C for 30 min, as evidenced by differences of *L*, *a*, and *b* values between *A. parasitcus* infected and sound kernels and between natural mold infected and sound kernels (Figure 2). After 15 min of roasting, infected kernels underwent considerably more changes in color than did the control (sound) kernels. Rapid discoloration of the mold-infected kernels facilitates sorting by hand or machine. Visually, the extent of color difference between infected and control kernels decreased after 15 min of roasting. Therefore, 15 min of roasting was designated an appropriate time to enable differentiation of infected and sound kernels.

Since peanut roasting characteristics are affected by their initial moisture content (Chiou et al., 1991), moistened and mold-infected peanut kernels were dried at 45 °C to their original moisture contents prior to roasting at 160 °C for 15 min. After roasting, peanuts were manually deskinned, sorted into sound (unblemished) and discolored sublots, and subjected to weight percentage determination and aflatoxin analysis (Table 3). Weight percentages of the sound sublots in *A. parasiticus* and natural mold infected lots were 7.7 and 38.3%, respectively. Aflatoxin was not detected in

Table 3. Weight Percentages and Aflatoxin Content in Sound and Discolored Sublots of Peanuts Sorted from *A. parasiticus* and Natural Mold Infected Lots and from Commercial Lots of Peanut Kernels after Light Roasting and Deskinning

			aflatoxin,[a] ppb				
sample lot	sublot	wt %	B_1	B_2	G_1	G_2	total
control I[b]	sound	100	nd[g]	nd	nd	nd	nd
	discolored	0					
control II[c]	sound	100	nd	nd	nd	nd	nd
	discolored	0					
Asp1–Asp5[d] (5 lots)	sound	7.7	nd	nd	nd	nd	nd
	discolored	92.3	5411 ± 2594	1200 ± 640	7045 ± 5614	4511 ± 4012	18200 ± 7280
NMI1–NMI15[e] (15 lots)	sound	38.3	nd	nd	nd	nd	nd
	discolored	61.7	1.00 ± 1.26	0.47 ± 0.62	4.78 ± 7.73	2.33 ± 3.11	8.58 ± 12.52
COM1–COM17[f] (17 lots)	sound	99.9	nd	nd	nd	nd	nd
	discolored	0.1	73.34 ± 97.60	68.56 ± 82.28	142.78 ± 104.53	122.17 ± 149.95	402.08 ± 302.99
COM18–COM30[f] (13 lots)	sound	99.9	nd	nd	nd	nd	nd
	discolored	0.1	nd	nd	nd	nd	nd

[a] Mean of values with standard deviation. [b] Control I, raw kernels stored at −25 °C. [c] Control II, nonmoistened kernels stored at ambient temperature. [d] Asp, *A. parasiticus* infected lots. [e] NMI, natural mold infected lots. [f] COM, commercial lots. [g] nd, not detected.

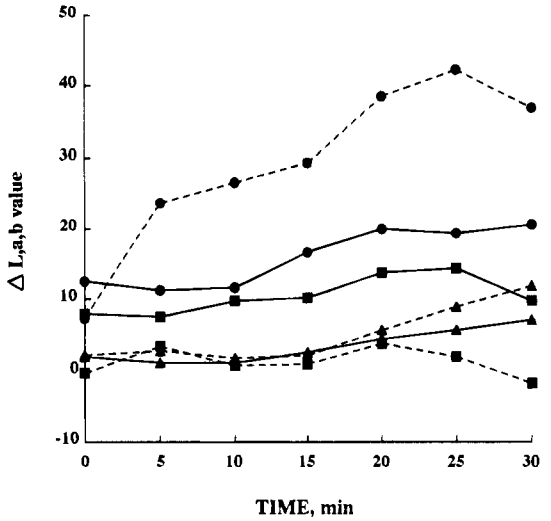

Figure 2. Color changes expressed as differences of L, a, and b values between *A. parasiticus* infected and sound kernels (—) and between natural mold infected and sound kernels (- - -) during roasting at 160 °C for up to 30 min: (●) L values; (▲) a values; (■) b values.

unblemished sublots. The average aflatoxin content was 12.5 ppb in natural mold infected and discolored sublots. In the *A. parasiticus* infected and discolored sublots, the average aflatoxin content was 18 200 ppb and varied considerably among lots. A comparison of changes in colors of *A. parasiticus* and natural mold infected peanuts (Figure 2) indicates that extensive mold infection caused rapid and significant discoloration after light roasting. However, discoloration was not accompanied by aflatoxin production unless aflatoxigenic molds were present.

When light roasting, deskinning, and color sorting was applied to a commercial scale using 30 lots (200 kg for each), aflatoxin was not detected in sound sublots. Aflatoxin content varied considerably among discolored sublots. Aflatoxin was not detected in 13 lots. In the remaining 17 lots, the average aflatoxin content was 402 ppb and ranged from nondetected to 976 ppb. Since the commercial peanut kernels were size graded, electronically sorted, and hand picked, the weight percentage of discolored peanut kernels after light roasting and deskinning was about 0.1%. On the basis of the fact that aflatoxin was not detected in the sound sublots, complete removal of aflatoxin by the application of light roasting, deskinning, and color sorting was nearly achieved.

In conclusion, mold-infected peanut kernels underwent color changes more rapidly during roasting than did sound kernels. Fifteen minutes of roasting was sufficient to differentiate and sort the discolored kernels. When kernels were roasted, deskinned, and sorted, aflatoxin was not detected in the sound sublots generated from laboratory lots or in commercial lots.

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