Table II. Flavonols in the Total Edible Portion of Onions (mg/kg Fresh Weight)[a]

variety	quercetin	kaempferol
Sweet Spanish Hybrid	62	3
Sweet Spanish Utah	61	ND
Carmen Hybrid	59	ND
Walla Walla	26	7
Yellow Globe Hybrid	25	ND
Early Yellow Globe	15	4
Red Hamburger	ND	ND
Evergreen Long White Bunching		
leaves	2	<1
bulbs	ND	ND

[a] ND = not detectable.

quercetin and kaempferol found in the edible portions of other fruits and vegetables known to be rich in flavonols (Herrmann, 1976), for example, sour cherry (80 and 17), black currant (68 and 10), apricot (53 and 2), apple peel (263 and 7), Brussels sprouts (25 and 40), and pea pods (130 and 5 mg of quercetin and kaempferol, respectively, per kg fresh weight).

Knowledge of varietal differences in the flavonol distribution in onions may be of potential value to breeders should they be required to select for reduced flavonol content. However, differences in flavonol content within a commodity need to be understood in terms of maturation and environmental effects before varietal differences can be exploited.

Registry No. Quercetin, 117-39-5; kaempherol, 520-18-3; myricetin, 529-44-2.

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Confirmation of Aflatoxins B_1 and B_2 in Peanuts by Gas Chromatography/Mass Spectrometry/Selected Ion Monitoring

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A rapid confirmatory method for aflatoxins B_1 and B_2 has been developed. The extract used for thin-layer chromatography quantitation is rapidly cleaned up by elution through a silica gel Sep-PAK cartridge and then analyzed by gas chromatography/mass spectrometry/selected ion monitoring (GC/MS/SIM) at 3000 resolution using a bonded-phase fused silica capillary column with on-column injection. Limits of detection for aflatoxins B_1 and B_2 in peanut samples were 0.1 ppb.

Aflatoxins are highly carcinogenic mold metabolites frequently found as natural contaminants in a variety of foods (Stoloff, 1976). Accordingly, foods are routinely analyzed for aflatoxin contamination by regulatory agencies throughout the world. In some cases, in part because the food is of considerable economic value, confirmation of identity is also required. Nesheim and Brumley (1981) reviewed a number of confirmatory techniques and concluded that a single, more certain method, such as mass spectrometry, was needed. In a later publication, Brumley et al. (1981) reported a confirmatory method for aflatoxins B_1 and M_1 based on negative ion chemical ionization mass spectrometry. This method employs a two-dimensional TLC cleanup step. Previously, Haddon et al. (1977) reported electron ionization analysis of aflatoxins B_1 and M_1 at resolution above 5000 in samples introduced through the direct insertion probe.

The method we describe is based on gas chromatography/electron impact mass spectrometry/selected ion monitoring at medium resolution and involves a more rapid and convenient cleanup step. In addition, while it is difficult to compare limits of detection when dealing with two different commodities (i.e., peanuts and peanut butter), our method may be 50–100 times more sensitive than the method of Brumley et al. (1981).

EXPERIMENTAL SECTION

Materials. Aflatoxin standards were obtained from the Bureau of Foods, FDA, and were checked for purity and analyzed for aflatoxin content according to established procedures (Horwitz, 1980).

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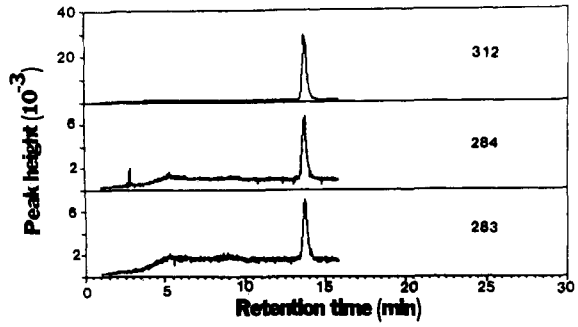

Figure 1. Three-ion selected ion monitoring chromatograms of aflatoxin B_1 standard (ca. 50 ppb equivalent). Linear carrier velocity was 30 cm/s.

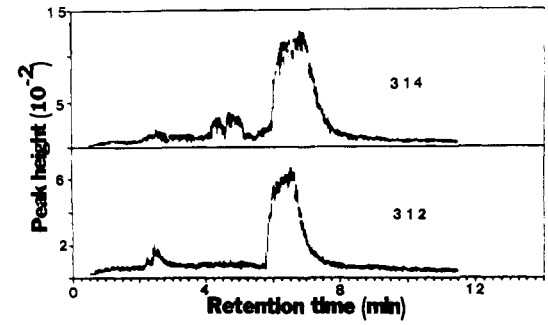

Figure 2. Aflatoxins B_1 and B_2 in peanuts (1 ppb each) at 1000 resolution. Under the GC conditions used, aflatoxin B_1 eluted at 6 min 32 s.

Instrumentation. The operations described were performed on a Finnigan MAT 212 mass spectrometer (San Jose, CA) interfaced to a Varian 3700 gas chromatograph (Palo Alto, CA) via a 1 m × 0.17 mm i.d. fused silica line that was fed through a standard, heated line-of-sight (LOS) inlet. The GC column was a 15 m × 0.32 mm i.d. J & W Scientific DB-5 (SE-54) fused silica capillary (Rancho Cordova, CA) having a film thickness of 0.25 μm. The linear carrier (helium) velocity was 50 cm/s, and the temperature was programmed from 200 to 290 °C at 15 °C/min and held at the final temperature for 10 min. Samples were injected with J & W Scientific standard 0.15–0.17-mm o.d. fused silica syringe needles through a J & W Scientific on-column injector. Mass spectrometer conditions were as follows: filament voltage 100 eV; filament current 2 mA; interface and ion source temperatures 310 °C; resolution 3000; multiplier voltage 2.4 kV (equivalent to a gain of ca. 5×10^6).

Selected ion monitoring (m/z 312.063 for aflatoxin B_1 and m/z 314.079 for aflatoxin B_2) was conducted at 3000 resolution and was controlled by a Finnigan SS-200 data system.

Qualitative MS on unspiked peanuts was conducted at 10 000 resolution by using peak matching techniques.

Analytical Procedure. Peanuts were analyzed for aflatoxins by both the CB and BF procedures (Horwitz, 1980) using thin-layer chromatography as the determinative step. Solutions found to be negative for aflatoxins (limit of detection <1 ppb) were evaporated to dryness under nitrogen at 60–85 °C. The residue was dissolved in 0.1 mL of benzene containing aflatoxin B_1 and/or aflatoxin B_2 in concentrations appropriate for 1–10-ppb spikes. For example, 0.1 mL of benzene containing 0.178 ng/μL aflatoxin B_1 was used to spike extracts cleaned up by the CB method in order to make up a 2-ppb spike. The solution was then placed onto a silica gel Sep-PAK cartridge (Waters Associates, Milford, MA). The residue vial was rinsed with 10 mL of benzene, and the rinse was used to elute the aflatoxins from the silica gel. The eluate was then evaporated to dryness under nitrogen at room temperature, and the resulting residue was taken up in 10 μL of methylene chloride. Selected ion monitoring was carried out with 2-μL injections of this solution.

RESULTS AND DISCUSSION

Analysis of aflatoxins by GC/MS has been impossible until recently because aflatoxin could not be chromatographed on packed or open tubular capillary columns, probably because of binding and/or decomposition by trace metals in the glass columns. With the advent of fused silica capillary columns (known to contain <1 ppm of metals), Friedli (1981) was able to chromatograph an aflatoxin B_1 standard. We have coupled this technological advance with medium-resolution selected ion monitoring to develop a method for confirmation of aflatoxins B_1 and B_2 in shelled peanuts at limits of detection (2.5 × noise) of approximately 0.1 ppb. The noise level was determined by integrating a portion of the base line where there were no peaks.

Our initial attempt focused on three-ion selected ion monitoring at low resolution (Figure 1) as this technique assures essentially absolute confirmation of identity (Millard, 1978). However, the m/z 284 ($M^+ - CO$) and the m/z 283 ($M^+ - CHO$) ions were of much lower intensity than the parent ion, a circumstance that resulted in a method with less than the desired sensitivity. More importantly, as the temperature of the GC column rose during temperature programming, the base lines for the m/z 284 and 283 ions also rose, giving a poor signal to noise ratio. Single ion detection at m/z 312 gave considerably improved sensitivity for aflatoxin standards. However, application of this method to a peanut extract spiked with the aflatoxins at the 1-ppb level resulted in the chromatograms shown in Figure 2. Clearly, naturally interfering material(s) with a mass spectrum that gave m/z 312 and 314 made this approach impractical.

A direct probe analysis of the peanut extract at 10 000 resolution indicated that the major interference at nominal m/z 312 had m/z 312.2183, corresponding to $C_{23}H_{36}^+$. Since aflatoxin B_1 has an exact mass of 312.0633, a resolution of 2013 is sufficient, theoretically, to separate the two ions. However, a resolution of 3000 was determined experimentally, because the peak from the interference ion was much larger and wider than the aflatoxin B_1 parent ion. Monitoring aflatoxin B_1 at m/z 312.063 and aflatoxin B_2 at m/z 314.079 at 3000 resolution thus effectively separates these ions from the interfering ions in peanuts. In addition, the high electron multiplier gain used to achieve high sensitivity results in a noisy base line because of residual pump oil in the system. Since these are hydrocarbon interferences, separation from the aflatoxins at nominal m/z 312 at 3000 resolution is achieved. Aflatoxins B_1 and B_2 both contain six oxygen atoms that contribute to a significant mass defect, and this is the reason why the aflatoxin molecular ions can be separated from hydrocarbon ions at only 3000 resolution. Other atoms with mass defects are halogens, phosphorous, sulfur, and silicon. Compounds containing these atoms could conceivably interfere with aflatoxin confirmation provided that their electron impact fragmentation gave m/z 312.063 ± 0.104 ions and the interferences had the same retention times as the aflatoxins. However, even should these unlikely events occur, interfering compounds containing bromine, chlorine, sulfur, or silicon would easily be detected because simultaneous monitoring of m/z 314.079 would show an unusually large response due to the isotopic distributions of these atoms. Interferences containing only one iodine

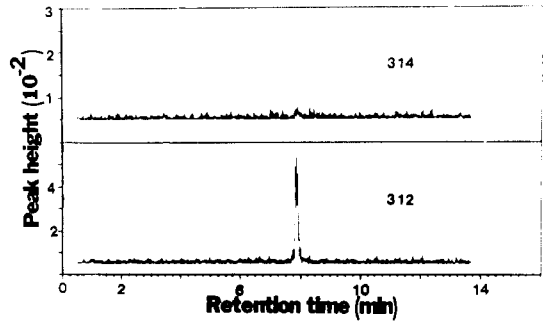

Figure 3. Aflatoxin B_1 in peanuts (1 ppb) at 3000 resolution.

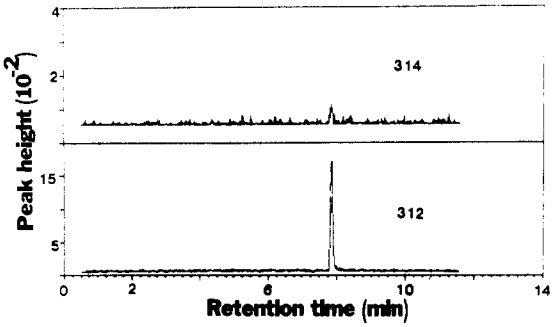

Figure 4. Aflatoxin B_1 in peanuts (2 ppb) at 3000 resolution.

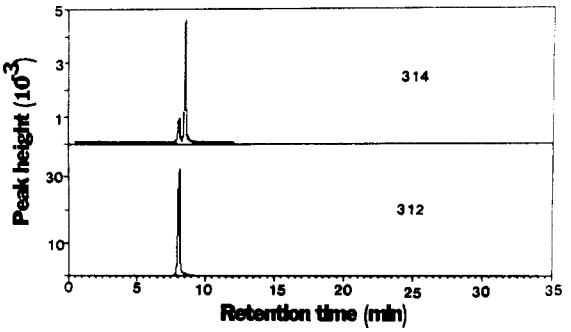

Figure 5. Aflatoxins B_1 and B_2 in peanuts (10 and 1 ppb, respectively) at 3000 resolution.

atom would not interfere because of a too large mass defect; the mass defect of fluorine is too small. The exact masses for a number of alkylphosphorus and alkyloxyphosphorus m/z 312 ions have been calculated and all are separable from 312.063 at 3000 resolution.

Figure 3 shows the selected ion monitoring chromatogram of aflatoxin B_1 spiked into peanut residue at 1 ppb after the peanuts were cleaned up by the BF method, a method that requires no column chromatography. The only further cleanup of the sample was a very rapid elution through a silica gel Sep-PAK. At the 1-ppb level, only a tiny m/z 314 is visible. Should an interference containing bromine, chlorine, sulfur, or silicon have been partially responsible for the peak at 7 min 50 s, then the peak at m/z 314.079 would be much larger. Figure 4 shows the results for a 2-ppb spike of peanut residue cleaned up by the CB method. The peak visible at m/z 314.079 is made up of instrument noise and the 1.2% of [^{18}O]aflatoxin B_1 present. Figure 5 illustrates the chromatographic separation of aflatoxin B_1 from aflatoxin B_2.

Addition of the appropriate internal standards could make this method quantitative for aflatoxins B_1 and B_2. However, regulatory decisions are made on total aflatoxins B and G, and aflatoxins G_1 and G_2 do not come through the column used in this work. A quantitative method for all the aflatoxins may be possible once the correct column conditions for chromatographic separation of all four aflatoxins is determined.

Registry No. Aflatoxin B_1, 1162-65-8; aflatoxin B_2, 7220-81-7.

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