°C, and filtering. Absolute ethanol (50 mL) was then added to the filtrate, followed by 1.3 g (0.013 mol) of methyl 2-acetamidoacrylate. The homogeneous solution was then heated for 24 h at 55 °C in a sealed vessel. After concentration in vacuo, the oil was redissolved in 100 mL of water and H_2S bubbled through the solution for 3 h. After treatment with charcoal and filtration, 100 mL of 12 N HCl was added to the colorless filtrate and the solution refluxed for 2 h. The crude lysinoalanine hydrochloride was then concentrated in vacuo and the resulting oil dissolved in 25 mL of H_2O and chromatographed on Dowex 50W-X8 (200–400 mesh, 2.0 × 42 cm). The column was washed, successively, with 4 L of water, 2 L of 1 N HCl, and 1 L of 2 N HCl, and the lysinoalanine hydrochloride eluted with 4 L of 4 N HCl. After concentration in vacuo, the resulting oil was redissolved and redried in vacuo with 2 × 50 mL of H_2O to assure removal of maximum HCl. The final oil, dissolved in 10 mL of H_2O, was treated with 1 g of activated carbon, filtered, and 10 mL of 1 M pyridine in 95% ethanol added. The solution was then diluted with 480 mL of 95% ethanol and cooled at −20 °C for 48 h. The lysinoalanine monohydrochloride was collected by filtration and recrystallized from ethanol–water at room temperature, collected by filtration, and dried in vacuo over P_2O_5 to yield 1.97 g (56%) of lysinoalanine monohydrochloride: mp 156 °C; ^{1}H NMR (D_2O) 2.45 (m, 6 H), 3.95 (t, 2 H, J = 4.0 Hz), 4.25 (d, 2 H, J = 3.5 Hz), 4.55 (t, 1 H, J = 3.5 Hz), 4.80 (t, 1 H, J = 3.5 Hz).

Anal. Calcd for $C_9H_{20}N_3O_4Cl$: C, 40.01; H, 7.52; N, 15.58; Cl, 13.14. Found: C, 40.06; H, 7.78; N, 15.67; Cl, 12.89.

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Native Iranian Peanut Resistance to Seed Infection by *Aspergillus*

The presence of aflatoxin in three varieties of peanuts that are currently cultivated in Iran has been examined by two methods. The results indicated that two varieties of the peanuts were contaminated with this toxin, but the native Iranian seeds, Local Gilan Iran, in comparison with standard, showed no contamination. The finding of this peanut which resists seed infection by *Aspergillus* is important from nutritional and economical points of view.

Aflatoxins, the possible etiological agents in certain human diseases (FAO–WHO–UNICEF, 1972; Newbern and Butler, 1969; Wogan, 1968; Shank et al., 1972; Austwick and Ayerst, 1963), have been detected in peanuts grown in various countries (Natarajan et al., 1975; Barnes, 1970). There are also several reports that showed peanut resistance to seed infection by *Aspergillus flavus* and aflatoxin production (Kulkarni et al., 1967; Roa and Tupuli, 1967). Further plantings of these two varieties mentioned by these authors and rehydration of the seeds cultivated in 1969 indicated very little resistance to *A. flavus* (Mixon and Rogers, 1973). The following studies were conducted to determine the presence of aflatoxin in peanuts which are currently cultivated in Iran.

EXPERIMENTAL SECTION

Peanuts. From 14 varieties of peanuts (mostly with foreign origin) that are currently cultivated in Iran, three varieties, namely, Local Gilan Iran (peanut I), Flori-Spanish 334-A (peanut II), and Georgia 119–20 (peanut III) were randomly chosen. The product of two reported years of 1974 and 1975 was obtained from the Seed and Plant Improvement Institute, Karaj, Iran, and used for this study. Samples were kept in the cold room (4 °C) during experiments, though all three varieties were stored in the same condition in warm humid weather of the northern part of Iran before starting the experiments, without any special care to prevent fungal growth. As far as history goes, peanut II and III were brought from the United States in about 1965 and were grown in Iran since that time. Peanut I is locally grown in Gilan Iran.

Examination and Concentration. Holaday and Barnes (1973) and AOAC (1970) methods were used to examine aflatoxin in peanuts. These experiments were repeated more than 20 times for each variety and product. To measure the concentration of aflatoxin in the samples, precoated plastic sheet Polygram Sil G (Machery Nagel and Co., Duren) plates and developing solution of methanol–chloroform (97:3 v/v) were used for TLC. Aliquots of 1-mL of the extract of peanut II and 60 μL

Table I. Aflatoxin Content of Some Peanuts that Are Currently Cultivated in Iran

Varieties	Millicolumn, ppb	Spectrophotometric, ppb
Local Gilan Iran	0	0
Flori Spanish 334 A	10	12.50 ± 0.18
Georgia 119-20	50	60.50 ± 0.91

(because of its higher concentration of aflatoxin) of peanut III prepared by the method described by Holaday and Barnes (1973) were applied to the plates, respectively. The chromatograms were developed and then observed by a mercury lamp. The blue fluorescent spots were scraped off, and the aflatoxin was extracted by ethanol, filtered, and dried. To the dried sample 5 mL of ethanol was added, and aflatoxin concentration was measured by an ultraviolet spectrophotometer (Beckman DB-GT) in 233 nm. To make a standard solution, a 0.1-μL aliquot of 5% aflatoxin solution (Makors Chemical LTD) was applied to the plates, chromatographed, extracted, and measured. The data are averages of three replicate analyses unless otherwise indicated.

RESULTS AND DISCUSSION

The blue fluorescent bands in the millicolumns prepared from peanut II and III extracts and the TLC prepared from the same extracts indicated the presence of the aflatoxin in these samples. Such spots, in comparison to the standard, were not observed on TLC plates prepared from extract of Local Gilan Iran variety (Figure 1). Besides the spectrophotometric data, some degrees of quantitation was possible with the method described by Holaday and Barnes. Table I shows the absence of aflatoxin in Local Gilan Iran and the concentration of the toxin in the other two samples. It should be mentioned that not only the kernel but also the shell of the samples from either 1974 or 1975 were not contaminated with aflatoxin.

Significance. Research on prevention (Beuchat and Smith, 1974) or removing (Goldblatt, 1969; Kensler and Natoli, 1969) of aflatoxin from peanuts and peanut products has not been successful. Further increases in commercially available peanut and peanut products (peanut butter, oil, and defatted meal) calls for detoxification of this nutrient. Thus, our finding, peanut resistance to seed infection, is important in viewpoint of yielding detoxified rich protein and sulfur amino acid peanut that could be used as such in nutrition without being considered a potential threat to food safety and human health, and problem for producers, handlers, and users of peanut and peanut products.

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Analysis of *N*-Nitrosoproline in Raw Bacon. Further Evidence that Nitrosoproline Is Not a Major Precursor of Nitrosopyrrolidine

Nitrosoproline (NPro) has been suggested as a precursor of nitrosopyrrolidine (NPyr) in fried bacon. We have developed a procedure for isolation of NPro from raw bacon for subsequent chromatographic analysis. Analysis has been done by both gas and high-performance liquid chromatography. The two methods have included different specific detectors. Results indicate that preformed NPro is not a major source of NPyr.

Nitrosopyrrolidine (NPyr) is a carcinogen which has been consistently found in fried, but not raw, bacon (Crosby et al., 1972; Fazio et al., 1973). The mechanism causing the formation of NPyr has been the subject of several investigations (Pensabene et al., 1974; Fiddler et al., 1974), but it is still unclear. Lijinsky and Epstein (1970) suggested that NPyr is formed by nitrosation and decarboxylation of proline, though not necessarily in that order. The presence of nitrosoproline (NPro) in raw bacon would suggest nitrosation of proline during bacon curing and storage, followed by decarboxylation to NPyr on frying. The substantial decrease of NPyr found when ascorbyl palmitate is added prior to frying, however, implies that nitrosation during cooking is responsible for much of the NPyr formed (Sen et al., 1976b).

A few studies have focused on either the presence of NPro in raw bacon (Kushnir et al., 1975; Nakamura et al., 1976) or the yield of NPyr from NPro in bacon or in model