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Ochratoxin A Found in Commercial Roast Coffee

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Of 68 samples of commercial roast coffee purchased in 1987, 5 were found to contain ochratoxin A at concentrations of 3.2–17.0 μg/kg by our previous HPLC method. This is the first report of the detection of ochratoxin A in commercial roast coffee samples.

Ochratoxin A (OCT-A) is a nephrotoxic fungal metabolite produced by *Aspergillus spp.* and *Penicillium spp.* and has a high acute toxicity in duckling, rats, mice, chick, rainbow trout, etc. (Krogh, 1978). This mycotoxin may be responsible for kidney disease of swine in Denmark (Krogh, 1978) and was shown to cause hepatoma and renal cell tumors in mice (Kanisawa and Suzuki, 1978).

Although several studies have demonstrated the natural occurrence of OCT-A in various agricultural products (Nakazato, 1983) and many strains of OCT-A-producing *Aspergillus ochraceus* have been isolated from green coffee beans (Stack et al., 1983; Tsubouchi et al., 1984), OCT-A has rarely been detected in green coffee beans, and there has been no report of its detection in roast coffee samples. The reasons for this were considered to be that caffeine in coffee beans had inhibited the growth of the fungi and the production of OCT-A (Buchanan and Fletcher, 1978) and that the roasting procedure had destroyed the toxin in the coffee beans (Levi et al., 1974). However, the analytical methods employed would not have been sufficiently sensitive to detect low levels of OCT-A.

Recently, we reported that some strains of *A. ochraceus* isolated from green coffee beans grew well and produced high levels of OCT-A in YES medium containing 0.1–1.0%

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Table I. Levels of Ochratoxin A Found in Samples of Commercial Roast Coffee

country of origin	no. of samples	no. containing ochratoxin	level of toxin, μg/kg
Ethiopia	2	0	
Costa Rica	1	0	
Guatemala	2	0	
Indonesia	7	2	3.2, 4.4
Columbia	8	0	
Brazil	9	0	
Jamaica	12	0	
Mexico	1	0	
U.S.	5	0	
Tanzania	9	0	
Yemen Arab Republic	10	3	17.0, 6.9, 6.5
Zimbabwe	2	0	
total	68	5	

caffeine and reduced almost all of the caffeine in the medium (Tsubouchi et al., 1985). Later, we reported that OCT-A in green coffee beans artificially contaminated with *A. ochraceus* was little reduced (0–12%) by heat treatment at 200 °C for 10–20 min, and almost all the toxin was infused into the coffee decoction when the roasted coffee beans were ground and extracted with boiling water (Tsubouchi et al., 1987). Moreover, we reported a new analytical HPLC method for OCT-A in coffee beans and coffee products (Terada et al., 1986). We have now surveyed OCT-A contamination in commercial roast coffee samples. This is the first report describing the detection of OCT-A in commercial roast coffee samples.

MATERIALS AND METHODS

Collection and Preparation of Samples. Sixty-eight samples of roast coffee beans and roast ground coffee were purchased from a market in Nagoya City, Japan, in January and February 1987. These beans had been imported from Ethiopia, Brazil, Guatemala, Columbia, Costa Rica, Indonesia, Jamaica, Mexico, the United States, Tanzania, the Yemen Arab Republic, and Zimbabwe. All samples were ground finely and analyzed for OCT-A by means of our HPLC method (Terada et al., 1986).

Confirmatory Tests. When a peak coinciding with OCT-A appeared on the chromatogram, confirmatory tests were carried out by an improved version of our method (Terada et al., 1986) as follows. Acetonitrile (10 mL) was added to the remaining sample solution, and the mixture was poured into a Sep Pak NH_2 cartridge (Millipore), preconditioned with 20 mL of 50% v/v aqueous acetonitrile and 20 mL of acetonitrile prior to use, at a rate of 2 mL/min. After the cartridge was washed with 5 mL of acetonitrile, OCT-A was eluted with 2 mL of a mixture of acetonitrile–water–0.2 M phosphate buffer (pH 7.4, 40:57:3) containing 3 mM cetyltrimethylammonium bromide. The elute was evaporated to dryness on a water bath (40 °C) under vacuum, then 5 mL of an alcohol (methanol, ethanol, 1-propanol) for esterification and 0.1 mL of H_2SO_4 were added to the residue, and the subsequent operations were the same as those described in our previous report (Terada et al., 1986).

RESULTS AND DISCUSSION

The results of our investigation of OCT-A in commercial roast coffee samples are summarized in Table I. A chromatogram obtained from a sample contaminated with OCT-A and chromatograms obtained in confirmatory tests are shown in Figures 1 and 2, respectively. Five of commercial roast coffee samples contained OCT-A. The confirmatory tests with use of the Sep Pak NH_2 cartridge procedure supported the presence of low levels of OCT-A

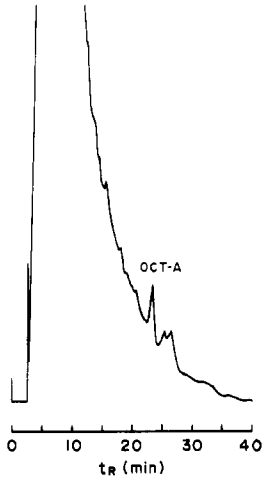

Figure 1. Chromatogram obtained from a commercial roast sample contaminated with ochratoxin A (17.0 μg/kg).

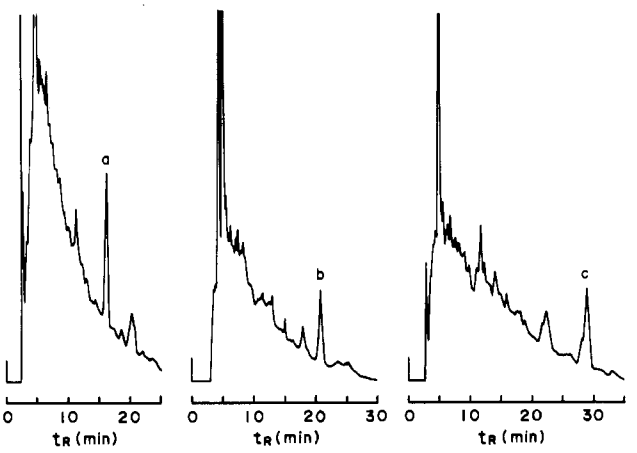

Figure 2. Chromatograms illustrating confirmatory tests for ochratoxin A. The sample was treated as described in Materials and Methods. Peaks a–c correspond to ochratoxin A methyl, ethyl, and 1-propyl esters, respectively.

in these samples. The OCT-A-containing samples had been imported from the Yemen Arab Republic (17.0, 6.9, 6.5 μg/kg) and Indonesia (4.4, 3.2 μg/kg).

Many strains of *A. ochraceus* producing high levels of OCT-A have been isolated from green coffee beans (Stack et al., 1983; Tsubouchi et al., 1984), but OCT-A has been found only rarely in green coffee beans (Levi et al., 1974; Tsubouchi et al., 1984). The reason for this may be that the analytical method for OCT-A in green beans or roast coffee samples was not sufficiently sensitive. Levi et al. (1974) reported that 3 of 68 commercial green coffee samples contained OCT-A (range trace–≤20–80 μg/kg), and Tsubouchi et al. (1984) reported that 4 of 22 commercial samples containing detectable OCT-A (range 9.9–46 μg/kg). This time, we detected OCT-A in 5 out of 68 roast coffee samples at concentrations in the range 3.2–17.0 μg/kg. These contamination levels are very similar to the previous results on green coffee beans.

The detection of OCT-A in roast coffee samples is not unexpected in light of our previous findings. That is, caffeine-resistant strains of OCT-A-producing *A. ochraceus* grow well and produce high levels of OCT-A in green coffee beans (Tsubouchi et al., 1985), and the toxin is not destroyed by heat treatment corresponding to that applied when green coffee beans are roasted (Tsubouchi et al., 1987). We also showed in the latter report that OCT-A is infused into coffee decoction when roasted beans contaminated with OCT-A are extracted with boiling water.

We conclude that surveys of mycotoxins in green coffee beans, as well as other agricultural products, should be continued.

Registry No. OCT-A, 303-47-9.

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Autolysis of α-Galactosides of Defatted Soy Flakes: Influence on Nutritive Value for Chickens[1]

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Autolysis of the α-galactosides of defatted soy flakes (soybean meal before toasting), accomplished through in vitro incubation of soy flakes (SF), increased reducing-sugar content from 12.6 to 57.6 mg/g and decreased raffinose and stachyose from 37% of soluble carbohydrates to 5.1%. A reference diet containing 30% soybean meal (SBM) and diets in which incubated SF, unincubated SF, or soy milk (steam-infusion processed SF, 87.5% moisture) replaced all or part of the SBM were fed to broiler chicks. Chicks fed the diet containing soy milk gained more weight than chicks fed the unincubated SF-containing diets. Otherwise, no diet effects on weight gain or feed efficiency were observed. Nitrogen-corrected metabolizable energies determined with chicks and roosters showed that incubation did not significantly change the energy value of SF diets. These results indicate that the conversion of raffinose and stachyose to monosaccharides by incubation did not measurably improve the nutritional qualities of SF for chickens.

Soybean meal (SBM) is an excellent source of dietary protein, but its energy is poorly utilized by poultry. Of the gross energy contained in SBM, poultry digest and metabolize only 52%, due in part to the low digestibility of its carbohydrates (Potter and Potchanakorn, 1985). Soybean meal contains substantial amounts of stachyose, raffinose, and complex polysaccharides that are poorly digested, and these saccharides have been implicated as the causes for the poor utilization of the energy of SBM (Potter and Potchanakorn, 1985). The presence in the small intestine of unabsorbable, water-soluble sugars of low molecular weight, such as raffinose and stachyose, also may result in an osmotic effect, leading to fluid retention and an increased rate of food passage that could adversely affect the absorption of nutrients (Wiggins, 1984).

Hydrolysis of raffinose and stachyose by endogenous α-galactosidase in beans has been shown to occur in vitro under specific conditions (Crocco, 1973; Kon et al., 1973; Becker et al., 1974; Olson et al., 1975). Incubation of ground soybeans in a 0.1 M sodium acetate buffer solution (pH 5.2) for 48 h at 45 °C resulted in an almost complete hydrolysis of raffinose and stachyose and an increase in galactose and sucrose (Olson et al., 1975). Exploitation of this endogenous enzyme to reduce the raffinose and stachyose contents of defatted soy flakes (dehulled SBM before toasting) may provide a means whereby the energy content of soybean meal could be made more available to poultry.

The objectives of the research reported here were to determine a set of incubation conditions that would facilitate autolysis of the raffinose and stachyose present in defatted soy flakes without loss of their constituent monosaccharides or disaccharides and to evaluate the soy flake material obtained from in vitro incubation as a feed ingredient for chickens. In the latter instance, weight gain, feed efficiency, and metabolizable energy values were the criteria used.

EXPERIMENTAL SECTION

Soy flakes (SF) were obtained from Archer-Daniels Midland, Des Moines, IA. The SF contained, on a dry-matter basis, 53.4% protein, 6.8% ash, 0.2% ether extract, and 33.5% neutral detergent fiber.

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