operation.

The experimental results show a considerable saving in caustic soda consumption when continuous operation is employed. The data indicate that a crude oil with 2.5% FFA would require only 36.5% excess caustic soda for continuous operation, whereas the same crude oil would require ca. 68% excess caustic soda if batch operation is used. For this particular case, a savings of ca. 84% in caustic soda consumption is realized.

The results of the bleachability tests indicate that continuously refined oil tends to acquire better bleaching properties. This result can be explained as being caused by 2 factors. First, the temperature during continuous operation is controlled in the range of 40-45 C. Thus, oil oxidation from overheating is avoided. Second, color fixation as a result of oxidation is not possible during continuous operation because the drying operation is carried out under vacuum.

Continuous operation gave significant savings in refined-oil losses and caustic-soda sonsumption as well as enhanced color and bleaching properties. The savings in refining losses were shown to be particularly significant.

Recent advances in continuous refining (13) indicate that refining losses can be markedly reduced by employing steam distillation rather than caustic refining. However, in such processes, degumming is usually included as a pretreatment step to remove phospholipids by water hydration and centrifugal separation.

From an operational point of view, the continuous process eliminates interstage storage (except surge storage). It is also readily susceptible to continuous automatic and on-line control, thus achieveing high throughputs with better product quality and at the same time reducing operational and supervisory labor, hence reducing production costs. A full description of a proposed on-line computer control system for an oil refining and hydrogenation plant has been compiled (14).

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Application of TLC Chemical Confirmatory Tests to Minicolumn Chromatography of Aflatoxins

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ABSTRACT

The minicolumn (MC) proposed by Holaday and Lansden was developed with standard aflatoxin solution and also with the extracts of corn, rice, wheat, cottonseed, peanut cake and black pepper; each having different levels of aflatoxins. One-half mL each of 2,4-dinitrophenylhydrazine, *p*-anisaldehyde, 20% H_2SO_4, 20% HCl and trifluoroacetic acid (TFA) with 25% HNO_3, which were used for confirming aflatoxins on TLC, were applied to the developed column. Among these, all the 3 acid reagents changed the blue fluorescence of aflatoxins to yellow and thus were found to be satisfactory confirmatory tests. The TFA with 25% HNO_3 had the lowest detection limit—5 ppb.

INTRODUCTION

Concern is growing about the aflatoxin contamination in foods and feeds because of their harmful biological effects and the consequent losses in agricultural trade. Thus, the routine screening of agricultural commodities, foods and feeds for aflatoxins has become very essential. Several minicolumn (MC) methods have, therefore, been developed during the last decade for avoiding lengthy, expensive thin layer chromatography (TLC) techniques (1). In these analytical methods, mistaking aflatoxins for naturally occurring blue fluorescent substances is always possible (2-4). Several chemical tests to confirm aflatoxins on TLC have been proposed in the past (5,6). Jemmali (7) made a pioneering attempt by suggesting a confirmatory test with 50% H_2SO_4 for aflatoxins in cellulose-tube MC. An attempt has been made here to introduce a chemical confirmatory test as a regular step in MC chromatography.

EXPERIMENTAL PROCEDURES

Materials

MC fabricated from a 20-cm length of 6 mm o.d. and 4 mm i.d. borosilicate wall tubing flared into a funnel top, as suggested by Holaday and Lansden (8), was used. Solvents, e.g., acetone, ethanol, methanol, benzene, hexane, acetonitrile, concentrated sulphuric acid, concentrated hydrochloric acid, trifluoroacetic acid (TFA), concentrated nitric acid and glacial acetic acid, used were of analytical reagent grade. Salt solution was prepared by dissolving 15 g sodium chloride and 15 g zinc acetate in 100 mL distilled water. *p*-Anisaldehyde reagent and 2,4-dinitrophenyl hydrazine reagent were prepared as described by Scott et al. (9) and Crisan (10). Aflatoxin reference standard containing 1 μg/mL was prepared in benzene/acetonitrile (98:2, v/v) as outlined in the official AOAC method for standards (11). Neutral alumina (100-200 mesh) activated by drying at 100 C for 2 hr and florisil (100-200 mesh) activated by drying at 100 C for 2 hr were used for packing the column.

Methods

The MC was packed as described by Holaday and Lansden (8) and stored overnight in a desiccator containing saturated NH_4Cl solution for equilibration with 80% RH.

TABLE I

Comparison of Three Chemical Confirmatory Tests for Aflatoxins in MC

	Aflatoxin levels in ppb	20% H_2SO_4		20% HCl		TFA + 25% HNO_3	
		Standard	Sample	Standard	Sample	Standard	Sample
Corn	5	+	−	−	−	+	+
	10	+	+	−	−	+	+
	20	+	+	+	+	+	+
Rice	5	+	+	−	−	+	+
	10	+	+	−	+	+	+
	20	+	+	+	+	+	+
Wheat	5	+	−	−	−	+	+
	10	+	+	−	−	+	+
	20	+	+	+	+	+	+
Cottonseed	5	+	−	−	−	+	−
	10	+	+	−	−	+	+
	20	+	+	+	−	+	+
Peanut cake	5	+	−	−	−	+	−
	10	+	+	−	−	+	+
	20	+	+	+	+	+	+
Black pepper	5	+	−	−	−	+	−
	10	+	−	−	−	+	−
	20	+	+	+	−	+	+

+ = Positive, − = negative.

Aflatoxin from naturally contaminated corn, rice, wheat, cottonseeds and peanut cake and artificially contaminated black pepper was extracted as in the procedure described by Barabolak et al. (12), using salt solution as precipitant instead of ammonium sulphate. The final benzene extract was evaporated to near dryness and dissolved in a known volume of benzene and quantitated on TLC by visual comparison with standard. Further, it was diluted with benzene to obtain the different concentrations of aflatoxins—5 ppb, 10 ppb and 20 ppb. One mL of each concentration, containing 50 ηg, 100 ηg and 200 ηg of aflatoxin, respectively, was added to the column. An additional 3 mL of hexane/acetone (80:20, v/v) was added to MC for the development and after draining it completely, the MC was viewed under longwave ultraviolet (UV) light. A blue fluorescent band at the interface of florisil and neutral alumina indicated the presence of aflatoxins.

Confirmatory Tests

The chemical reagents, e.g., 2,4-dinitrophenylhydrazine, *p*-anisaldehyde, 20% H_2SO_4 (20 mL conc. H_2SO_4/80 mL water), 20% HCl (20 mL conc. HCl/80 mL water), trifluoroacetic acid/benzene (1:1, v/v) with 25% HNO_3, which have been used earlier (5,6) for confirming aflatoxins on TLC were applied to MC. One-half mL of each reagent was added to standard column developed in reference standard aflatoxin solution having 1 μg/mL concentration diluted to give 5 ppb, 10 ppb and 20 ppb concentrations as described by Cucullu et al. (13) and observed for the fluorescent band, changing to yellow within 30 sec. Reagents yielding positive results were applied to the columns developed with extracts of naturally contaminated rice, wheat, corn, cottonseeds and peanut cake and artificially contaminated black pepper at different levels of aflatoxin—5 ppb, 10 ppb and 20 ppb. All tests were done in duplicate.

RESULTS AND DISCUSSION

When 2,4-dinitrophenylhydrazine was added, the entire florisil zone turned orange, whereas with *p*-anisaldehyde reagent the blue fluorescent band in the florisil layer of the developed column was quenched. On adding 20% H_2SO_4 to the column, the blue fluorescent band at the interface of florisil and neutral alumina changed to yellow. Similar change was observed with 20% HCl. With TFA reagent, the fluorescence became bright greenish-blue and, on adding 25% HNO_3, it changed to yellow. In all 3 cases, when columns were dried at 60 C for 30 min after adding the reagents, the yellow fluorescence changed back to the original blue.

The sensitivity of the test was found to vary with different reagents for samples as well as standards (Table I). The tests indicated that TFA with 25% HNO_3 could be used to confirm the lowest level of aflatoxins, i.e., 5 ppb. 20% H_2SO_4 was found to be the next best reagent, having a 10 ppb detection limit. The MC method will become more sensitive and reliable by the use of these confirmatory tests.

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