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Chemical Studies on Corn Embryos Infected by Various Fungi

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ABSTRACT

The occurrence of various fungi in corn kernels obtained from eight localities in Egypt in two successive years was studied. Values for refractive index, color, acid value, saponification value, iodine value, peroxide value and unsaponifiable matter content of oils extracted from corn embryos that were deliberately infected by various fungi were compared to those for oil extracted from healthy embryos. Spectrometric analyses (UV, visible and IR) were done to deduce differences in the functional groups of the oils. Corn oil extracted from embryos infected with various fungi contained the same lipid classes as the oil extracted from healthy embryos. Contents of mono- and diglycerides and free fatty acids were much smaller for the oil extracted from healthy embryos. The fatty acid and unsaponifiable compositions of oils were studied by gas liquid chromatography. The fatty acid composition of corn oil extracted from infected embryos showed that some new and short-chain fatty acids had appeared and that some of the 18:2 was converted to 18:0. Analysis of the hydrocarbon fraction of the unsaponifiables showed also that some new compounds had appeared and others disappeared. The sterols were greatly influenced by the fungi and the ratio between different sterols might be used to characterize the effect of fungi. Aflatoxin B_1 content of oil extracted from corn embryos infected by *A. flavus* was 300 μg/kg.

INTRODUCTION

Many seed crops are subjected to contamination by fungal metabolites such as aflatoxins, creating serious problems for food and feed for humans and animals. Aflatoxins, when fed to rats, cause a high incidence of hepatomas and renal damage (1). *Aspergillus flavus* is the major cause of corn grain deterioration in storage (2). A good correlation was observed between the presence of *A. flavus* and aflatoxins in naturally contaminated white corn (3).

Previous researchers have focused on the presence of aflatoxins, the highly toxic fungus metabolites present in several crops. Very limited studies were done to show the change of commodity constituents due to infection by fungi. This investigation demonstrated changes in physical and chemical properties, lipid classes, free fatty acids and fatty acid composition, and unsaponifiables of oil extracted from corn embryos that were deliberately inoculated with various fungi commonly present in grain during storage.

MATERIALS AND METHODS

Occurrence, Isolation and Identification of Seed-Borne Fungi

Naturally infected corn kernels obtained from various experimental stations (Table I) were employed to recover fungi (4). Colonies developed were observed during 3-4 days of incubation and colony counts were recorded. Distribution (%) of six fungal species associated with corn kernels from eight localities during 1975 and 1976 was calculated. Fungi were isolated by a single spore technique (5). They were grown on potato dextrose agar (PDA) at 25 C. The isolates were identified by the Commonwealth Mycological Institute (Ferry Lane, Surrey, England).

TABLE I

Distribution (%) of Six Fungal Species Associated with Corn Kernels Obtained from Eight Localities during the 1975-76 Seasons

Fungus	Localities and seasons																	
	Bahteim		CAR[a]		El-Gemmeiza		Monifyia		Sakha		Sedes		Shandaweel		UFC[b]		Average	
	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
A. flavus	27.2	28.2	26.8	29.1	24.2	22.2	30.3	27.3	17.4	20.0	26.2	28.1	23.4	20.0	17.6	23.9	24.1	24.8
A. melleus	13.3	9.2	8.7	7.8	7.4	6.0	–	–	12.1	10.0	9.1	–	12.1	11.5	–	11.1	7.9	7.0
A. nidulans	9.1	10.4	7.4	9.2	10.6	10.1	16.2	5.4	7.6	8.6	12.2	14.8	16.3	7.8	–	8.6	9.9	9.1
A. niger	13.3	16.6	20.8	20.5	14.1	14.1	21.8	16.0	19.7	16.4	11.6	14.1	–	11.5	27.9	19.0	16.1	16.0
F. moniliforme	19.6	22.7	20.1	19.9	24.2	27.5	31.7	25.3	26.5	25.7	23.2	25.0	28.4	18.5	19.8	18.4	24.2	22.8
P. oxalicum	17.5	12.9	16.2	13.5	19.5	20.1	–	26.0	16.7	19.3	17.7	18.0	19.8	30.7	35.1	19.0	17.8	20.0

[a]CAR = the Center of Agriculture Research.
[b]UFC = the University Farm, Cairo.

Preparation of Spore Suspensions

Spore suspensions were prepared from 15-day-old cultures of the fungi: *A. flavus, A. melleus, A. nidulans, A. niger, Fusarium moniliforme* and *Penicillium oxalicum*, grown on PDA. The plates were flooded with sterile, distilled water containing 1% agar, shaken for 1-2 min. The suspensions were filtered through sterile cheese cloth.

Artificial Inoculation of Corn Embryos

Disease-free embryos, manually separated from the kernels, were inoculated with each isolated fungus (6).

Lipid Extraction

Lipid materials of corn embryos were extracted using a chloroform/methanol mixture (2:1, v/v) (7).

Characteristics of Crude Lipids

Refractive index, color, acid value, saponification value, iodine value, peroxide value and unsaponifiable content were determined by AOAC methods (8).

Spectrometric Analysis

The absorbance spectra in the ultraviolet (UV), visible and infrared (IR) regions were obtained (9).

Identification of Lipid Classes

Lipid classes were separated and identified by thin layer chromatography (TLC) using the solvent system petroleum ether/diethyl ether/acetic acid (90:10:1, v/v) (9). Oils extracted from healthy and infected corn embryos were dissolved in chloroform to give a solution of 30% (w/v), and 50 μl of this solution was spotted on TLC plates coated with Silica Gel G (0.25 mm thick) using a microdoser.

Separation of Free Fatty Acids by TLC

Free fatty acids were separated from the oils using the solvent system petroleum ether/acetic acid/diethyl ether (87:1:12, v/v) (10).

Sources of Standard Materials

A set of standard fatty acids of 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3 and 20:0 with a stated purity of 99% by gas liquid chromatography (GLC) was purchased from Nu-Chek-Prep. Pure saturated hydrocarbons (*n*-eicosane, *n*-docosane, *n*-triacontane and *n*-dotriacontane), cholesterol, campesterol, stigmasterol and β-sitosterol were Sigma grade. The purity of each standard compound was checked by GLC and gave one peak.

Extraction of Unsaponifiables

Lipids were saponified overnight with methanolic potassium hydroxide (20%, w/v) at room temperature. The unsaponifiables were extracted three times with petroleum ether (40/60 C), the combined extract was washed several times with distilled water and dried over anhydrous sodium sulfate.

Methylation of Lipid Materials

The conversion of oils and free and standard fatty acids to fatty acid methyl esters suitable for GLC analysis was done by methanolysis using methanolic potassium hydroxide (11). Unsaponifiables and standard sterols were dissolved in a little anhydrous ether and the methyl esters were prepared using an ethereal solution of diazomethane (12).

Separation of Fatty Acid and Unsaponifiable Methyl Esters by GLC

The methyl esters of the fatty acids obtained from free fatty acids and oils, the unsaponifiables and authentic compounds were analyzed with a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detector and dual channel recorder. The separation of fatty acid methyl esters was conducted using a coiled glass column (1.5 m x 4 mm) packed with Diatomite C (100-120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column was operated isothermally at 170 C with nitrogen at 30 ml/min. Detector and injection temperatures were 220 and 200 C, respectively. The unsaponifiables were also separated on a coiled glass column (2.8 m x 4 mm) packed with Diatomite C (100-120 mesh) and coated with 1% OV-17. The column was maintained at 270 C and the flow rate of nitrogen was 30 ml/min. Detector and injection temperatures were 300 and 280 C, respectively. Hydrogen and air flow rates were 33 and 330 ml/min, respectively. The range was 32×10^2.

Identification and Determination of Fatty Acids and Unsaponifiables

Peak identification was performed by comparing the relative retention times of each peak with those of standard materials. The relative retention times for methyl stearate and β-sitosterol are given a value of 1.00. The linear relationship between log retention times of the standard fatty acids or the hydrocarbons and the number of carbon atoms of these compounds was used to characterize the odd-carbon fatty acids or the unavailable authentic hydrocarbons (C_{21}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29} and C_{31}). The peak area was measured by triangulation and the relative proportions of the individual compounds were therefore obtained by determining the partial areas in relation to total areas.

Extraction of Aflatoxins

Extraction of aflatoxins from corn embryos was done using chloroform according to the method described by Parker and Melnick (13). The oil so obtained was used for the determination of aflatoxins.

Fractionation and Determination of Aflatoxins

A standard solution of aflatoxins, B_1, B_2, G_1 and G_2 (10 μg/ml) was prepared, fractionated and spectrophotometrically determined as described in AOAC methods (8). A preliminary run using diethyl ether as a mobile solvent had been done for oils before developing in 5% acetone in chloroform. The plates were examined under long-wave UV light (365 nm) and the blue and violet fluorescence of aflatoxins was located. The spots for the aflatoxin standard and samples were scraped from the plates then placed in small test tubes and eluted with benzene/acetonitrile (98:2, v/v). The absorbance of the filtrate was measured at 365 nm. A graphic plot of absorbance of the standard aflatoxins over the range 0.1 to 10 μg was linear. The concentration of individual aflatoxins in the oil was read from the corresponding standard curve.

RESULTS AND DISCUSSION

The genera of fungi associated with corn kernel samples obtained from various localities for two successive years in Egypt are presented in Table I. Results indicated the common occurrence of *A. flavus, A. niger, A. nidulans, A. melleus, Fusarium moniliforme* and *Penicillium oxalicum*. Distribution of the genera (*Aspergillus, Fusarium* and *Penicil-*

lium) in 1975 and 1976 was as follows (%): 58 and 56.9; 24.2 and 22.8; and 17.8 and 20, respectively. These fungi were used to infect corn embryos (Shedwan 3 variety) to study the physical and chemical changes of corn oil with the fungal metabolites.

Table II shows the refractive index of the oils extracted from healthy and infected embryos, which ranges from 1.4680 to 1.4730 at 25 C. Because there is no significant difference between the oils of healthy and infected embryos, one would expect that the infected oils had nearly the same composition of unsaturated fatty acids as that in the control oil.

The color determination was done with a fixed yellow slide of value 35 on the Lovibond scale, and the difference in color between oils was assessed by matching its color against the red slides. The color of crude healthy corn oil was yellow 35 and red 3.3 on the Lovibond scale. On the other hand, the crude oils extracted from embryos infected by *P. oxalicum, A. melleus, A. nidulans, A. niger, A. flavus* and *F. moniliforme* were 8.4, 9.3, 18.9, 24, 33 and 44, respectively, on the red slides. The remarkable difference between the infected oils and the healthy oil indicates a pronounced effect of fungi on the oil color. Also, the color results indicate that the species of fungi influenced the color of the oils extracted from infected embryos. The deepened color could stem from the pigments in mycelium of fungi extracted with the oils. The results of the color determination suggest that it may not be possible to use the oils infected by fungi either industrially (in the manufacture of hydrogenated oils) or for edible purposes, and that several bleaching or decolorization processes would be necessary for both purposes. This would mean that an additional cost would be involved in the use of infected oils.

The acid value of crude oil extracted from healthy embryos was 3.48. The relatively high acid value is due to the moisture content of the embryos, which was raised to ca. 20% to obtain maximal fungal growth and to encourage the lipase to hydrolyze some triglycerides to give free fatty acids. The acid value ranged from 38.31 to 97.81 for oils extracted from infected embryos. The variation in acid values for oils extracted from corn embryos infected by different fungi may reflect the difference in ability of fungi to produce lipases which carry out lipid hydrolysis. The efficiency for producing lipase by the fungi according to this hypothesis would be in the following decreasing order: *A. melleus, A. niger, F. moniliforme, A. flavus, A. nidulans, P. oxalicum.* The high acid value for the oils extracted from infected embryos may prevent their direct application for edible purposes and neutralization of acidity would be necessary before use.

The saponification and iodine values of corn oil obtained from healthy embryos were 201.43 and 109.34, respectively. The above chemical constants for oils extracted from infected embryos were in the range of 202.92 to 208.81, and 100.12 to 108.02, respectively. These results demonstrated that there was little difference in the saponification and iodine values for oils obtained from healthy and infected corn embryos.

The peroxide value of the oil extracted from healthy corn embryos was 4.95. Higher peroxide values were found for the oils extracted from infected embryos, ranging from 11.4 to 50.52. These results indicate that the fungi used in our study had a deleterious effect on the oils.

The oil extracted from uninfected corn embryos contained 3.02% unsaponifiables (Table II). The oil obtained from embryos infected by various fungi contained unsaponifiables ranging from 7.10% in oil infected with *A. flavus* to 10.66% in oil infected with *A. nidulans*, which was twice and three times that in uninfected oil. The increase in the amount of the unsaponifiable matter might have come from the extraction of the fungal pigments and metabolites which were not affected by alkali. These results agreed quite well with the oil color determination using a Lovibond tintometer, since the color of the oil extracted from infected embryos was much darker than that of uninfected ones, as shown in Table II.

The higher amounts of unsaponifiables in the oil obtained from embryos infected by various fungi suggests that they can withstand oxidative rancidity for a considerable time. The addition of total unsaponifiables extracted from crude sesame oil and soybean oil to both linoleic acid and refined cottonseed oil raised their stability toward oxidative rancidity (14). However, high peroxide values for the oils extracted from embryos infected with fungi were found, despite the fact that they contained higher amounts of unsaponifiables than the healthy oil. It has been reported that certain microorganisms have the ability to cause the oxidative reactions normally associated with rancidity, i.e., the formation of peroxides and their subsequent decomposition into carbonyls (15,16). These results support our findings. However, further work is needed to elucidate the possible role of fungal metabolites, e.g., aflatoxins in the prevention of the unsaponifiables' antioxidative effect.

Spectrometric analyses such as IR, UV and visible (vis) spectroscopy have been applied to illustrate the changes that could occur in the oil obtained from corn embryos infected by various fungi, taking the uninfected oil as a control. A broad absorption between 250 and 380 nm was observed and that may be attributed to the carbonyl groups of the fatty acids and the unsaturated double bonds (17). The absorption spectra for corn oil extracted from embryos infected with fungi did not differ greatly from that of the healthy oil. Therefore, the absorption spectra in the UV and vis regions do not elucidate the changes that might have occurred in healthy oil and in the oil extracted from infected embryos.

The IR spectrum for uninfected corn oil exhibits only a strong peak at 1740 cm^{-1}, whereas all the oils obtained

TABLE II

Some Physical and Chemical Constants of Oil from Healthy and Infected Corn Embryos

Property	Healthy	*A. flavus*	*A. melleus*	*A. nidulans*	*A. niger*	*F. moniliforme*	*P. oxalicum*
Refractive index (25 C)	1.4730	1.4680	1.4680	1.4680	1.4690	1.4710	1.4670
Color:							
Yellow	35	35	35	35	35	35	35
Red	3.5	33.0	9.3	18.9	24.0	44.0	8.4
Acid value	3.4	76.22	97.81	68.46	92.63	91.14	38.31
Saponification value	201.43	204.82	205.81	204.43	204.34	208.81	202.92
Iodine value	109.34	100.87	104.64	106.64	108.02	105.11	100.12
Peroxide value	4.95	15.67	50.52	11.40	50.07	37.97	35.45
Unsaponifiables (%)	3.02	7.10	7.26	10.66	9.30	7.74	7.45

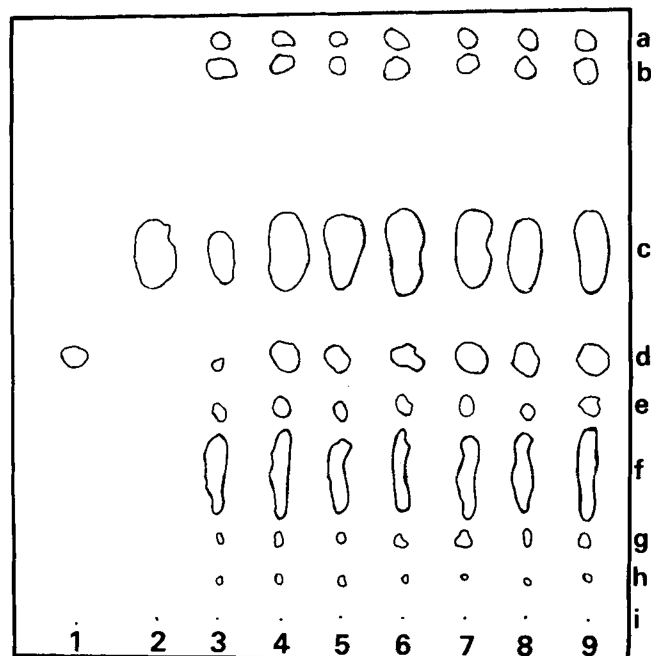

FIG. 1. Thin layer chromatoplate of lipid classes. 1: Standard free fatty acid; 2: standard triglyceride; 3: healthy corn oil; 4,5,6,7,8 and 9 represent corn oil extracted from embryos infected by various fungi. a: Hydrocarbons; b: sterolesters; c: triglycerides; d: free fatty acids; e: free sterols; f: unidentified; g: diglycerides; h: monoglycerides; i: phospholipids.

from infected embryos gave two peaks at 1710 and 1740 cm^{-1}, indicating the presence of free fatty acids. These results confirm the acid values for the oils obtained from infected embryos. Uninfected corn oil showed a very weak absorption band at 3500 cm^{-1}, indicating the presence of trace hydroxyl groups resulting from the presence of trace amounts of free fatty acids. This finding coincides with the acid value for the uninfected corn oil. On the contrary, a moderately intense absorption band appeared in this region for all infected oils, indicating that they are prone to hydrolysis and that free fatty acids were present at significant concentrations. Here again, the IR results for the oils extracted from embryos infected by fungi confirm their acid values.

Lipid Classes

Figure 1 shows the lipid classes for the oils obtained from healthy embryos and from embryos infected with various fungi. Nine spots were found on the TLC plate and their relative R_f values are presented in Table III. The lipid classes migrated in the following order (from solvent front to origin): hydrocarbons, sterol esters, triglycerides, free fatty acids, free sterols, diglycerides, monoglycerides and phospholipids. This sequence for lipid classes was reported by several authors (9,17). It was found that the oil extracted from embryos infected with fungi contained the same lipid classes as the uninfected oil. The apparent differences between the oils obtained from uninfected embryos and those infected with fungi were the spot size on TLC chromatoplate corresponding to mono- and diglycerides and free fatty acids. These lipid fractions were much smaller for the uninfected oil than for the oil extracted from embryos infected with various fungi, bearing in mind that each spot contained the same amount of oil. These results demonstrated that the fungi used in the present investigation hydrolyzed some of triglycerides to give a mixture of mono- and diglycerides and free fatty acids. TLC analysis

TABLE III

Thin Layer Chromatography of Lipid Classes

Lipid	RR_f[a]
Hydrocarbons	1.00
Sterol esters	0.94
Triglycerides	0.64
Free fatty acids	0.44
Free sterols	0.37
Unidentified	0.28
Diglycerides	0.14
Monoglycerides	0.12
Phospholipids	0.00

[a]RR_f refers to relative R_f, and the R_f value of hydrocarbons is given a value of 1.00.

for the oils confirms the results obtained from the acid values.

Fatty Acid Composition

Figure 2 is a representative chromatogram of standard fatty acid methyl esters used to characterize the unknown fatty acids. Table IV shows the fatty acid composition of the oil samples in this investigation. Uninfected oil contained 10:0, 12:0 and 18:0 fatty acids in trace amounts, myristic acid (14:0) occurred as a minor component, and 16:0, 18:1 and 18:2 fatty acids were present as major constituents. Uninfected oil contained two unidentified fatty acids with relative retention times of 0.15 and 0.36. The authors concluded that the unidentified fatty acids have 12 and 14 carbon atoms, respectively, and more than one double bond or some other functional groups that influence their retention times.

Several remarks have to be made about the fatty acid analysis in relation to the uninfected oil. Myristic acid content of oil obtained from infected embryos was much higher than that of the uninfected oil. On the contrary, the amount of 16:0 in uninfected oil was higher than the 16:0 of oil infected with various fungi. The increase in myristic

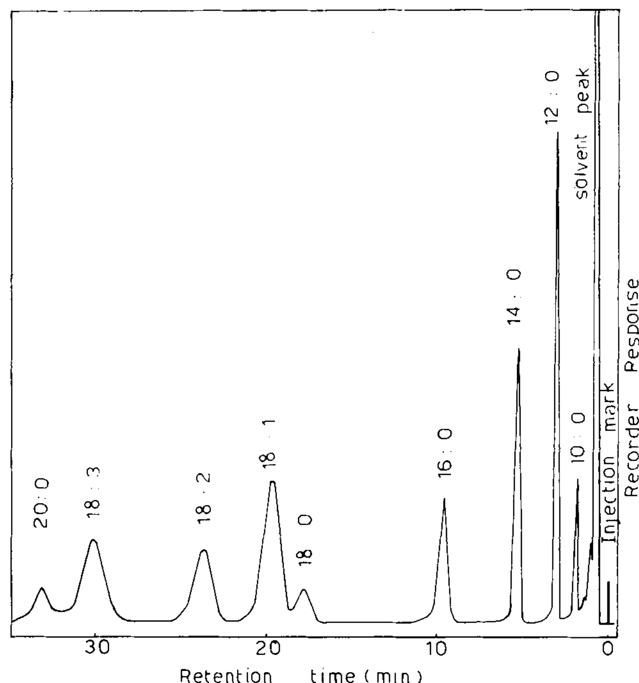

FIG. 2. GLC chromatogram of standard fatty acid methyl esters.

TABLE IV

Percentage Fatty Acid Composition of Uninfected and Infected Corn Oil[a]

Fatty acid	RRT[b]	Uninfected oil	Infected oil *A. flavus*	*A. melleus*	*A. nidulans*	*A. niger*	*F. moniliforme*	*P. oxalicum*
8:0	0.03	–	–	0.60	–	1.80	–	–
10:0	0.06	0.60	2.60	–	–	–	3.20	2.60
11:0	0.10	–	1.20	3.30	7.40	8.30	4.40	3.50
12:0	0.12	0.50	–	–	–	–	–	0.60
Unknown	0.15	2.80	0.20	2.60	0.70	0.70	3.10	3.10
13:0	0.17	–	5.10	–	–	–	4.30	–
14:0	0.24	1.50	3.30	6.50	4.10	8.70	5.40	7.90
Unknown	0.36	0.60	0.60	–	0.60	–	–	–
16:0	0.48	16.40	9.80	7.20	10.80	7.40	8.40	8.80
Unknown	0.55	–	1.10	4.40	–	–	–	–
Unknown	0.58	–	–	–	0.50	–	–	–
Unknown	0.64	–	2.30	3.80	–	1.80	–	–
Unknown	0.74	–	–	–	2.90	1.50	–	–
18:0	1.00	0.90	8.20	5.60	8.50	6.70	8.40	6.90
18:1	1.10	32.30	31.20	31.60	31.40	30.50	30.60	32.40
18:2	1.34	44.40	34.40	34.40	33.10	32.10	32.60	34.20
TU/TS[c]		3.87:1	2.17:1	2.86:1	2.09:1	1.92:1	1.93:1	2.24:1

[a]Infected corn oil refers to the oil extracted from infected corn embryos.
[b]RRT indicates the relative retention times; relative retention time for methyl stearate is given a value of 1.00.
[c]TU/TS refers to the ratio between the total unsaturated fatty acids (18:1 + 18:2) to the total saturated fatty acids (8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 16:0 and 18:0).

acid concentration of infected oils and the decrease in palmitic acid concentration suggest that some 16:0 is converted to 14:0 and possibly to 10:0 and 8:0 fatty acids, also due to the fungi splitting off two carbon fragments. The amount of 18:2 in uninfected oil was ca. 1.3 times as high as in the infected oils. The ratio between 18:1 and 18:2 in the infected oils was ca. 1:1, whereas in the uninfected oil, it was 1:1.37. The ratio for the infected oil illustrates that some 18:2 is converted to saturated fatty acids. The ratios of the unsaturated fatty acids (18:1 + 18:2) to saturated fatty acids (8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 16:0 and 18:0) were calculated for the uninfected and infected corn oils and the results are given in Table IV. From the ratios of TU/TS, it is clear that uninfected corn oil contained as much as 1.3 to 2 times the amount of unsaturated fatty acids as the infected oil, depending on the fungal species.

Several unknown fatty acids with relative retention times of 0.15, 0.36, 0.55, 0.58, 0.64 and 0.74 were found in infected corn oil. The unknown fatty acids with relative retention times of 0.15 and 0.36 have 12 and 14 carbon atoms, respectively. The other unknown fatty acids have 16 carbon atoms with one or more than one double bond. The presence of the last three unknown fatty acids in infected oils suggests that the fungi might synthesize new fatty acids or introduce other functional groups, e.g., OH or double bonds that would influence their retention times.

These results illustrate significant effects of the fungus on the fatty acid composition of corn oil. One might suggest that such changes stem from the fungal fatty acids. The authors found that the lipid content of these fungi was too low (1-2%) and their fatty acid composition was quite different in comparison to the fatty acid composition of the infected oils. Hence, the change in the fatty acid composition of the oil is a genuine effect resulting from fungal infection and not from the fungal lipids.

Free Fatty Acid Composition of Uninfected and Infected Corn Oils

GLC was also employed to study composition of the free fatty acids separated from the oils by TLC. The fatty acid composition of the free fatty acids extracted from the uninfected and infected oils is shown in Table V.

Uninfected corn oil was shown to contain the following free fatty acids: 14:0, 16:0, 18:0, 18:1 and 18:2, in the ratio of 4.3:3.1:1:6.95:7.33, respectively. These ratios demonstrated that the corn embryos contained lipase, which showed a preference to hydrolyze the unsaturated fatty acids having high molecular weights. Also, the ratio between total unsaturated fatty acids (TU) and total saturated fatty acids (the unknown fatty acids were not included in these calculations) indicated that the lipase preferably hydrolyzed the unsaturated fatty acids. It was found that 18:1 and 18:2 acids were present in a ratio of 1:1. This ratio revealed that the lipase hydrolyzed these fatty acids in equimolar ratio.

The fatty acids 8:0, 10:0, 12:0, 11:0 and 13:0 were present as members of the free fatty acids of infected corn oil and not in the uninfected oil. This means that these fatty acids, under the influence of fungi, stem from fatty acids with high molecular weights. The ratio of 14:0 to 16:0 in infected oil was ca. 1:1. This indicates that lipase hydrolyzes these fatty acids at a constant rate. It was found that 18:0 fatty acid in the free fatty acids of the uninfected oil was ca. half that in infected oil. Oleic acid (18:1) and linoleic acid (18:2) were present in equal proportions and their percentages were almost the same for the uninfected oil as for oils infected by fungi except in the free fatty acids associated with the oil infected by *F. moniliforme.* Because 18:1 and 18:2 fatty acids constituted more than 50% of the total free fatty acids, one might conclude that the lipase in corn embryo cells has the ability to hydrolyze the unsaturated fatty acids much faster than the other fatty acids. The results for 18:1 and 18:2 acids indicate that the lipase has no specificity toward the number of double bonds in fatty acid moieties.

Unsaponifiable Composition

Crude unsaponifiables extracted from healthy and infected corn oil were identified by GLC against authentic compounds. Figure 3A shows the chromatogram of the standard which was used to characterize the unknown hydro-

TABLE V

Percentage Free Fatty Acid Composition Extracted from Uninfected and Infected Corn Oil[a]

Fatty acid	RRT[b]	Uninfected oil	Infected oil *A. flavus*	*A. melleus*	*A. nidulans*	*A. niger*	*F. moniliforme*	*P. oxalicum*
8:0	0.03	–	–	0.80	–	1.70	–	–
10:0	0.06	–	3.40	–	–	–	4.00	2.30
11:0	0.10	–	1.70	4.60	3.60	2.20	4.70	6.40
12:0	0.12	–	–	–	–	–	–	1.60
Unknown	0.15	–	0.60	3.80	3.80	4.40	–	3.30
13:0	0.17	–	4.80	–	–	–	4.40	–
14:0	0.24	14.80	8.10	10.0	10.90	11.50	10.50	9.50
Unknown	0.36	11.60	0.90	–	0.50	–	–	–
16:0	0.48	14.00	10.30	10.30	11.10	13.30	9.10	8.90
Unknown	0.55	–	1.50	4.70	2.50	–	–	–
Unknown	0.64	–	2.90	3.80	–	–	–	–
Unknown	0.74	–	–	–	1.90	3.50	–	–
18:0	1.00	3.90	6.90	6.60	7.70	8.20	7.10	7.60
18:1	1.10	27.10	29.40	26.80	29.90	26.70	34.00	29.30
18:2	1.34	28.60	29.50	28.50	28.10	28.50	26.40	30.90
TU/TS[c]		1.70:1	1.67:1	1.70:1	1.74:1	1.52:1	1.70:1	1.65:1

[a]Infected corn oil refers to oil extracted from infected corn embryos.
[b]RRT means the relative retention times; the relative retention time for methyl stearate is given a value of 1.00.
[c]TU/TS refers to the ratio between the total unsaturated fatty acids (18:1 + 18:2) to the total saturated fatty acids (8:0, 10:0, 11:0, 12:0, 13:0, 14:0, 16:0 and 18:0).

carbons and sterols in corn oil samples. Table VI presents the retention times (RT) and relative retention times (RRT) of standard hydrocarbons and sterols. Table VII and Figures 3B, 3C, 3D, 4A, 4B, 4C and 4D present the unsaponifiable compounds of oils extracted from healthy embryos and embryos infected with various fungi. Each chromatogram of the unsaponifiables could be divided into two clear parts. The first part consisted of 14 compounds corresponding to the saturated and unsaturated hydrocarbons. These compounds seemed to constitute an important part of the unsaponifiables and the absence of some authentic substances prevent the complete identification of these compounds.

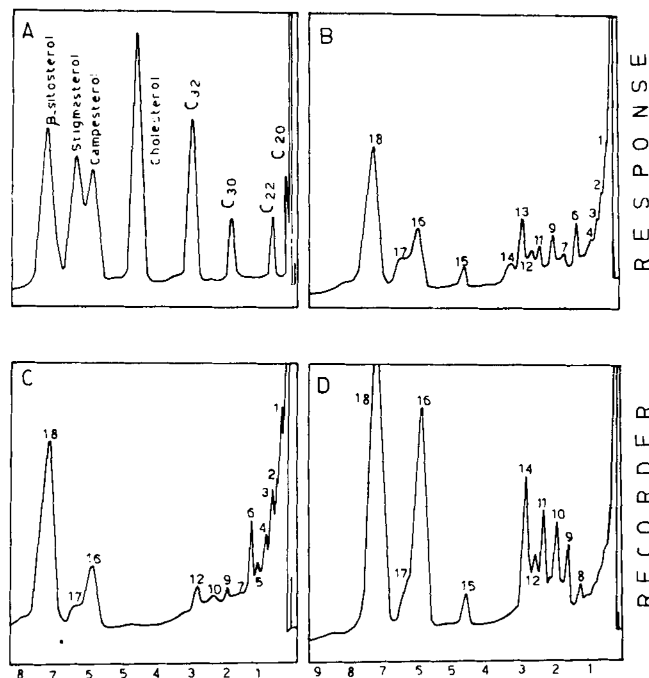

FIG. 3. GLC chromatograms of standards and the unsaponifiables of corn oil. A: Standard materials; B: healthy corn oil; C and D: corn oil extracted from embryos infected by *A. flavus* and *A. melleus*, respectively. Peak numbers refer to the components listed in Table VII.

The results of this work demonstrated that each fungus had an independent effect on the hydrocarbon fraction constituents. Hence, no general trend can be outlined expressing the role of the fungi used in this study. Also, the results for the hydrocarbon showed that some new compounds had appeared and some compounds had disappeared. An obvious change in the concentration of these compounds in all corn oil samples was found as a result of the infection by various fungi.

Sterol fractions of the oils were much simpler in composition than the hydrocarbons. Only four sterols were found in the uninfected oil, i.e., cholesterol, campesterol, stigmasterol and β-sitosterol. The ratio of the four sterols was greatly influenced by the fungi and may be useful in the characterization of the effect of fungi. Also, the results for sterol fraction of corn oil extracted from embryos infected

TABLE VI

Retention Times (RT) and Relative Retention Times (RRT) of Standard Hydrocarbons and Sterols

Component		RT (min)	RRT[a]
n-Eicosane	(C_{20})	0.37	0.05
n-Heneicosane	(C_{21})	0.46	0.06
n-Docosane	(C_{22})	0.59	0.08
n-Tricosane	(C_{23})	0.77	0.10
n-Tetracosane	(C_{24})	0.93	0.13
n-Pentacosane	(C_{25})	1.08	0.15
n-Hexacosane	(C_{26})	1.23	0.17
n-Heptacosane	(C_{27})	1.38	0.19
n-Octacosane	(C_{28})	1.54	0.21
n-Nonacosane	(C_{29})	1.69	0.23
n-Triacontane	(C_{30})	1.85	0.25
n-Hentriacontane	(C_{31})	1.98	0.27
n-Dotriacontane	(C_{32})	2.15	0.29
n-Tritriacontane	(C_{33})	2.29	0.31
Cholesterol		4.7	0.63
Campesterol		6.2	0.84
Stigmasterol		6.6	0.89
β-Sitosterol		7.4	1.00

[a]Relative retention time for β-sitosterol is given a value of 1.00.

TABLE VII

Percentage Composition of the Unsaponifiable Matter of Uninfected Oil and Corn Oil Extracted from Corn Embryos Infected with Various Fungi

Component	Peak number	RRT[a]	Uninfected oil	Corn oil infected with					
				A. flavus	*A. melleus*	*A. nidulans*	*A. niger*	*F. moniliforme*	*P. oxalicum*
Unknown	1	0.05	0.78	1.98	–	0.82	0.82	0.86	4.23
C_{20}	2	0.06	0.43	0.92	–	0.61	0.61	0.24	–
C_{22}	3	0.07	0.47	2.77	–	0.20	–	0.26	–
C_{24}	4	0.08	0.23	0.92	–	0.55	0.41	0.39	–
C_{26}	5	0.14	–	0.66	–	0.41	0.56	0.06	–
C_{28}	6	0.15	3.90	5.12	–	4.39	0.21	5.89	0.07
Unknown	7	0.20	0.78	0.13	–	0.94	3.76	0.94	3.85
C_{30}	8	0.25	–	–	0.40	–	0.99	0.64	1.41
Unknown	9	0.27	2.81	0.89	2.07	2.37	–	4.95	4.02
C_{31}	10	0.28	–	0.92	2.15	–	3.09	0.11	1.02
Unknown	11	0.37	1.56	–	3.01	1.53	1.71	2.25	3.74
Unknown	12	0.39	0.70	2.64	0.52	0.41	7.98	1.89	3.33
C_{32}	13	0.44	1.83	–	–	5.55	–	24.31	9.25
C_{33}	14	0.48	1.41	–	6.00	0.86	1.66	1.67	–
Cholesterol	15	0.63	3.56	–	1.12	–	2.62	4.26	21.11
Campesterol	16	0.84	15.75	13.44	30.22	17.81	12.88	8.02	9.87
Stigmasterol	17	0.89	6.56	4.62	7.10	7.15	13.38	2.25	8.43
β-sitosterol	18	1.00	59.23	64.59	47.41	56.40	49.32	41.01	29.67

[a]Relative retention time for β-sitosterol (retention time = 7.4 min) taken as 1.00.

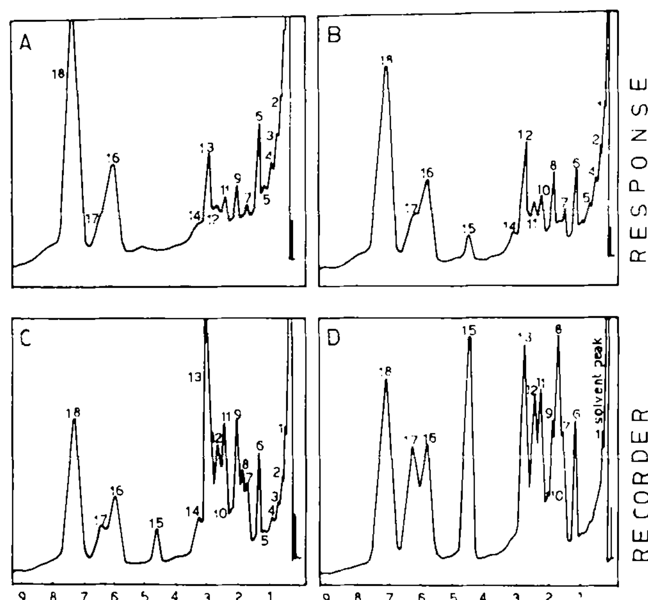

FIG. 4. GLC chromatograms of the unsaponifiables of corn oils. A: Infected by *A. nidulans*; B: infected by *A. niger*; C: infected by *F. moniliforme*; D: infected by *P. oxalicum*. Peak numbers refer to the components listed in Table VII.

with *A. flavus* and *A. nidulans* suggested that the absence of cholesterol was due to its transformation to the other sterols and only to campesterol, respectively. Also, the increase in cholesterol and stigmasterol content of corn oil extracted from embryos infected by *P. oxalicum* and *A. niger* stems from conversion some of β-sitosterol and stigmasterol, respectively. These data suggest that interconversion between the sterols may occur and further work is necessary to study the mechanism by which interconversions might take place.

Aflatoxins in Corn Oil from Embryos Infected by *A. flavus*

A portion of corn embryos that were deliberately infected by *A. flavus* was extracted with chloroform/methanol mixture (2:1, v/v). TLC analysis indicated only the presence of aflatoxin B_1 in the oil extract. Spectrophotometric determination of aflatoxin B_1 in oil extracted from embryos infected by *A. flavus* was 300 μg/kg.

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