Improved Procedure for Titrating Cyclopropene Esters with Hydrogen Bromide

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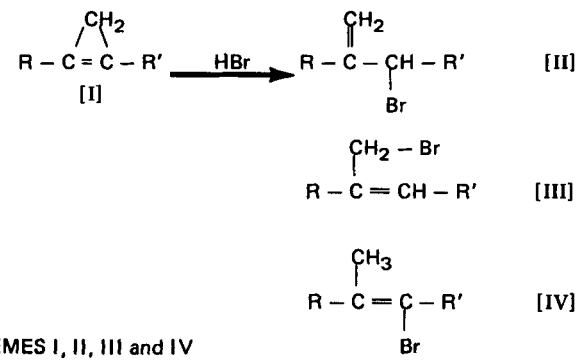
ABSTRACT

The titration of cyclopropenes in cottonseed and other oils with hydrogen bromide was improved by changes in the preparation of the sample and the titration. Methanolysis of the oil under selected conditions with a large excess of alkaline catalyst (sodium methoxide or tetramethylammonium hydroxide) yielded methyl esters free of oxidation products and partial glycerides and eliminated the need for treatment with a large amount of activated alumina, which disproportionates methyl esters. The color indicator, 4-phenylazodiphenylamine, exhibited great sensitivity in toluene at 25 C. The best procedure consisted of titrating methyl esters with hydrogen bromide at 60-65 C to just past the end-point, cooling to about 25 C, and back-titrating with aniline in toluene. With 10- to 2-g samples containing less than 1% cyclopropenes, reproducibility usually was within ± 0.003% of the average value.

INTRODUCTION

Glycerides of sterculic acid (9,10-methano-9-octadecenoic acid) and its homolog, malvalic acid (8,9-methano-8-heptadecenoic acid) occur in cottonseed oil and other seed oils of the order Malvales. Because these cyclopropene acids possess unusual physiological properties, considerable effort has been devoted to developing methods of analyzing for small amounts. Analytical methods based on reactions with such compounds as sulfur and hydrogen bromide have been devised (1-4), as have primarily physical methods (5-8). When the samples being tested are free of interfering substances, most of the methods will determine cyclopropene content more or less accurately. But unfortunately, they frequently require considerable judgment and skill on the part of the analyst. A simple, accurate and inexpensive method is still needed.

Titration of cyclopropenes with an anhydrous solution of hydrogen bromide possesses most of the qualifications desired of a good analytical method, and a number of methods based on such a titration have been described (4,9-11). In these methods, the cyclopropene moiety (I) reacts quantitatively with hydrogen bromide to yield three types of compounds (12).



SCHEMES I, II, III and IV

At 20 C, the reaction product contains ca. 69% of II and 21% of III; at higher temperatures more of III is formed, principally at the expense of II.

The first methods proposed for the titration of cyclopropenes with hydrogen bromide consisted of dissolving the sample of triglycerides or methyl esters in a solution of benzene and glacial acetic acid and titrating at 55 C with hydrogen bromide in glacial acetic acid. A practically closed system was used; mixing was performed with a magnetic stirrer and a Teflon-coated bar. The end-point was determined with the color indicator crystal violet.

One objectionable feature of these methods was the hydrogen bromide-catalyzed reaction of the acetic acid with ca. 15% of the cyclopropenes (13). Later methods that used acetic acid as solvent employed a correction factor to compensate for this side reaction (9). A minor problem with this titration was judging the blue-green end-point as the crystal violet indicator changed from violet to blue for the singly protonated form and then to yellow for the double protonated form.

The problem presented by the hydrogen bromide-catalyzed addition of acetic acid to a portion of the cyclopropenes being titrated can be overcome by replacing the acetic acid-benzene solution with pure toluene, which like other aromatic hydrocarbons, exhibits some of the properties of a Lewis base and forms loose addition compounds with hydrogen bromide (10). But crystal violet and the other color indicators that had been used function poorly in toluene. A titration procedure was devised in which an excess of hydrogen bromide/benzene reagent is allowed to react for 30-40 min with the cyclopropene-containing sample at 20 C, then water is added and the mixture is back-titrated with sodium hydroxide solution to the phenolphthalein end-point (11).

Another objectional feature of the hydrogen bromide method was the technique recommended for removing interfering impurities from the samples. Peroxides, monoglycerides, and diglycerides titrate and are present at least in trace amounts in most samples of triglycerides and methyl esters. These impurities were removed by passing a hexane solution of the sample through a large proportion of alumina. Effective removal of impurities required at least partially activated alumina and its use resulted in a sizable sample loss by adsorption. Retention of the sample components on the alumina was somewhat selective and produced some disproportionation.

This communication describes improvements in the preparation of samples for titration and improvements in the titration procedure, including use of the color indicator 4-phenylazodiphenylamine.

EXPERIMENTAL PROCEDURES

Materials

Dry toluene used in the standard solutions and as a solvent was prepared from a reagent-grade product (ca. 0.03% water) by stripping with dry nitrogen under vacuum. The 0.1 N hydrogen-bromide-in-toluene solution was prepared by passing hydrogen bromide (99.8% purity) from a gas cylinder into the dry toluene. Approximately 0.05 N aniline in toluene was prepared with reagent-grade aniline.

The refined and refined and bleached cottonseed oils

were typical commercial products; the refined and bleached oil had been made from the refined oil.

The refined peanut oil was a high-grade commercial product. The oxidized peanut oils were derived from a commercial product which had been stored in the laboratory in a partially filled container for several years and then was further oxidized by passing air through the oil at 100 C. Final peroxide value of one of the oxidized oils was 25.8 meq/kg and that of the other was 38.6 meq/kg.

Refined and bleached *Sterculia foetida* oil was prepared in the laboratory from crude oil extracted from ground seed with hexane.

The color indicators were reagent-grade chemicals used as 0.05% solutions. Crystal violet was dissolved in butyric acid and the other indicators were dissolved in toluene.

Compounds used to standardize the hydrogen bromide solution were of primary standard quality with the exception of the 1,3-diphenylguanidine, which was of reagent grade. The following proportions of solvents were employed to dissolve the quantity of standard needed to react with ca. 8 ml of 0.1 N hydrogen bromide: tris(hydroxymethyl)aminomethane (THAM), 20 ml toluene and 5 ml butyric acid; potassium hydrogen phthalate, 14 ml glacial acetic acid and 7 ml toluene; sodium carbonate, 20 ml glacial acetic acid; and 1,3-diphenylquanidine, 20 ml toluene.

Alumina used to purify the oils and methyl esters was chromatographic grade (Alumina F-20, Alcoa 80-200 mesh) and either was used as received or was freshly activated by heating at 200 C for several hours.

Methanolysis of the oils was catalyzed by either tetramethylammonium hydroxide (Aldrich, 20% solution in methanol) or sodium methoxide, obtained as an alcohol-free powder (Mathieson) or prepared in the laboratory from metallic sodium.

Equipment

For the titrations with hydrogen bromide, a 10-ml, automatic burette with a Teflon plug (Kimax No. 17138-F) was fitted with a Leur tip to which was attached a Teflon needle (no. 26 or 28, 30 cm long). During titration, only one of the two breather tubes on the burette was opened to the atmosphere and then moisture was prevented from entering by attaching a Drierite tube and keeping the size of the opening small. The lower end of the Teflon needle was passed through a no. 4 neoprene stopper used to seal the titration flask (a 50-ml extraction flask with a 24/40 standard taper joint). The length of the Teflon needle in the flask was adjusted to reach the bottom of the flask and release titrant below the surface of the solution to be titrated. A no. 15, stainless-steel, hypodermic needle also was inserted through the neoprene stopper to serve as a breather and as an opening through which to insert the Teflon needle from the aniline-containing burette. A Teflon-coated stirring bar was placed in the titration flask to mix the contents. The assembly was placed in a silicone oil bath on a magnetic stirrer-hot plate combination.

The burette containing the aniline-toluene solution was similar to that containing the hydrogen bromide solution, except that the capacity was 5 ml instead of 10.

Procedures

Methyl esters were prepared by the alkali-catalyzed interesterification of oil and methanol in a round-bottom, distilling flask immersed in a silicone oil bath at 50 C. Reaction time was usually 30 min. At the start of each reaction, the head space was flushed with dry nitrogen, and the flask was sealed with a glass stopper. A Teflon-coated stirring bar was used to mix the reactants. The ratio of oil to catalyst-containing methanol (adopted after some testing) was 1 g to 1 ml. The weight proportions of catalyst in methanol finally adopted were 4% sodium methoxide or 20% tetramethylammonium hydroxide. At the completion of the reaction, the product was cooled while stirring was continued and a volume of hexane equal to 5 times that of the reactants was added. After thorough stirring, the mixture was transferred to a separatory funnel with the aid of a small additional amount of hexane. After settling, the clear alcohol layer was drawn off. The first washing of the hexane solution was made with water containing 2% acetic acid. Subsequent washings were performed with water only. Hexane was removed from the washed solution by stripping with dry nitrogen under reduced pressure at temperatures up to 70 C.

When loss of sample and disproportionation were immaterial, alumina treatment of the oils and methyl esters consisted of passing a solution of the sample in hexane (1:1, w/w) through a ca. 40-cm column of alumina (4 parts alumina to 1 part sample, w/w). At lower alumina-to-sample ratios, shorter columns, down to ca. 20 cm in length, with smaller cross-sectional areas were used. Hexane was removed from the sample by stripping with nitrogen under vacuum at temperatures below 70 C.

Several titration procedures were tried before the following was adopted: The sample of methyl esters (varying in size up to 25 g and containing cyclopropenes equivalent to no more than 250 mg of sterculic acid) was weighed into the 50-ml titration flask; usually, enough dry toluene was added to bring the sample in solution to between 20 and 30 ml; the Teflon-coated stirring bar was added; air space above the sample was flushed with nitrogen; and the rubber stopper through which the Teflon and stainless steel needles had been passed was put into place. The flask was mounted in the silicone bath, maintained at 60-65 C, and allowed to equilibrate. About one-half of the titration with 0.1 N hydrogen bromide was completed before the color indicator (0.2 ml of 0.05% 4-phenylazodiphenylamine in toluene) was added. The titration was continued about 0.1 ml beyond the point at which the indicator changed from light yellow to rose and remained a rose color for 1 min. The flask was removed from the silicone bath and plunged into an ice and water mixture for 1-2 min to reduce the temperature to ca. 25 C and change the indicator color from rose to a deep rose-purple. The Teflon needle from the 0.05 N aniline burette was inserted through the stainless steel needle in the stopper of the titration flask until the tip of the Teflon needle reached the bottom of the solution in the titration flask. The assembly was placed over a magnetic stirrer and the solution was back-titrated with the aniline to a light yellow color.

RESULTS AND DISCUSSION

Purification

Most of the oxidation products in oils react readily during the titration of cyclopropenes at 60-65 C. A peanut oil with a peroxide value of 25.8 meq/kg (14) consumed hydrogen bromide equivalent to 0.82% methyl malvalate. Purified monoglycerides titrated readily to the equivalent of ca. 60% methyl malvalate. Purified 1,3-distearin also could be titrated, but the reaction rate was relatively slow. After 1 ml of 0.1 N hydrogen bromide had reacted with 0.5 g 1,3-distearin at 65 C, a 0.05-ml excess of titrant required ca. 2 min to react.

The alkali-catalyzed methanolysis of oils was examined to establish to what extent this reaction could be used to simultaneously eliminate peroxides, monoglycerides and diglycerides from cyclopropene-containing samples. Such interesterifications have no effect on the cyclopropenes.

Methyl esters prepared from peanut oil by interesterification at 50 C with 1.5 equivalents of methanol in the presence of 0.2% sodium methoxide, followed by cooling, settling, removal of the glycerol-rich layer, and washing with water, yielded a finished product containing over 15% glycerides (mono-, di- and tri-) when analyzed by thin layer chromatography.

A methanolysis catalyzed with tetramethylammonium hydroxide (TMAH) as described by Metcalfe (15) was adapted to produce methyl esters free of glycerol. For this reaction, 20 g of oxidized peanut oil (peroxide value, 38.5 meq/kg) in 300 ml diethyl ether was reacted with 20 ml of 20% TMAH in methanol for 10 min at 25 C. The peroxide value was reduced to 2.0 meq/kg, but persistent emulsions formed during water-washing of the reaction product, and the methyl esters obtained were distinctly yellow in color. Modification of this methanolysis by substituting 30 ml hexane for the 300 ml diethyl ether and conducting the reaction for 20 min at 50 C resulted in no emulsions upon washing and yielded esters very light in color and having a peroxide value of 1.6 meq/kg. But these esters and other esters prepared in hexane solution always contained detectable amounts of mono-, di- and triglycerides.

A series of interesterifications was conducted with oxidized peanut oil to establish the effect of type and concentration of catalyst and ratio of oil to methanol on reduction of peroxide value (Table I). In each run hexane was added after a reaction time of 30 min at 50 C. The reaction product from run TMAH-1 contained ca. 50% glycerides, but the other products were free of glycerides. Reactions conducted with relatively low ratios of methanol to oil or low concentrations of catalyst effectively destroyed peroxides, but the lowest peroxide values were obtained with an oil:methanol ratio of 1 g:1 ml and the higher catalyst concentrations.

Twenty percent TMAH in methanol is the mol equivalent of 11.85% sodium methoxide in methanol. However, a reaction conducted with 20% TMAH at an oil:methanol ratio of 1 g:1 ml resulted in two liquid phases free of solids, whereas a similar reaction conducted with 12% sodium methoxide in methanol yielded a large volume of solids in addition to the two liquid phases. The highest concentration of sodium methoxide in methanol that did not produce solids under these conditions was ca. 4%.

The peroxide values of methyl esters made from highly oxidized peanut oil could be reduced to ca. 0.3 by further mixing and heating the methyl esters with powdered sodium hydroxide or sodium methoxide, but the values never could be reduced to zero.

Of course, the need for a procedure to analyze highly oxidized or rancid oils for cyclopropenes is not great. The idea of using excess catalyst to destroy peroxides was tested with a peanut oil having a peroxide value of 2.6. The peroxides in this oil were destroyed completely.

Interesterification of an oil for 30 min at 50 C with either 4% sodium methoxide or 20% TMAH in methanol (1 g:1 ml) followed by mixing with hexane (1:5, v/v) resulted in a quantitative conversion to methyl esters. Settling and removal of the methanol layer followed by washing with hexane layer, first with 2% acetic acid in water and then with water, could be performed readily with practically no loss of methyl esters. No problems with emulsions were encountered.

Even though oils of reasonably good quality could be interesterified to obtain methyl esters apparently free of oxidation products, monoglycerides and diglycerides, the elimination of all forms of alumina treatment for removing such impurities from samples is inadvisable. If an analytical procedure is desired which determines cyclopropene content down to ca. 0.01%, precautions need to be taken to remove possible traces of interfering impurities.

The alumina treatment employed heretofore specified 4 parts alumina to 1 part sample. Freshly activated alumina, activity 1.5 (16), used in this manner to treat oxidized peanut oil (peroxide value, 38.6) reduced the peroxide value to 1.6 but retained 49% of the sample. Methyl esters from the oil were retained to the extent of 34%. Such heavy treatment with freshly activated alumina always increased the cyclopropene content. For example, a mixture of purified methyl esters derived from peanut and *S. foetida* oils analyzed 9.62% cyclopropenes (calculated as sterculic acid) before treatment and 10.58% after treatment. At levels of ca. 0.5% cyclopropenes, such increases caused by disproportionation are, of course, more difficult to detect. Reducing the ratio of alumina to sample from 4:1 to 0.4:1 does not affect the ability to remove traces of mono- and diglycerides, and the change in cyclopropene content can be tolerated.

Another approach to the alumina treatment might be employed. In this and previous work, it was noted that decreasing the activity of the alumina by allowing it to absorb moisture reduced the capacity to retain methyl esters but within broad limits, did not significantly affect the ability to remove objectionable impurities.

TABLE I

Destruction of Peroxides during Methanolysis of Oxidized Peanut Oil[a]

Catalyst, run no.	Concentration, catalyst in CH_3OH (wt %)	Ratio, oil to CH_3OH solution (g:ml)	Peroxide value of methyl esters (meq/kg)
TMAH			
1	20	10:2	2.3
2	6.67	10:30	2.7
3	10	10:10	1.3
4	20	10:10	1.3
$NaOCH_3$			
1	1	10:10	6.2
2	3	10:10	3.4
3	4	20:20	1.9
4	6	10:10	1.8
5	12	20:20	0.5

[a]Peanut oil had a peroxide value of 38.6 meq/kg. Methyl ester products diluted with 100 ml hexane before washing.

Titrations

Tests with a number of color indicators in toluene solution revealed that those of the azo type performed best. However, such compounds as *p*-phenylazoaniline and *p*-dimethylaminoazobenzene were relatively insoluble in the protonated form and the acid-base colors did not reverse readily.

Trans-Pyridine-2-azo-*p*-dimethylaniline was found to be usable. In toluene alone and in toluene solutions containing less than 5% butyric or longer chain fatty acids, it formed a yellow solution. In butyric acid, the color was deep red. As increasing amounts of a strong acid were added slowly to a toluene solution of this indicator, the color changed from yellow to red to yellow so rapidly that the end-point could easily be missed. This indicator could be used to titrate cyclopropenes at a slow rate.

The best indicator found was 4-phenylazodiphenylamine. It dissolved readily in toluene, the complex with hydrogen bromide appeared to be soluble, and the color reversed readily. A few drops (0.2 ml) of 0.05% solution, when added to 20 ml of toluene at room temperature, formed a light yellow solution. Addition of 0.001 ml of 0.1 N hydrogen bromide in toluene produced a deep purple

color. This amount of hydrogen bromide is equivalent to 0.0003% cyclopropenes in a 10-g sample. Also, this amount of hydrogen bromide was ca. 1/15th the amount required to change the color of crystal violet in 20 ml glacial acetic acid to the blue-green end-point. Ordinarily, color indicators are relatively insensitive in nonaqueous solvents, and the high sensitivity of 4-phenylazodiphenylamine was unexpected. Further testing revealed that a toluene solution of protonated 4-phenylazodiphenylamine changed from a deep purple to a rose color as the temperature was raised from 25 to 60 C.

Standardization of hydrogen bromide was performed satisfactorily with each of the primary standards. The indicator crystal violet was used with the sodium carbonate in acetic acid solution. Tris(hydroxymethyl)aminomethane (THAM) was the preferred standard because it is readily available in highly purified form and the required amount could be dissolved in a predominantly toluene solution (20 ml toluene plus 5 ml butyric acid). The aniline-in-toluene solution was standardized by titrating with the hydrogen bromide in toluene to the 4-phenylazodiphenylamine end-point.

Titrations of cyclopropenes with hydrogen bromide were conducted at temperatures between 40 and 80 C. At 40 C and 20 ml of cyclopropene-containing solution, the rapid addition of ca. 0.1 ml of titrant during the early stages caused the indicator to turn red and then revert back to yellow in ca. 3 sec. At 60 C, the hydrogen bromide reacted as rapidly as the titrant could be added, ca. 0.8 ml/min. The reaction rate slowed considerably near the end-point when only very low concentrations of hydrogen bromide and cyclopropenes were present. The problem was solved by adding a slight excess of titrant (0.1 to 0.2 ml), heating and mixing for 1 min, cooling to intensify the acid color of the indicator, 4-phenylazodiphenylamine, and back-titrating with the aniline solution. During these manipulations, the unwanted transfer of solution from the Teflon needles was negligible. The no. 28 needles used had an internal vol of ca. 0.0011 ml/cm.

The method of titration given in the section on experimental procedures was compared with a proven method (10) by analyzing highly purified methyl esters made from the *S. foetida* oil, which contained ca. 57% cyclopropenes (calculated as sterculic acid). No differences were found among the results.

A purified sample of methyl esters from peanut oil that contained no cyclopropenes, also was analyzed and found to titrate as though it contained 0.005% cyclopropenes when the values were corrected for titrants consumed in a blank titration.

When mixtures of methyl esters derived from cottonseed and peanut oils were titrated, the contents of cyclopropene esters agreed with those calculated from the percentages of methyl esters of cottonseed oil present. One set of such titrations is recorded in Table II.

As reported previously (10), the sharpness of the crystal violet end-point in the titration of cyclopropenes with hydrogen bromide in toluene decreased as the oil (triglyceride) content of the sample increased above 12 g/20 ml toluene. Such an effect was not noticed with the indicator 4-phenylazodiphenylamine, which performed well even with neat samples. But when large samples of oil were titrated without first adding a small amount of toluene, the cyclopropene contents usually were 0.01 or 0.02 unit above those obtained when toluene was added (Table III). Starting a titration with relatively viscous triglycerides may permit a minute amount of hydrogen bromide to escape from the liquid phase.

No such problems were encountered with methyl esters.

TABLE II

Analysis of Mixtures of Methyl Esters Preprared from Peanut and Cottonseed Oils[a]

Titration no.	Cyclopropenes[b] Found (%)	Cyclopropenes[b] Calculated (%)	Methyl esters from cottonseed oil (%)
1	0.568	–	100.0
2	0.568	–	100.0
3	0.294	0.284	50.00
4	0.288	0.284	50.00
5	0.109	0.114	20.00
6	0.108	0.114	20.00

[a]Methyl esters were made from commercially refined cottonseed and peanut oils, purified by passage through alumina, and mixed in the indicated proportions. All samples weighed 20 g and were diluted with 10 ml toluene before titration.

[b]Calculated as malvalic acid, wt %.

TABLE III

Effect of Solvent on Cyclopropenes Found in Cottonseed Oil[a]

Cottonseed oil (g)	Toluene added (g)	Cyclopropenes found[b] (%)
10	15	0.592
10	15	0.592
25	0	0.609
25	0	0.610

[a]Commercially refined and bleached oil subsequently purified by passage through alumina.

[b]Calculated as malvalic acid, wt %.

TABLE IV

Effect of Sample Size and Amount of Toluene on Cyclopropenes Found in Methyl Esters Made from Refined Cottonseed Oil[a]

Methyl ester (g)	Toluene added (g)	Methyl ester concentration (%)	Cyclopropenes found[b] (%)
5	20	20	0.578
10	15	40	0.570
15	10	60	0.572
20	5	80	0.568
25	0	100	0.572

[a]Methyl esters were made from commercially refined oil and purified by passage through alumina.

[b]Calculated as malvalic acid, wt %.

The same results were obtained when sample size and proportion of toluene were varied over a considerable range (Table IV). With methyl ester samples of 10-25 g containing less than 1% cyclopropenes, reproducibility was usually within ± 0.003% of the average percentage.

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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.