Fatty Acid and Vitamin E Content of Nutrimaiz, a Sugary/Opaque-2 Corn Cultivar

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Comparative analyses were carried out on Nutrimaiz (a corn cultivar with Sugary/Opaque-2 endosperm), derived from Opaque-2 and Sugary varieties, on its progenitors, and on a common corn (Maya XII Normal) for tocopherols, tocotrienols, and fatty acids. A shorter procedure was developed for extracting nonsaponifiable material (containing the tocols) after direct saponification of the sample, instead of first extracting the lipids. Individual tocopherols and tocotrienols were separated by TLC on silica gel, extracted, and determined by a new reaction with cupric ion and a complexing agent, either cuproine or bathocuproine. Fatty acids were determined by GLC of their methyl esters. Nutrimaiz was found to be more similar to Sugary than to Opaque-2 in total lipids, fatty acids, and total tocols, indicating that the Sugary gene is epistatic over that of Opaque-2 for these traits. Results expressed on a dry basis showed that all varieties increased in total lipids and total tocols in going from the fresh to the mature state.

Research on the genetic improvement of protein in corn was stimulated by the discovery of the Opaque-2 gene effect and that the introduction of the Opaque-2 gene into other varieties produces a higher lysine content in the endosperm. The development of a new variety, called Nutrimaiz, homozygous for both Sugary/Opaque-2 genes (*suo2*) resulting from the cross between an Opaque-2 (*o2*) and a Sugary (*su*) cultivar has been studied extensively at the State University of Campinas by Sgarbieri et al. (1977) and da Silva et al. (1978). These authors found exceptionally high nutritional value for *suo2*, in comparison with both the normal corn Maya XII (*M*) and the progenitors *su* and *o2*.

A typical characteristic of the variety *suo2* is the rapid increase in the size of the germ that, only 24 days after pollination, shows a lipid content comparable with normal corn (*M*) in its mature state (60 days after pollination). This concentration of lipids awakened interest in knowing the content of linoleic acid and tocopherols, all important compounds in the nutritional performance of the cereals. These compounds were determined in *suo2*, in its progenitors *su* and *o2*, and, for comparison, in the common corn *M*. The samples were examined in both the fresh and mature states in order to learn about changes during maturation and to look for some information about heredity with respect to tocopherols.

Tocopherols and tocotrienols can be determined by HPLC, GLC, and TLC. The last method is within reach of any laboratory and serves admirably for separating typical complex mixtures of tocopherols and tocotrienols that occur during the development of cereals. Evaluation of the separated compounds in the TLC chromatogram was done by a new reaction (Contreras-Guzmán and Strong, 1982b) that involves the reduction of cupric ions to cuprous by the tocopherols and subsequent complexation with 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine).

EXPERIMENTAL SECTION

Samples. All the corn samples (*Zea mays* L.) were grown at the Experimental Area of the Universidade Estadual de Campinas. Four varieties of corn, harvested in both 1978 and 1979, were studied: Sugary (*su*), Opaque-2 (*o2*), Nutrimaiz (*suo2*), and Maya XII Normal (*M*). Samples for chemical determinations were collected in the fresh or milky state (24 days after pollination) and in the mature state (60 days after pollination). Aproximately 200 kg of cobs, either fresh or mature of each variety, was collected and 10 kg (5%) sampled for studies. The fresh corn was promptly frozen and lyophilized on the cob. After dehydration, the grains were separated and stored in sealed flasks in a freezer. The grains of mature corn were removed and stored in the same manner.

Reagents. The following reagents were used: 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline (bathocuproine), reagent grade (Carlo Erba); 2,2′-biquinoline (cuproine) (Baker); methyl esters of fatty acids (Applied Science Laboratories); *all-rac*-α-tocopherol, for biochemical use 99% (Merck); *d*-γ-tocopherol, and *d*-δ-tocopherol isolated from natural sources (Contreras-Guzmán, 1981); ethanol, heptane, hexane, and toluene, analytical grade, treated to remove reducing impurities according to Contreras-Guzmán (1981). TLC plates were aluminum sheets 20 × 20 cm, precoated with silica gel 60 G, with a layer thickness of 0.2 mm (Merck).

Solutions used were as follows (all weight per volume except as noted): 0.50% $Cu(NO_3)_2 \cdot 3H_2O$ in distilled water (I); 0.10% $Cu(NO_3)_2 \cdot 3H_2O$ in absolute ethanol (II); 0.50% cuproine in toluene (III); 0.50% bathocuproine in toluene (IV); 2.5% urea in absolute ethanol (V). Complexing reagent for total tocols was 10 mL of I and 20 mL of III, diluted to 50 mL with V (VI). Complexing reagent for individual tocopherols consisted of 10 mL of II and 2.0 mL of IV, diluted to 50 mL with V (VII). The standard mixture of tocopherols (T) was 0.4 mg of αT plus 1.6 mg of γT plus 0.2 mg of δT per mL. Solutions II–IV should be kept in a refrigerator. Solutions VI and VII were prepared just before using.

Procedures. *Extraction of Tocopherols by Direct Saponification.* Samples were ground at the time of the analysis to totally pass sieve no. 60 (0.25-mm openings). A sample of 3.50 g was weighed in a 125-mL flask, to which was added 200–250 mg of solid ascorbic acid and 20 mL of absolute ethanol. The condenser was connected to the flask and the system heated in a boiling water bath until the ethanol began to boil. Then 5 mL of 50% KOH (w/w) was added through the condenser, and the reflux was maintained for 30 min. The mixture was cooled and transferred quantitatively to a 100-mL glass-stoppered, graduated cylinder, washing the flask several times with 2.5% NaCl and diluting the total volume to 75 mL. Unsaponifiable material was then extracted in the cylinder by shaking, with 15-, 15-, 10-, and 10-mL portions of hexane, and transferring each extract to a separatory

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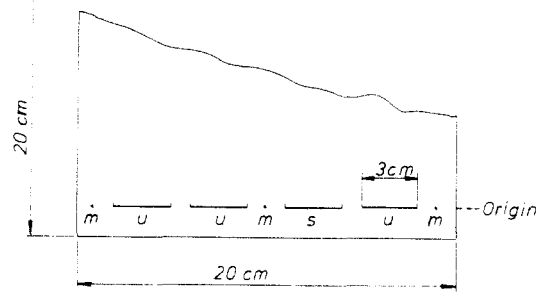

Figure 1. Arrangement of samples, standards, and monitor for the quantitative determination of tocopherols and tocotrienols by TLC. u = unsaponifiable material from corn; s = standard mixture; m = monitor.

funnel. The hexane was washed twice with distilled water and dried with anhydrous Na_2SO_4. The hexane extract was then transferred quantitatively to a 125-mL flask and the solvent removed at 40 °C in a rotatory evaporator. The residue was dissolved in 10 mL of hexane. A standard consisting of 100 μg of αT was also processed along with the samples, recovered in 10 mL of hexane, and used for calculations. For comparative purposes, the lyophylized samples of corn were extracted and saponified according to the AOAC (1980) methods. The unsaponifiable fraction was dissolved in 10 mL of hexane. Again 100 μg of αT was submitted to the whole process and used for calculations.

Determination of Total Tocols, Expressed as αT. Volumes of 2.5 mL of unsaponifiable fraction, αT standard, and blanks of hexane were diluted with 2.5 mL of heptane. To these were added 5 mL of the complexing reagent VI and the mixtures were agitated vigorously (mechanically or manually) for 2.5 min. They were allowed to settle and 3.0 mL of the lower layers was measured. To these was added 0.5 mL of absolute ethanol to clarify them. After 5 min, the absorbances were read at 545 nm. Additional details are given in a preliminary paper (Contreras-Guzmán and Strong, 1982a).

Composition of Fatty Acids. An aliquot of total lipid extract, obtained by the method of Bligh and Dyer (1959) and containing approximately 100 mg of total lipids, was evaporated under vacuum, and the lipids were methylated according to Metcalfe et al. (1966).

A Perkin-Elmer Model 900 gas chromatograph was used for the separation. The stainless steel column, 3.6 m in length and 3 mm in inside diameter, was packed with 3% by weight of diethylene glycol succinate on Chromosorb GAW M 80/100. A sample of 10 μL containing approximately 100 μg of methyl esters was injected. The percentages of fatty acids were obtained by direct comparison of areas under peaks of a standard mixture containing typical fatty acids found in corn oil, having the following composition: methyl palmitate ($C_{16:0}$) 12%; methyl stearate ($C_{18:0}$), 3%; methyl oleate ($C_{18:1}$), 25%; methyl linoleate ($C_{18:2}$), 58%; methyl linolenate ($C_{18:3}$), 2%.

Separation of Tocopherols and Tocotrienols by TLC in One Dimension. (1) Application of the Samples and Standards. The silica gel sheets were used without activation. The plate was divided as shown Figure 1. A volume of 50 μL of standard (VIII) was applied in zone S in the form of a line band 3 cm long. Preparation of sample of unsaponifiable material for application to the plate was as follows: 5 mL of hexane solution was evaporated to dryness in a small tube by bubbling nitrogen through it. The residue was redissolved in a few drops of petroleum ether and transferred to the plate in the form of a 3-cm band. Rinsings of the tube and the micropipet were also applied to the same band.

At the points m, a monitoring solution was applied whose only function was to locate the positions of tocopherols after chromatography. It was prepared by mixing equal volumes of the hexane solutions of unsaponifiable material from the four corn samples and concentrating them to 1/4 of the initial volume. A drop of 5 μL was applied at each point m.

(2) Chromatography. The procedure of Müller Mulot (1976) was followed. This consists basically of double development of the chromatograms (16 cm in the same direction 2 times) using hexane–ethyl acetate, 92.5:7.5 v/v. Two plates were placed in each chamber, which permitted three samples and the standard mixture in duplicate to be done at the same time.

(3) Detection. Only the monitor samples were treated to render the separated components visible, the other areas being protected with glass plates. Solution VI was used for spraying, producing purple spots on a white background with tocopherols and tocotrienols. These served to dilineate the zones where the components would exist in the untreated regions of the plate. These zones were then separated by cutting the aluminum sheet with scissors.

(4) Elution. Each segment, measuring approximately 5 × 35 mm, was carefully scraped, and the scraping were collected in a small tube with a screw cap. Desorption was carried out with 2.0 mL of absolute ethanol, with gentle shaking for 30 min. A segment of the plate that was not used for samples served as a blank, treating it like a sample.

(5) Chemical Reaction. The tube were centrifuged, and to 1.0 mL of the supernatant liquid was added 3.0 mL of solution VII. After mixing well and allowing the solution to stand for 5 min, we measured the absorbances at 478 nm in 1-cm cells. Conversion of absorbances into micrograms of tocopherol was accomplished by using the absorbances of the eluted standard zones that contained, respectively, 20 μg of αT, 80 μg of γT, and 10 μg of δT. The two bands for corn that did not correspond to tocopherols in the standard mixture were tentatively identified as α-tocotrienol (αT_3) and γ-tocotrienol (γT_3). For lack of a standard for these compounds, the absorbances were converted into micrograms by means of the αT and γT standards, respectively.

Separation of Tocopherols and Tocotrienols by Two-Dimensional TLC. These experiments were carried out on a qualitative level as an additional criterion for the identification of the bands of tocopherols and tocotrienols of corn. The method of Whittle and Pennock (1967) was used, in which chloroform is employed in the first dimension and a mixture of petroleum ether and isopropyl ether (80:20 v/v) in the second. Under these conditions, tocopherols arrange themselves more or less in the same straight line (T) and the tocotrienols in another parallel line (T_3) as shown in the schematic diagram (Figure 2). Samples of unsaponifiable matter from palm oil and wheat bran were chromatographed, along with the corn samples. A new standard mixture containing βT was chromatographed. Visualization of all chromatograms was carried out by spraying with solution VI.

Expression of Tocopherols and Tocotrienols as International Units of Vitamin E (IU/mg). The quantity of individual tocols (milligrams per 100 g of dry corn) was multiplied by different factors given in "Humans Needs for Vitamin E" (1977); these factors are 1.49, 0.30, 0.15, and 0.45 for αT, γT, δT, and αT_3, respectively. The factor for γT_3 has not been published; any case, it should be small enough so as not to produce significant changes in the results.

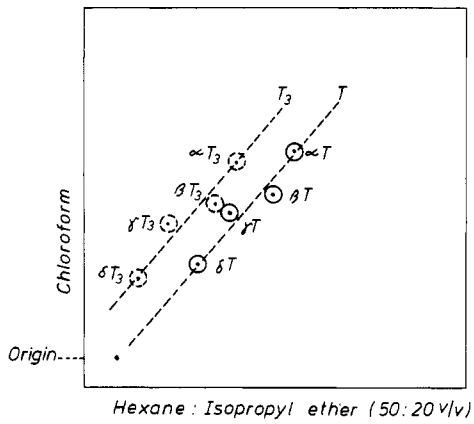

Figure 2. Typical results of two-dimensional TLC for the identification of tocopherols and tocotrienols. Line T contains tocopherols from the standard mixture, while line T_3 contains the tocotrienols (Whittle and Pennock, 1967).

Table I. Comparison between Duplicate Results for Total Tocol Determinations in Unsaponifiable Materials from Fresh Corn by (1) Direct Saponification of the Total Sample and (2) Saponification of Extracted Lipids

sample	total tocols, mg of αT/100 g of corn (dry basis)	
	by direct saponification	by saponification of extract
Maya XII Normal	4.6, 4.7	4.6, 4.6
Sugary (*su*)	6.1, 6.2	6.1, 6.2
Opaque-2 (*o2*)	4.2, 4.3	4.2, 4.2
Nutrimaiz (*suo2*)	5.1, 5.2	5.1, 5.1

RESULTS

Figure 3 shows a typical unidirectional chromatogram for corn tocopherols. Table I shows the comparison between the content of total tocols obtained by the conventional method and by the method of direct saponification of the ground corn. Table II summarizes the results for total lipids, total tocols, linoleic acid, and composition of fatty acids for the four corn varieties in the fresh and mature states. Percentages of tocopherols and tocotrienols obtained by TLC and spectrophotometric measurement in the fresh and mature corn varieties are given in Table III. Table IV shows the Vitamin E content, in internationals units, calculated on the basis of the individual tocols separated by TLC.

DISCUSSION

The values of total tocols for directly saponified corn samples were similar to those for saponification of previously extracted lipids (Table I). The latter method occasionally gave somewhat lower values, probably due to the necessity for increased handling of the samples.

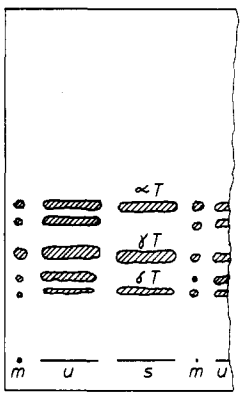

Figure 3. TLC separation of tocopherols and tocotrienols from corn on silica gel G for quantitative analysis. Solvent: hexane–ethyl acetate, 92.5:7.5 v/v. Double development, 16 cm each time in the same direction. u = unsaponifiable material from corn; s = standard mixture; m = monitor.

Analysis of both extracts by TLC showed that no difference existed in the number and position of reducing compounds. These findings agree with those of McMurray et al. (1980), who reported higher extraction of αT from animal feedstuffs when samples are saponified before extraction.

The first column in Table II shows the change in total lipids of the four corn varieties in going from the fresh to the mature state in the field. Samples of *su* and *suo2* contained more lipids than *o2* and *M*, and they increased more on reaching the mature state (74 and 91% vs. 43 and 42%, respectively).

The composition of fatty acids changed significantly with maturing linoleic acid ($C_{18:2}$), expressed as percentage of fatty acids, decreased in all samples (5.6, 11.9, 11.8, and 15.2% for *o2*, *su*, *suo2*, and *M*, respectively). In contrast, oleic acid ($C_{18:1}$) increased (22.4, 26.3, 29.8, and 30.2% in the same order). With respect to these two acids, *suo2* is more similar to *su* than to *o2*. On a basis of sample weight (column 3), linoleic acid increased 68% in *suo2* and 53% in *su* on maturing. Smaller increases were recorded for the other two varieties. These variations in the content of lipids and in the composition of the fatty acids are in complete agreement with the work of Weber (1969, 1973).

In the process of maturing, the total tocol content of the four varieties, in the order in Table II, increased 98, 71, 85, and 61%. In the varieties *su* and *suo2*, there was a parallel increase in total tocols and total lipids, the quotient of these two determinations remaining almost constant in the fresh and in the mature condition: 1.10 and 1.08 for *su* and 1.07 and 1.04 for *suo2*. Samples *o2* and *M* show variations that were less clear. Levy (1974) demonstrated that, when vitamin E is expressed as quantity per 100 g of corn, there is a positive correlation with the percent of lipids, and when vitamin E is expressed on the basis of

Table II. Lipid Compound Content of Corn Varieties in Fresh and Mature States

sample	total lipids, g/100 g of corn[a]	total tocols, mg/100 g of corn[a]	linoleic acid, g/100 g of corn[a]	fatty acid composition, g/100 g of acids				
				$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
o2: fresh	4.20	4.17	2.01	15.62	1.69	26.07	54.79	1.82
mature	6.00	8.27	2.71	14.12	1.27	31.90	51.71	1.00
su: fresh	5.25	5.79	2.53	13.58	2.05	27.68	55.11	1.58
mature	9.14	9.90	3.88	13.20	2.07	34.95	48.51	1.23
suo2: fresh	4.80	5.15	2.27	13.11	2.27	27.99	54.15	2.47
mature	9.15	9.54	3.82	12.69	1.91	36.34	47.74	1.31
M: fresh	3.95	4.39	1.92	15.07	1.96	25.07	55.67	2.23
mature	5.62	7.08	2.32	13.98	1.73	36.20	47.19	0.90

[a] Dry basis.

Table III. Changes in the Distribution of Tocols with the Maturing of Corn Varieties Opaque-2 (*o2*), Sugary (*su*), Nutrimaiz (*suo2*), and Maya XII Normal (*M*)

corn variety	composition of tocopherols and tocotrienols, %[a]					
	αT	αT_3	γT	γT_3	δT	δT_3
o2: fresh	15.0	19.5	38.2	25.5	1.8	trace
mature	17.9	8.4	59.6	14.0	trace	
su: fresh	12.5	21.8	44.0	19.7	2.0	trace
mature	17.2	7.2	61.7	7.9	6.0	
suo2: fresh	12.9	20.9	47.1	18.0	1.1	trace
mature	16.4	7.0	63.6	8.9	4.1	
M: fresh	10.2	30.2	27.4	30.0	2.2	trace
mature	16.4	14.2	49.0	15.2	5.2	

[a] Percent of total tocols.

Table IV. Percent Increase in Total Tocols and in Vitamin E with the Maturing of the Corn Varieties

corn	vitamin E		total tocols	
	IU/100 g[a]	increase, %	mg/100 g[a]	increase, %
o2: fresh	1.78		4.17	
mature	4.04	127	8.27	98.3
su: fresh	2.41		5.79	
mature	4.76	97.3	9.90	71.0
suo2: fresh	2.20		5.15	
mature	4.51	105	9.54	85.2
M: fresh	1.64		4.39	
mature	3.29	101	7.08	61.3

[a] Dry basis.

total lipids, there is a positive correlation with the percent of linoleic acid.

With respect to the composition of tocopherols and tocotrienols, the chromatograms showed five components for all the corn samples. The following R_f values were obtained by using the chromatographic conditions of Müller Mulot (1976): 0.50, 0.44, 0.34, 0.28, and 0.25. Müller Mulot reported that αT, αT_3, γT, γT_3 and δT had R_f values of 0.50, 0.43, 0.34, 0.29, and 0.26, respectively. From the comparison between R_f values and results with authentic standards the definite presence of αT, γT, and δT was established plus the doubtful presence of αT_3 and γT_3. This doubt was dispelled by analyzing the samples by two-dimensional TLC in which all corn varieties showed the presence of three tocopherols and two tocotrienols. Finally, when two-dimensional chromatograms of palm oil and wheat bran, which are known to be rich in tocotrienols, with those for corn, were compared, the presence of αT_3 and γT_3 was confirmed. It is important to repeat that, due to the lack of αT_3 and γT_3 standards, the absorbances of these tocotrienols were expressed in micrograms by use of the αT and γT standards, respectively.

Table III shows the percentages of tocopherols and tocotrienols obtained through separation by TLC and chemical evaluation by reaction with cupric ion and bathocuproine. The most notable transformation is the great change in the tocol components during maturing of the corn grains. The percentages of the unsaturated members (tocotrienols) decreased while the saturated ones (tocopherols) increased. This situation can be noted in all four corns, but the extent of the change appears to be a function of the biological variety.

The contribution of the sum αT + γT increases substantially in the mature samples, reaching 77.5, 78.9, 80.0, and 65.4% of the total tocol in *o2*, *su*, *suo2*, and *M*, respectively. In the fresh state, the sum αT + γT contributes only 53.2, 56.5, 60.0, and 37.5%. Small quantities of δT were detected in the fresh corn samples and these also increased in the mature corn. Traces of δT_3 were observed in all the chromatograms of fresh corn, conforming to a pattern suggested by Slover et al. (1969). These authors claim that, in the majority of cereals (corn, wheat, oats, and rye), a given tocopherol is always accompanied by the corresponding tocotrienol. In this research, the four samples certainly contained $\alpha T/\alpha T_3$, $\gamma T/\gamma T_3$, and δT. Although δT_3 was observed, it was in quantities insufficient for its determination.

The *su* and *suo2* varieties, genetically high in oil, showed 41.5 and 38.9% tocotrienols in the fresh state, decreasing to 15.1 and 15.9% when mature. In contrast, the *M* variety, genetically low in oil, had 60.2% tocotrienols in the fresh state and still maintaining a relatively high 29.4% in the mature state. This percentage *o2* formed by αT_3 and γT_3. The corn *o2* shows 45.0% tocotrienols in the fresh state and 22.4% when mature. These values differ from those found in Nutrimaiz (*suo2*), which shows a pattern closer to that of its other progenitor, *su*.

McLaughlin and Weihrauch (1979) compiled data for 127 samples of normal mature corn, obtaining an average value of 5.81 mg of total tocols/100 g, with values fluctuating between 0.9 and 11.6%. They found that the total of tocols consisted of 8.4% αT, 3.6% αT_3, 78.5% γT, and 7.9% γT_3. Our data for normal Maya XII do not resemble these average values very much but are close to the individual values of Grams et al. (1970, 1971), who analyzed normal corn, Opaque-2, and other varieties. According to Slover (1971), the content and composition of vitamin E depend not only on the genetic pattern but also on climatic conditions and the degree of maturity. The variations of the content of tocopherols and tocotrienols with maturation are reflected in the content of the active vitamin E because different factors are used for each member of the family in its calculation ("Human Needs for Vitamin E", 1977).

It can be seen in Table IV that active vitamin E in corn increases an average of 107% during the change from the fresh to the mature state, while the total tocol content increases an average of 80%. Such results emphasize the necessity to know the tocopherol and tocotrienol composition in order to calculate the true ingestion of vitamin E in dietetic evaluations.

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Determination of Pentachlorophenol in Hardwood Chips

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A rapid and sensitive procedure is described to determine pentachlorophenol (PCP) in hardwood chips or cardboard. The PCP is isolated via liquid–liquid partitioning steps. Pentachlorophenyl acetate is formed by acetylation using pyridine and acetic anhydride, and the derivatized material is partitioned into hexane for electron-capture GC analysis. Hardwood chips, spiked at five levels with PCP, had recoveries of 83 ± 6%. Results from 86 hardwood chip samples and 11 cardboard samples that were analyzed between 1977 and 1981 had PCP residue levels that ranged from <5 to 240 ppb and from 0.13 to 4.4 ppm, respectively.

Pentachlorophenol (PCP) is a registered fungicide used in slime control and the preservation of wood products. This pesticide is environmentally persistent and poses a potential health problem to animals exposed to PCP-treated products or to toxic PCP contaminants, such as dioxins as reported by Plimmer et al. (1973), Dickson (1980), and Lamberton et al. (1979). Hardwood chips are used as bedding for laboratory research animals while sawdust and wood shavings are used to control litter in chicken and pig pens and in holding areas for cattle. Since the presence of excessive PCP in these wood byproducts may be deleterious to animals, it is important to monitor the PCP levels in such products. Wood chips or cardboard having PCP levels above 2 ppm are not used in our laboratory to avoid biasing results from animal studies. Thus, a simple and accurate method was needed to analyze wood products for PCP at levels below 2 ppm. Establishment of the 2.0-ppm limit of PCP was to prevent biological response and restrict exposure of experimental animals to potentially deleterious chemicals that may be present in ancillary animal supplies as discussed by Greenman et al. (1980). In addition, supplies could be obtained that satisfied the arbitrary PCP limitation.

Extensive reviews of the environmental hazards and analytical procedures to determine residues of PCP in various substrates have been published by Bevenue and Beckman (1967) and by Ahlborg and Thunberg (1980). Since the introduction of PCP in the 1930s, methods developed to conduct PCP analysis include colorimetric, UV, and IR absorption plus paper, thin-layer, gas, and liquid chromatography. Many of these procedures are tedious and lack the necessary selectivity and/or sensitivity required for residue analysis. The HPLC method reported by Daniels and Swan (1979) does provide a more rapid procedure but lacks the sensitivity we require. Most of the methods reported have been for the analysis of wastewater, animal tissues, urine, air, and soil. Ting and Quick (1980) reported a TLC method to determine PCP in sawdust and wood shavings that is capable of detecting about 2 ppm of PCP. A method reported by Rudling (1970) to analyze for PCP in animal tissue or water consisted of extracting the acidified sample with *n*-hexane followed by acetylation. The method presented herein also acetylates the PCP for subsequent electron-capture GC analysis. The analytical procedure to determine PCP in hardwood chips or cardboard is sensitive and can be completed in less than 1 day. In addition, the method has good precision as demonstrated by the standard deviation obtained for the spiked recoveries, and it is free from interference by other residues that may be present in the samples.

EXPERIMENTAL SECTION

Reagents. The PCP standards (No. 3462, Eastman Kodak Co., Rochester, NY, and Reference Standard, EPA, Perrine, FL) were used as received because they contained no extraneous peaks when analyzed by flame ionization detection (FID) and electron-capture (EC) gas chromatography (GC). In addition, confirmation and purity analyses were obtained on the two standards by mass spectrometry.

The acetylating reagent was prepared by mixing 2 mL of pyridine (No. 27530, Pierce Chemical Co., Rockford, IL) with 0.8 mL of acetic anhydride (No. A-10, Fisher Scientific Co., Fair Lawn, NJ). Fresh reagent was prepared weekly and kept under refrigeration.

All solvents were Nanograde (Mallinckrodt, Inc., St. Louis, MO), and all reagents were CP grade.

Materials. *Hardwood chip* samples were taken from batches of hardwood chips purchased commercially for animal bedding. The chips were manufactured only from maple, birch, beech, or mixtures thereof with particle size specifications requiring that over 95% pass through a no. 8 mesh screen and less than 6% pass through a no. 20 mesh screen; moisture content did not exceed 8%. Shipments of hardwood chips were received in 40-lb bags, and 250-g aliquots of material were taken randomly from nine different bags. The individual collections were composited in a "V" blender to produce a homogeneous sample from each shipment for PCP analysis. In a similar fashion, commercially supplied cardboard samples were taken from a batch of gray-mist chipboard sheets (about 30 × 30 cm and 0.07 cm thick) that are used to form feeder boxes. Six

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