methyldodecylamine in air and the reversal of this trend in the case of maleic hydrazide. However, this observation is in agreement with the previously reported effect that air had on the production of the mixture of isomeric benzopyrenes during the pyrolysis of *N,N*-dimethyldodecylamine and maleic hydrazide (Patterson et al., 1972).

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Quantitation of Hexane-Extractable Lipids in Serial Samples of Flue-Cured Tobaccos

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Solanesol, fatty acids, sterols, neophytadiene, and hydrocarbon waxes were quantitated in two flue-cured varieties of tobacco obtained at six intervals: (1) green, before harvest; (2) ripe, at harvest; (3) cured; (4) redried; (5) aged 1 year; and (6) aged 2 years. Solanesol and neophytadiene concentrations increased during processing, while fatty acid concentrations generally decreased and sterol and hydrocarbon wax content remained essentially unchanged in both varieties. Lipid concentrations also varied with crop year. PAH content of smoke condensate was related to concentration of lipids in the cigarette tobacco.

The hexane-extractable fraction of tobacco contains lipids that are important to leaf quality and are precursors of polynuclear aromatic hydrocarbons (PAH) in smoke (Chortyk and Schlotzhauer, 1973; Schmeltz and Hoffmann, 1973). Most quantitative studies of tobacco lipids have been limited to individual compounds or classes of compounds. Thus, fatty acids and their esters (Chu et al., 1972; Chu and Tso, 1968; Tso and Chu, 1970; Hoffmann and Woziwodzki, 1968; Ellington et al., 1977), sterols and steryl esters (Ellington et al., 1977; Grunwald, 1975; Schmeltz et al., 1975; Davis et al., 1970; Cheng et al., 1971; Grunwald, 1970), neophytadiene (Ellington et al., 1977; Bilinsky and Stedman, 1962; Chortyk et al., 1975), hydrocarbon waxes (Ellington et al., 1977; Chortyk et al., 1975; Stedman and Rusaniwskyi, 1959), and solanesol (a C_{45} unsaturated alcohol) (Irvine et al., 1972; Severson et al., 1977), which constitute the major tobacco lipids, have been quantitated by various research groups. In most instances only one or two of these components have been analyzed in the same tobacco sample (Chu et al., 1972; Cheng et al., 1971).

Pyrolysis of specific components of the hexane extract of tobacco by Schlotzhauer and Schmeltz (1969) indicated that straight-chain aliphatic compounds, such as hexane, stearic acid, and dotriacontane, yielded significant quantities of aromatic products with alkyl side chains.

Table I. Total and Free Solanesol and Sterols

	% dry leaf			
	Total[a]		Free[b]	
	NC95	PY	NC95	PY
	Solanesol			
G	0.986 ± 0.04	0.88	0.91	0.41
R	1.73 ± 0.06	0.93	1.57	0.81
C	2.60	1.45	1.99	1.02
RD	2.59	1.34	1.86	1.13
1 yr	2.07	1.52	2.00	1.34
2 yr	2.29	1.74	1.65	1.15
	Sterols[c]			
G	0.135 ± 0.006	0.17	0.09	0.08
R	0.161 ± 0.002	0.19	0.11	0.08
C	0.17	0.15	0.13	0.07
RD	0.17	0.17	0.10	0.10
1 yr	0.16	0.16	0.09	0.09
2 yr	0.16	0.24	0.08	0.09

[a] Standard deviations are given where three replicate samples were analyzed; other values represent averages of two analyses. [b] Analyses were performed on single samples only. [c] Cholesterol, campesterol, stigmasterol, and sitosterol.

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Table II. Major Fatty Acids

	% dry leaf					
	Total[a]		Palmitic		C18 Unsaturated[b]	
	NC95	PY	NC95	PY	NC95	PY
G	1.87 ± 0.060[c]	1.95	0.43 ± 0.029	0.46	1.13 ± 0.072	1.24
R	1.66 ± 0.088	1.56	0.42 ± 0.038	0.40	0.97 ± 0.026	0.86
C	0.89	1.22	0.27	0.33	0.43	0.62
RD	1.09	1.33	0.31	0.33	0.57	0.66
1 yr	0.84	1.01	0.26	0.27	0.44	0.56
2 yr	0.76	1.24	0.22	0.32	0.40	0.64

[a] Myristic, palmitic, stearic, oleic, linoleic, and linolenic. [b] Total of oleic, linoleic, and linolenic. [c] Standard deviations are given where three replicate samples were analyzed; other values represent averages of two analyses.

Table III. Neophytadiene and Hydrocarbon Waxes[a]

	% dry leaf			
	Neophytadiene		Hydrocarbon Waxes C27-C34	
	NC95	PY	NC95	PY
G	0.02	0.02	0.16	0.17
R	0.05	0.02	0.18	0.19
C	0.08	0.07	0.17	0.16
RD	0.14	0.06	0.19	0.14
1 yr	0.12	0.10	0.22	0.22
2 yr	0.15	0.09	0.19	0.26

[a] Analyses were performed on single samples only.

Table IV. Hexane Extractables and Total Measured Lipids

	% dry leaf					
	Hexane extractables in tobacco[a]		Hexane extractables in hydrolyzate[b]		Total measured lipids in hydrolyzate[c]	
	NC95	PY	NC95	PY	NC95	PY
G	5.99	5.54	5.31	3.84	2.97	2.73
R	7.77	5.28	6.25	5.03	3.44	2.61
C	8.30	4.92	6.38	4.42	3.33	2.58
RD	8.30	5.64	6.47	4.50	3.56	2.56
1 yr	7.80	6.22	5.77	4.65	2.92	2.92
2 yr	7.58	6.46	6.27	5.06	3.06	3.44

[a] Analyses were performed on single samples only. [b] Averages of duplicate analyses. [c] Summation of values from Tables I, II, and III.

Compounds such as sitosterol, phytol, and linolenic acid, which contain double bonds, produced the greatest yields of benzo[*a*]pyrene (B*a*P). Schlotzhauer et al. (1976) reported recently that solanesol may contribute 40% of the B*a*P and more than 30% of the total PAH in the pyrolyzate of the extract.

Agricultural practices, environmental conditions, and postharvest handling have been reported to affect the levels of selected tobacco constituents (Tso and Chu, 1970; Grunwald, 1973). We thought it would be of interest to monitor the lipid levels of flue-cured tobacco at the various stages of processing from green leaf through aging. A recently reported (Ellington et al., 1977; Severson et al., 1977) quantitative method for solanesol, fatty acids, sterols, neophytadiene, and the hydrocarbon waxes was used in the analyses.

MATERIALS AND METHODS

Serial samples of two flue-cured tobacco varieties [NC95 and NC95 × TI1372 (pale yellow, PY)] were obtained from the Tobacco Research Laboratory, Oxford, N.C. The samples from the 1973 crop were obtained at six intervals: (1) green (G), 2 weeks before harvest; (2) ripe (R), at harvest; (3) cured (C); (4) redried (RD); (5) 1-year aged (1 yr); and (6) 2-years aged (2 yr). The green and ripe samples were freeze-dried, ground to pass a 32-mesh screen and stored at approximately −15 °C. All other samples were ground to 32 mesh and refrigerated. Moisture content of each sample was determined and used for correction of all analytical data to a dry weight basis.

The analytical procedures used were those reported by Ellington et al. (1977) and Severson et al. (1977). Briefly, these techniques consisted of direct hydrolysis of tobacco combined with Soxhlet extraction and silicic acid column chromatography for fractionation of component lipids. Final quantitation was accomplished by means of gas chromatography. Analyses were performed in duplicate or greater replication for total solanesol, total fatty acids,

Table V. Yearly Lipid Variation in Cured Tobacco

	% dry leaf					
	NC95			PY		
	1973	1974	1975	1973	1974	1975
Solanesol	2.60[a]	1.37	2.02	1.45[a]	1.10	1.65
Total major Fatty acids	0.88[a]	1.07	1.26	1.22[a]	1.06	1.46
Total sterols	0.17[a]	0.12	0.14	0.15[a]	0.12	0.12
Neophytadiene	0.08	0.06	0.08	0.07	0.06	0.07
Hydrocarbon Waxes	0.17	0.07	0.07	0.16	0.11	0.10

[a] Average of duplicate analyses; all others are values from single analyses.

Table VI. PAH and Lipid Levels for NC95 and Pale Yellow Cigarettes

	NC95	PY
PAH[a]	μg/100 cigarettes	
Phenanthrene-Anthracene	36.2	31.8
Methylphenanthrene-methylanthracene	83.5	66.8
Dimethylphenanthrene-anthracene	58.1	55.3
Fluoranthene	11.3	10.5
Acephenanthrene	13.1	8.9
Pyrene	11.4	10.1
Methylfluoranthene	24.7	19.8
Methylpyrene	25.0	19.7
Chrysene	8.2	6.6
Methylchrysene	7.6	7.2
Benzofluoranthene	3.3	2.7
Benzopyrene	2.2	1.7
Total PAH	284.6	241.1
Lipids	% dry leaf	
Total solanesol	1.37	1.10
Total fatty acids	1.07	1.06
Total sterols	0.12	0.12
Neophytadiene	0.06	0.06
Hydrocarbon waxes	0.07	0.10

[a] PAH levels are from Severson (1976).

Table VII. PAH and Lipid Levels for Kentucky Reference and Experimental Burley Cigarettes

	1R1[b]	2R1[c]	Burley control[d]	Burley HLC sheet tobacco[d]
PAH[a]		μg/100 cigarettes		
Phenanthrene-anthracene	44.0 ± 6.5	36.4	35.0	14.8
Methylphenanthrene-methylanthracene	101.8 ± 6.8	109.3	51.4	28.8
Fluoranthene	14.8 ± 1.0	14.6	11.8	6.9
Pyrene	13.9 ± 1.1	15.6	9.1	4.7
Methylpyrene	19.9 ± 2.9	26.6	13.8	5.5
1,2-Benzanthracene-chrysene-triphenylene	8.6 ± 1.3	8.3	7.7	4.4
Benzo[e]pyrene-benzo[a]pyrene	2.5 ± 0.3	2.2	2.0	1.6
Total PAH	205.5	213.0	130.8	66.7
Lipids		% dry leaf		
Total solanesol	1.19	1.31	1.38	0.94
Total fatty acids	0.75	0.83	0.43	0.50
Total sterols	0.13	0.14		
Neophytadiene	0.04	0.04	0.08	0.03
Hydrocarbon waxes	0.09	0.11		

[a] Severson et al. (1976). [b] Five determinations. [c] Three determinations. [d] Two determinations.

interval used (Ellington et al., 1977). All gas chromatographic quantitations were performed in duplicate.

RESULTS AND DISCUSSION

The levels of total and free solanesol and sterols are given in Table I. As in samples examined previously (Severson et al., 1977), most of the solanesol was present in the free form. Solanesol content of the NC95 variety increased substantially in the 2 weeks of growth before harvest and during curing, while the solanesol level in PY tobacco increased during the curing stage. During aging solanesol content of the PY continued to increase while that of the NC95 decreased. In both varieties sterol levels remained fairly constant throughout the processing of the tobaccos.

The fatty acid analyses are given in Table II. The total major fatty acid levels of both varieties were essentially the same in the green tobacco and decreased substantially through redrying. The level of total acids in NC95 and PY continued to decrease at a slower rate during aging. Palmitic and the C_{18} unsaturated acids decreased from green through aging in both varieties; however, the decreases in the unsaturated acids were more pronounced.

Data for the hydrocarbon waxes and neophytadiene are given in Table III. Neophytadiene generally increased at each stage, and, after 2-years aging, were four to seven times the levels in green tobacco. The percentages of saturated hydrocarbons remained fairly constant at each stage but did increase substantially in the 2-year sample of the PY. The levels of hexane extractables in tobacco and tobacco hydrolyzate are compared in Table IV. Also given in the table are the sums of the percentages of solanesol, fatty acids, sterols, neophytadiene, and hydrocarbon waxes in each sample. In almost every case the NC95 values were higher than the PY values. The total lipids measured constituted from 50 to 60% of the hexane extractables in the hydrolyzate.

We also determined yearly lipid variations in cured NC95 and PY tobaccos for the 1973, 1974, and 1975 crop years (Table V). The largest variations were noted in solanesol, fatty acids, and hydrocarbon waxes.

Several types of experimental cigarettes were also analyzed and Tables VI and VII show their lipid levels and the levels of selected PAH in their smoke condensates. Table VI shows data for cigarettes made from 1974 cured but not redried NC95 and PY tobaccos. The NC95 cigarettes produced 15% more of the selected PAH than the PY cigarettes. The levels of three of the measured lipids were essentially the same, but the percentages of solanesol and hydrocarbon waxes differed significantly between the two cigarettes. All the PAH levels in the condensate and solanesol in the leaf were higher in NC95 than in the PY; and these data agree with the solanesol pyrolysis data of Schlotzhauer et al. (1976).

Table VII shows the same comparisons for Kentucky Reference 1R1 and 2R1 cigarettes as well as cigarettes made from Burley 21 and Burley 21 HLC (homogenized leaf curing) sheet tobacco. Several PAH and lipid levels for the 2R1 cigarettes were only slightly higher than in the 1R1. The Burley control produced twice as much of the selected PAH as HLC sheet tobacco. The higher PAH values for the Burley control could not be attributed entirely to the higher solanesol and neophytadiene levels, since the 1R1 and 2R1 cigarettes, with slightly less solanesol, produced more PAH than the Burley control. The 1R1 and 2R1 samples had approximately twice the fatty acid content of the Burley control, possibly accounting for the PAH differences. The lower solanesol and neophytadiene levels in the HLC Burley sheet, as compared to the Burley control, are in agreement with lower values reported for other components in the HLC Burley (Tso et al., 1975). This same effect was observed in the analysis of reconstituted air-cured and homogenized Burley tobacco samples. The homogenized sample contained 0.67 and 0.05% total solanesol, and neophytadiene, while the air-cured contained 1.09 and 0.06%, respectively. The homogenized sample was slightly higher in total fatty acids (0.48%) than the air-cured sample (0.30%).

Although all organic compounds containing carbon and hydrogen may serve as precursors of PAH, tobacco is rich in compounds known to be precursors of specific PAH. These analytical data will be used in the development of methods designed to lower the concentrations of known PAH precursors in the tobacco leaf.

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COMMUNICATIONS

Stability of Carboxymyoglobin in Refrigerated Ground Beef

This paper describes the uptake of carbon monoxide by myoglobin in beef patties exposed to a 1% CO atmosphere and the subsequent loss of carbon monoxide when samples are placed in an air atmosphere under fluorescent illumination. The half-life for the loss of carbon monoxide from such samples is about 2 days.

Modified atmospheres for storage of fresh meats containing high concentrations of carbon dioxide (CO_2) and low concentrations of carbon monoxide (CO) are currently being studied. Atmospheres containing 1% CO and 50% CO_2 have been shown to extend both the microbiological shelf life as well as the color shelf life of refrigerated ground beef (Gee and Brown, 1978). With the incorporation of CO in gas mixtures, there is formed carboxymyoglobin (MbCO), a pigment that appears to be more resistant to oxidation than is oxymyoglobin (MbO_2) in the presence of high concentrations of CO_2 (Wolfe et al., 1976). This report summarizes a study of the stability of MbCO formed in a 1% CO atmosphere in refrigerated ground beef stored in air.

METHODS AND MATERIALS

Lean ground beef was purchased at a local retail chain store. Circular ground beef disks of uniform weight (50 ± 0.1 g), diameter (8.0 cm), and thickness (1 cm) were formed using a die and piston. The meat was stored on stainless steel mesh shelves in a 10-L desiccator jar in a 1% v/v carbon monoxide atmosphere at 2 °C for approximately 3 days. This atmosphere was chosen because of its potential of color shelf life extension without hiding bacterial spoilage or producing overly bright-red meat color (Gee and Brown, 1978). The meat was then held under a normal air atmosphere with continuous fluorescent illumination (15 W at 50 cm). The average temperature at the surface of the meat samples (Mettler TM 15 thermometer) was 1.7 °C, with or without illumination.

Extractions of myoglobin were performed by blending each ground beef patty at high speed for 60 s in a total of 100 mL of 0.2 M phosphate buffer, pH 5.92. The extract was centrifuged for 30 min at 2 °C and 14 000*g*. The supernatant was filtered through a glass wool plug and the volume measured. A 20-mL aliquot of the supernatant was recentrifuged for 15 min at 2 °C and 37 000*g*. Aliquots of this extract were scanned from 700 to 450 nm vs. water in a Cary Model 15 spectrophotometer. The sample was rescanned following saturation with CO. Using the method of Wolfe et al. (1978), data from the two scans were used to calculate the percentages of metmyoglobin (MetMb), MbCO, and MbO_2 plus deoxymyoglobin (Mb). At each sampling point, three sets of scans of extracts from each of four ground beef patties were made.

RESULTS AND DISCUSSION

A summary of the data is presented in Figure 1. Each point is the average of 12 scans. Initially, about 36% of the myoglobin in the beef patties was in the MetMb state with the remainder in the reduced forms (MbO_2 + Mb). Following a 3.4 day exposure to 1% CO, the level of MetMb fell to about 23% while the total reduced myoglobin percentage increased. The MbCO percentage initially following treatment was about 17%. Subsequent sampling of CO-treated patties exposed to air showed a steady decrease in the levels of MbCO and an increase in levels of MetMb. The half-life of MbCO in samples stored in air was found to be 2.1 days. Determination of the half-life of MbCO was important because levels of carbon

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