Influence of Temperature and Humidity on the Accumulation of Tobacco-Specific Nitrosamines in Stored Burley Tobacco

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Burley tobacco was stored in a constant-temperature humidity chamber at either 32 °C/83% RH or 32 °C/90% RH. Sampling of the tobacco over a 3-week period and analyses of the lamina and midvein showed that at 90% RH there was a significant increase of nitrite, N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), and 4-[N-(nitrosomethyl)amino]-1-(3-pyridyl)-1-butanone (NNK) within 7 days, which was followed by decreased amounts of these constituents. Alkaloid and nitrate concentrations were dramatically decreased during the 21-day storage at 32 °C/90% RH. Treatment of the tobaccos with streptomycin and rifampicin did not inhibit nitrite or nitrosamine formation. During this 21-day period, approximately half of the original weight was lost due to microbial activity and leaching. Storage at 32 °C/83% RH resulted in a reduction of alkaloids but no increase of nitrite or tobacco-specific nitrosamines (TSNA) and no change in nitrate concentration over a 28-day period.

Recent studies by Andersen et al. (1987) and Burton et al. (1986, 1988a) found that air-curing of burley tobacco at high temperature and humidities resulted in the accumulation of very high concentrations of N'-nitrosonornicotine (NNN), N'-nitrosoanatabine, and 4-[N-(nitrosomethyl)amino]-1-(3-pyridyl)-1-butanone (NNK). The high levels of the tobacco-specific nitrosamines (TSNA) were accompanied by enhanced concentrations of nitrite. During curing, TSNA and nitrite increases were observed to occur in a very short time span of 3 days (Burton et al., 1989). The large increase of TSNA accumulation over the short time span was not expected; therefore, it was decided that there should be a more detailed study of this phenomenon under controlled conditions and more uniform sampling. This was accomplished by subjecting a representative sample of air-cured burley tobacco to 32 °C and either 83% or 90% RH to determine whether nitrosamines can accumulate in cured tobacco at the above temperature and relative humidities and under aerobic conditions.

EXPERIMENTAL SECTION

Nicotiana tabacum L. cv. KY 14 was grown in 1986 at the University of Kentucky Agricultural Experiment Station farm under standard cultural practices. To investigate the influence of humidity on the air-cured tobacco, 24 bunches (four leaves/bunch) of tobacco from an upper stalk position were suspended in a Aminco climate lab constant-humidity cabinet. For the first study, the temperature and relative humidity (RH) were maintained at 32 °C and 83% RH (treatment 1). Three replicate samples (eight leaves/replicate) were taken at 0 time and 7, 14, 21, and 28 days. Samples were separated into midvein and lamina, stored at −40 °C, and freeze-dried. In most cases the samples were reweighed to determine the moisture content of the leaf. Samples were ground to pass a 40-mesh screen and stored at −40 °C until analyzed. Treatment 2 involved subjecting 24 bunches of air-cured tobacco leaves in the same manner as treatment 1, except the relative humidity was increased to 90% RH. Samples were taken at days 4, 7, 14, and 21 and handled in the same manner as treatment 1. Treatment 3 was the same as treatment 2, except the leaves were dipped (1–2 s) in 0.0125% streptomycin sulfate and 0.01% rifampicin aqueous solution. The tobacco was dipped again the third day after the initial treatment with the same solution. After that time, the tobacco leaves were sprayed with the antibiotic and antifungal solution twice a week for the duration of the experiment. Samples were taken at 4, 7, 14, and 21 days. In treatment 4 the leaf was first separated into lamina and midvein, and then 24 bunches of lamina and 24 bunches of midvein were hung in the environmental chamber for 21 days. Again, samples were taken at the same intervals as treatments 2 and 3.

Chemical Analysis. Total nitrogen was determined via the Kjeldahl method described by Bradstreet (1965). Calcium and potassium were determined by atomic absorption on the 9:1 nitric acid to perchloric acid digest of the tobacco samples. Alkaloids were analyzed by a Technicon autoanalyzer procedure described by Harvey et al. (1969). Nitrate was determined colorimetrically by a procedure described by Lowe and Gillespie (1975). Nitrite was determined in a procedure described by Crutchfield and Burton (1989). Individual alkaloids were analyzed by a procedure described by Severson et al. (1981) and Burton et al. (1988). The pH of tobacco was determined by the procedure described by Brunnemann et al. (1985). NNN, NAT, and NNK were quantified by a gas chromatographic procedure described by Djordjevic et al. (1989). All nitrosamine values are based on response and recovery factors of authentic standards.

RESULTS AND DISCUSSION

Influence of 32 °C and 83% RH on Changes in Tobacco Composition. Influence of long-term storage on the composition of burley tobacco has been reviewed (Bates et al., 1974; Andersen et al., 1982); however, the influence of adverse storage conditions on changes in chemical composition has not been studied in detail. This is particularly true for the influence of temperature and humidity on the decrease or increase of the nitrogen-containing constituents in burley tobacco. Data for burley tobacco that was maintained at 32 °C and 83% RH (treatment 1) in a constant-humidity chamber are presented in Tables I–III. On a constant-weight basis based on calcium content there were no statistically significant changes in the levels of total nitrogen, nitrate N, or calcium of the lamina (Table I). The only significant statistical changes were the decrease of lamina nicotine and total alkaloids, whereas anatabine and anabasine increased (Table II). The apparent increases are smaller when adjusted for the dry matter loss and are not significant when 0- and 28-day values are compared. Data for the midrib show the same trend, loss of nicotine during weeks 2 and 3 with no significant change in the other nitrogen constituents during this time.

TSNA content of treatment 1 decreased between the initial time and the first week. There was no decrease of NNN, whereas NAT and NNK decreased in both lamina and midrib (Table III). This result is interesting because this is the first time it has been reported that the nitrosamines decreased with respect to storage time. The mean moisture content of the lamina during this experiment was

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Table I. Influence of Storage Environment on Moisture Content, pH, Nitrogen Constituents, and Calcium[a]

	moisture, %	total N, mg g^{-1}	NO_3 N, mg g^{-1}	NO_2 N, mg g^{-1}	pH	Ca, mg g^{-1}	Ca(x)/Ca(0)
Treatment 1 (32 °C/83% RH)							
Lamina							
0		43.10 (43.10)[b]	4.15 (4.15)	0.02 (0.02)		35.33	1.00
7	19.20	46.13 (46.97)	3.65 (3.64)	0.02 (0.02)	5.28	35.17	1.00
14	12.67	44.10 (39.67)	4.46 (4.00)	0.01 (0.01)	5.27	39.30	1.11
21	12.30	43.50 (44.35)	3.62 (3.62)	0.01 (0.01)	5.46	35.60	1.01
28	19.07	40.80 (38.06)	4.04 (3.69)	0.01 (0.01)	5.63	38.67	1.09
LSD (0.05)	2.35	5.25 (11.13)	0.78 (0.48)	0.00 (0.00)	0.10	5.82	0.16
Midrib							
0		39.20 (39.20)	20.37 (20.37)	0.01 (0.01)		30.00	1.00
7	21.00	35.87 (36.73)	18.82 (19.06)	0.01 (0.01)	6.17	29.77	0.99
14	32.67	39.43 (36.69)	22.50 (20.95)	0.01 (0.01)	6.18	32.23	1.07
21		34.77 (32.77)	19.08 (17.90)	0.01 (0.01)	6.38	32.13	1.07
28	18.70	31.93 (28.57)	20.83 (18.61)	0.01 (0.01)	6.47	33.83	1.13
LSD (0.05)	4.93	3.76 (6.14)	2.53 (2.56)	0.01 (0.01)	0.15	4.87	0.16
Treatment 2 (32 °C/90% RH)							
Lamina							
0		43.10 (43.10)	4.15 (4.15)	0.02 (0.02)		35.33	1.00
4	65.33	34.67 (27.33)	6.75 (5.34)	0.18 (0.15)	7.48	45.23	1.28
7	71.67	36.60 (27.35)	6.42 (4.78)	2.27 (1.68)	8.15	47.33	1.34
14	73.00	37.67 (22.43)	3.38 (2.01)	6.83 (4.07)	8.60	59.37	1.68
21	70.03	38.00 (19.63)	2.93 (1.51)	7.33 (3.78)	8.85	68.47	1.94
LSD (0.05)	4.51	3.47 (3.62)	0.85 (0.76)	0.73 (0.76)	0.22	4.37	0.12
Midrib							
0		39.20 (39.20)	20.37 (20.37)	0.01 (0.01)		30.00	1.00
4	65.33	35.60 (31.42)	19.03 (16.81)	0.40 (0.35)	7.25	34.00	1.13
7	72.00	32.83 (28.29)	15.03 (12.97)	2.80 (2.41)	8.00	34.80	1.16
14	78.00	32.33 (22.41)	3.92 (2.69)	11.83 (8.21)	8.60	43.33	1.44
21	68.33	30.00 (17.51)	0.67 (0.40)	10.33 (6.01)	9.38	51.50	1.72
LSD (0.05)	2.94	1.98 (1.31)	1.94 (0.93)	0.75 (0.45)	0.25	2.87	0.19
Treatment 3 (32 °C/90% RH, Treated with Antibiotic)							
Lamina							
0		43.10 (43.10)	4.15 (4.15)	0.02 (0.02)		35.22	1.00
4	71.00	41.50 (38.32)	6.19 (5.78)	0.11 (0.10)	6.32	38.33	1.09
7	67.67	36.50 (28.76)	4.28 (3.38)	3.40 (2.66)	8.17	45.00	1.27
14	78.33	38.33 (25.04)	0.50 (0.32)	4.47 (2.89)	8.62	54.33	1.54
21	73.33	14.97 (−0.01)	−0.01 (−0.01)	0.60 (0.60)	9.15	73.33	2.08
LSD (0.05)	5.30	2.95 (1.07)	1.01 (0.53)	0.20 (0.18)	0.18	4.30	0.12
Midrib							
0		39.20 (39.20)	20.37 (20.37)	0.01 (0.01)		30.00	1.00
4	69.67	31.50 (32.19)	14.23 (14.54)	0.27 (0.27)	6.97	29.33	0.98
7	73.33	28.17 (26.49)	9.27 (8.71)	4.98 (4.70)	7.90	32.00	1.07
14	77.00	26.83 (20.85)	1.77 (1.36)	6.17 (4.77)	8.73	38.67	1.29
21	76.00	25.17 (14.80)	−0.27 (−0.16)	1.55 (0.91)	9.54	51.00	1.70
LSD (0.05)	5.01	2.99 (2.93)	2.25 (1.97)	1.23 (0.94)	0.22	2.45	0.08
Treatment 4 (32 °C/90% RH, with Lamina and Midribs Separated before Treatment)							
Lamina							
0		52.50 (52.50)	3.20 (3.20)		5.85	23.00	1.00
4		48.83 (43.44)	3.06 (2.72)	0.14 (0.12)	6.44	25.93	1.13
7		39.83 (26.89)	0.62 (0.64)	3.07 (2.07)	7.85	34.13	1.48
14		38.67 (21.43)	0.20 (0.11)	1.08 (0.60)	8.85	41.60	1.81
21		39.83 (19.34)	0.37 (0.17)	0.50 (0.28)	9.03	48.00	2.09
LSD (0.05)		3.09 (4.29)	0.49 (0.36)	0.45 (0.25)	0.17	4.98	0.21
Midrib							
0		33.67 (33.67)	10.38 (10.38)	<0.01 (<0.01)	6.72	22.40	1.00
4		31.50 (30.00)	13.33 (12.71)	1.80 (1.69)	7.29	23.60	1.05
7		27.50 (23.22)	10.92 (9.20)	9.58 (8.07)	8.25	26.53	1.18
14		27.83 (19.40)	0.73 (0.53)	6.33 (4.41)	9.33	32.27	1.44
21		26.50 (16.16)	0.52 (0.30)	3.92 (2.39)	9.50	36.80	1.64
LSD (0.05)		2.60 (2.61)	1.69 (1.68)	1.95 (1.35)	0.14	3.20	0.14

[a] All values reported on dry matter basis. [b] Values in parentheses are adjusted for dry matter loss with the calcium ratio.

15.8%. It appears that the moisture and temperature affected the 30% disappearance of nicotine in the lamina. From this experiment it is not possible to establish whether the nicotine decrease was due to microbial metabolism or to degradation.

Influence of 32 °C and 90% RH on Changes in Tobacco Composition. From the initial study it was obvious 83% RH had little short-term effect on the degradation of nitrogenous tobacco constituents. Treatment 2 maintained the same temperature (32 °C), but the relative humidity increased to 90%. This 7% increase in relative humidity caused the moisture content of the lamina and midrib to increase to 70% and 71%, respectively (Table I). This high moisture content had a dramatic influence on the changes in the composition of both lamina and midrib. Total nitrogen decreased in the lamina in treat-

Table II. Influence of Storage Environment on Individual Alkaloids[a]

	nicotine, mg g^{-1}	nornicotine, mg g^{-1}	anabasine, mg g^{-1}	anatabine, mg g^{-1}	total alkaloids, mg g^{-1}
			Treatment 1		
			Lamina		
0	40.63 (40.63)[b]	2.09 (2.09)	0.24 (0.24)	1.25 (1.25)	44.21 (44.21)
7	43.00 (43.20)	1.30 (1.31)	0.35 (0.35)	1.62 (1.63)	46.27 (46.48)
14	40.14 (36.08)	1.39 (1.25)	0.31 (0.28)	1.66 (1.49)	43.49 (39.10)
21	38.44 (38.15)	1.33 (1.32)	0.34 (0.33)	1.76 (1.74)	41.86 (41.54)
28	28.51 (26.05)	2.00 (1.83)	0.31 (0.28)	1.63 (1.49)	32.45 (29.65)
LSD (0.05)	5.45 (7.81)	1.17 (1.34)	0.05 (0.09)	0.22 (0.38)	5.64 (8.79)
			Midrib		
0	7.60 (7.60)	0.51 (0.51)	0.04 (0.04)	0.15 (0.15)	8.31 (8.31)
7	11.73 (11.82)	0.40 (0.40)	0.07 (0.07)	0.26 (0.26)	12.46 (12.56)
14	12.04 (11.21)	0.61 (0.57)	0.06 (0.06)	0.27 (0.25)	12.99 (12.09)
21	10.04 (9.37)	0.43 (0.40)	0.07 (0.06)	0.25 (0.23)	10.79 (10.07)
28	7.49 (6.64)	0.54 (0.48)	0.07 (0.06)	0.25 (0.22)	8.35 (7.40)
LSD (0.05)	1.98 (2.22)	0.36 (0.38)	0.01 (0.02)	0.04 (0.04)	1.84 (2.24)
			Treatment 2		
			Lamina		
0	40.63 (40.63)	2.09 (2.09)	0.24 (0.24)	1.25 (1.25)	44.21 (44.21)
4	33.37 (26.06)	1.98 (1.54)	0.40 (0.31)	1.63 (1.27)	37.37 (29.19)
7	11.08 (8.27)	1.01 (0.75)	0.27 (0.20)	1.02 (0.76)	13.38 (9.99)
14	1.60 (0.95)	1.88 (1.12)	0.11 (0.06)	0.34 (0.20)	3.94 (2.34)
21	0.96 (0.50)	1.04 (0.54)	0.04 (0.02)	0.09 (0.04)	2.12 (1.10)
LSD (0.05)	3.14 (3.70)	0.93 (0.80)	0.03 (0.03)	0.10 (0.13)	2.78 (3.83)
			Midrib		
0	7.60 (7.60)	0.51 (0.51)	0.04 (0.04)	0.15 (0.15)	8.31 (8.31)
7	9.24 (8.15)	0.61 (0.54)	0.07 (0.06)	0.27 (0.24)	10.19 (8.99)
14	5.46 (4.71)	0.36 (0.31)	0.07 (0.06)	0.25 (0.22)	6.15 (5.30)
21	0.81 (0.56)	0.53 (0.37)	0.04 (0.03)	0.11 (0.08)	1.50 (1.04)
28	0.64 (0.37)	0.43 (0.25)	0.02 (0.01)	0.04 (0.02)	1.12 (0.65)
LSD (0.05)	1.61 (1.43)	0.27 (0.23)	0.01 (0.01)	0.03 (0.03)	1.51 (1.36)
			Treatment 3		
			Lamina		
0	40.63 (40.63)	2.09 (2.09)	0.24 (0.24)	1.25 (1.25)	44.21 (44.21)
4	28.18 (25.97)	1.84 (1.70)	0.26 (0.24)	1.24 (1.15)	31.52 (29.05)
7	12.83 (10.08)	1.76 (1.38)	0.24 (0.19)	0.99 (0.78)	15.82 (12.42)
16	2.18 (1.42)	0.70 (0.46)	0.12 (0.08)	0.37 (0.24)	3.38 (2.20)
21	0.75 (0.36)	0.34 (0.16)	0.01 (0.00)	0.03 (0.01)	1.14 (0.55)
LSD (0.05)	2.55 (2.80)	1.02 (0.90)	0.02 (0.02)	0.08 (0.09)	2.43 (2.83)
			Midrib		
0	7.60 (7.60)	0.51 (0.51)	0.04 (0.04)	0.15 (0.15)	8.31 (8.31)
4	10.12 (10.35)	0.78 (0.79)	0.06 (0.06)	0.27 (0.28)	11.23 (11.49)
7	6.07 (5.69)	0.59 (0.55)	0.07 (0.06)	0.24 (0.23)	6.96 (6.53)
14	1.07 (0.83)	0.23 (0.18)	0.05 (0.04)	0.11 (0.09)	1.46 (1.13)
21	0.47 (0.28)	0.16 (0.09)	0.01 (0.01)	0.01 (0.01)	0.65 (0.38)
LSD (0.05)	1.31 (1.31)	0.47 (0.48)	0.01 (0.01)	0.03 (0.05)	1.28 (1.34)
			Treatment 4		
			Lamina		
0	47.73 (47.73)	0.96 (0.96)	0.18 (0.18)	2.31 (2.31)	51.18 (51.18)
4	34.89 (30.95)	0.64 (0.56)	0.16 (0.15)	1.90 (1.68)	37.59 (33.34)
7	14.25 (09.60)	0.64 (0.43)	0.17 (0.12)	1.99 (1.34)	17.04 (11.48)
14	3.47 (01.92)	0.20 (0.11)	ND (ND)	0.56 (0.31)	3.97 (2.20)
21	2.15 (01.03)	0.13 (0.06)	ND (ND)	ND (ND)	2.24 (1.07)
LSD (0.05)	4.25 (04.20)	0.44 (0.40)	0.10 (0.05)	0.45 (0.28)	4.51 (4.46)
			Midrib		
0	19.48 (19.48)	0.33 (0.33)	ND (ND)	0.45 (0.45)	20.12 (20.12)
4	9.83 (09.33)	0.30 (0.28)	ND (ND)	0.39 (0.37)	10.39 (9.86)
7	2.84 (02.40)	0.10 (0.08)	ND (ND)	0.19 (0.16)	3.00 (9.86)
14	1.85 (01.29)	ND (ND)	ND (ND)	ND (ND)	1.85 (1.29)
21	1.71 (01.04)	ND (ND)	ND (ND)	ND (ND)	1.71 (1.04)
LSD (0.05)	2.01	0.14		0.29	1.88

[a] Values reported on dry matter basis. [b] Values in parentheses are adjusted for dry matter loss with the calcium ratio.

ment 2. When adjusted for dry matter disappearance by using the calcium ratio, there was a 50% decrease of nitrogen. This decrease of nitrogen in lamina was not due to combined loss of nitrite or nitrate since there was significant increase of combined values of NO_3^- and NO_2 even when adjusted for dry matter loss. Some of the increase in lamina was due to migration of the nitrate–nitrite from the midrib.

The changes in nitrate and nitrite levels were dramatic during the 3-week storage (Table I). Within 1 week the nitrite levels had increased from 0.02 to 2.27 mg/g. On a constant-weight basis the maximum accumulation of nitrite occurred within 2 weeks after initiation of the experiment. At this point, the nitrite N content of the lamina is twice the content of nitrate N. The nitrate concentration was calculated by subtracting the nitrite value obtained

Table III. Influence of Storage Environment on the Accumulation of Nitrosamines

	NNN	NAT	NNK	TSNA
		Treatment 1		
		Lamina		
0	3.00[a]	19.70	1.37	24.06
7	2.29	10.55	0.75	13.59
14	3.80	11.06	0.99	15.86
21	1.58	5.14	0.41	7.12
28	2.40	8.08	0.43	10.91
LSD (0.05)	2.51	2.67	0.43	4.91
		Midrib		
0	9.08	20.94	6.66	36.68
7	4.94	13.14	2.33	20.40
14	14.99	6.97	0.31	22.26
21	5.08	4.70	0.42	10.20
28	8.51	9.62	0.86	18.99
LSD (0.05)	16.16	9.68	1.24	23.72
		Treatment 2		
		Lamina		
0	3.00	19.70	1.37	24.06
4	6.21	20.65	10.86	37.72
7	35.49	129.80	56.27	221.56
14	124.72	131.48	15.82	272.03
21	54.13	68.24	3.34	125.71
LSD (0.05)	21.25	36.42	20.65	62.85
		Midrib		
0	9.08	20.94	6.66	36.68
3	7.40	9.73	10.34	27.47
7	46.10	102.43	56.14	204.68
14	97.12	159.52	24.78	281.42
21	59.99	78.84	3.63	142.46
LSD (0.05)	60.32	70.13	30.44	149.21
		Treatment 3		
		Lamina		
0	3.00	19.70	1.37	24.06
4	6.33	21.93	5.89	34.06
7	273.67	824.37	75.11	1173.15
14	48.83	156.49	7.52	212.84
21	20.32	48.89	0.81	70.02
LSD (0.05)	118.05	30.70	8.63	116.75
		Midrib		
0	9.08	20.98	6.66	36.68
4	14.06	18.17	8.83	41.06
7	475.62	723.96	106.15	1305.73
14	68.19	121.85	9.84	199.88
21	14.45	44.43	1.44	60.33
LSD (0.05)	121.85	149.18	29.68	25.15
		Treatment 4		
		Lamina		
0	1.07	11.10	ND[b]	12.17
4	3.11	27.80	2.80	33.71
7	16.99	139.56	5.47	162.01
14	4.96	41.52	ND[b]	46.48
21	2.27	11.79	0.26	14.15
LSD (0.05)	6.67	54.38	4.83	63.03
		Midrib		
0	1.79	5.51	ND[b]	7.31
4	8.43	36.44	6.07	50.93
7	22.67	93.93	1.34	117.94
14	2.34	10.54	ND[b]	12.94
21	1.01	2.55	0.27	3.83
LSD (0.05)	2.69	10.69	2.48	11.30

[a] All values in micrograms per gram. [b] Not detected.

from the Griess procedure from the values obtained from the enzymatic reduction of nitrate to nitrite followed by the Griess colorimetric procedure. Under this storage condition, nitrate was reduced to nitrite since there was an overall decrease of nitrate content during the 3-week period. The decrease of nitrate after 1 week was due its reduction to nitrite rather than leaching based on the observations that the nitrite concentration increased significantly during that 3-week period. Based on relative changes of calcium and potassium in the lamina, the theoretical concentration of potassium should have been 85.7 mg/g by the third week. The measured value for potassium was 71.8 mg/g, and this 16.2% decrease was assumed to have been due to leaching of soluble K (Table IV). Actual loss of nitrate from the lamina was 64%, and approximately 16% of this could have been leaching from the lamina and midrib.

Table IV. Relative Influence of Moisture Content on Leaching of Plant Constituents As Determined by Calcium and Potassium

day	K, mg/g	K_{calcd}[a]	% loss
	Treatment 2		
0	44.2	44.2	0
3	41.3	56.6	
7	55.5	59.3	−7
14	67.1	74.3	−10
21	71.8	85.7	−16
	Treatment 3		
0	44.2	44.2	0
3	48.2	46.0	5
7	56.1	55.0	−2
14	68.1	61.5	−10
21	62.8	91.9	−32
	Treatment 4		
0	20.1	20.1	0
3	26.4	22.7	16
7	25.8	29.8	−13
14	34.1	36.3	−6
21	31.4	42.0	−25

[a] $K_{calcd} = [Ca(x)/Ca(0)]K_x$, where x = day of sampling and 0 = day 0.

For treatment 2 the alkaloids were almost totally destroyed in 3 weeks (Table II). Nicotine values showed a 98% decrease during this short-term study, whereas the nornicotine decreased by only 75%. Anabasine and anatabine had the same magnitude of loss as occurred for nicotine.

At the 32 °C/90% RH storage conditions, individual nitrosamines and total nitrosamines increased significantly. Maximum concentration of NNN occurred by the 14th day, and by the 3rd week there was a significant decrease of the nitrosamine (Table III). Leaching could only partially account for the decrease of nitrosamines; therefore, there was destruction of nitrosamines between the second and third weeks. It should also be noted there was a positive correlation between concentrations of nitrite and the nitrosamines.

High moisture content of the lamina also affected a significant change of the pH from 5.3 to 9.2. Except for the last sampling date, the changes of pH paralleled the increase of nitrosamine accumulation in tobacco. This is of interest since nitrosamine formation is favored under more acid conditions (Mirvish, 1975). However, our study indicates the nitrosamine accumulation occurred under alkaline conditions. This would indicate they were formed via nonclassical intermediate (Mirvish, 1975). Hoffmann et al. (1975) and Hecht et al. (1978) reported nicotine was converted to NNN in the presence of nitrite at pH 7.9. This was repeated on purified nicotine (Neurath et al., 1976), and in the presence of NO_2^- for the first 20 h of treatment, it was reported that NNN was detected only in solutions buffered at pH 6.6. These conflicting studies show that the influence of pH on formation of nitrosamines is not well-defined and their formation in a biological matrix is more poorly understood.

Influence of Bactericide and Fungicide on Changes in Tobacco Constituents (Treatment 3). To explain results of the 32 °C/90% RH treatment, it was assumed that accumulation of nitrite was due to the microbial reduction of nitrate. It was thought that treatment of tobacco with a bactericide (streptomycin) and fungicide (rifampicin) should retard or inhibit production of nitrite and therefore reduce the accumulation of the nitrosamines and perhaps retard the other changes in the composition of the lamina. In our study the tobacco was treated with the bactericide–fungicide two times per week by either dipping or spraying. Because of the high moisture content of treatment 3, it would be possible to argue the losses of alkaloids, nitrate, and total N could be due to leaching of these soluble constituents from the lamina. An attempt was made to calculate the potential for leaching the soluble constituents from the tobacco with potassium as the model. The calculated values of potassium leaching were obtained by using the equation in Table IV. This was based on the assumption that calcium will be in an insoluble form (i.e., oxalate) and will not be leached from tobacco. Potassium would be soluble and would be leached from tobacco. Since it cannot be metabolized, it was an excellent means for determining the leaching of soluble constituents from tobacco. Among the second, third, and fourth treatments, the maximum leaching occurred for treatment 3. Approximately one-third of the potassium was leached from tobacco when the lamina was dipped or sprayed with aqueous solution twice per week. Data for the potassium values indicated that loss of alkaloids, nitrate, nitrite, TSNA, and nitrogen may not be due totally to the leaching of these soluble organic constituents from tobacco.

Treatment 3 demonstrated that application of streptomycin and rifampicin to the tobacco did not inhibit nitrate loss. Comparison of the nitrate values between treatments 2 and 3 indicates that the bactericide and fungicide enhanced the decrease of nitrate. Nitrite values were lower in treatment 3 than treatment 2; however, a significant quantity of nitrite was found in the presence of the antibiotics. These data suggest that topical addition of antibiotics was not sufficient to inhibit microbial nitrate reductase activity.

High levels of nitrite concentration were paralleled by high levels of individual nitrosamines. By day 7 of treatment 3, the samples contained the highest concentration of individual nitrosamines. There was a significant decrease of these nitrosamines by the 14th day. From the values obtained for potassium, it would be difficult to attribute the losses of nitrosamines to be totally due to leaching. Therefore, the significant decreases of the nitrosamines during the later storage times were due in part to other losses (i.e., metabolism). This is of interest since there has been no report that TSNAs are destroyed during storage.

The nitrates and alkaloids in the midrib decreased (Tables I and II) during storage, which paralleled the changes in the lamina. There was a significant increase of nitrite and the nitrosamines in the midvein (Table III). As in the lamina, there was greatest accumulation of these constituents within the first week of this treatment, followed by a significant loss of nitrite and TSNA. Because of the same trends observed for both lamina and midvein, accumulation of nitrite and nitrosamines did not appear to be dependent on the specific leaf part.

From data obtained with treatments 2 and 3, it appeared that nitrate migrated from the midvein to the lamina (Table I) since there was a significant increase of nitrate in the lamina and a significant decrease of nitrate in the midrib by the third day of treatment. Therefore, another treatment (treatment 4) was initiated to determine whether there was migration of the nitrate between lamina and midvein.

Midrib was removed from the lamina, and the appropriate leaf parts were subjected to the same environment as treatment 2. It should be noted that the tobacco used for this treatment was from a different sampling than the tobacco of the first three treatments. Initial values are different, but the changes during storage at these conditions should be valid treatment effects. There was no increase of nitrate in lamina, and there was no significant decrease of nitrate in the midvein by the third day (Table I). This result supports the earlier hypothesis that the observed increases in nitrate in treatments 2 and 3 were due to migration of nitrate from the midrib to the lamina and were not due to microbial nitrification.

Nitrite first increased and then decreased during the 3-week storage rather than just increasing as observed for the midvein tissue in treatment 2. Individual nitrosamines also increased and decreased during this storage period for both midrib and lamina tissues. The trend of treatment 4 paralleled treatments 2 and 3, which showed that separation of lamina from the midvein did not alter the relative accumulation patterns of nitrosamine or nitrite.

In summary, several environmental conditions have been defined as requirements for accelerated accumulation of TSNA. Moisture content in leaf affected accumulation of the TSNA. When lamina moisture was 70%, there was a rapid accumulation of TSNA and nitrite. Maximum accumulation of the nitrosamine and nitrite occurred within 7 days, after which there was a significant decrease of both constituents. Decreases of the constituents can only partially be attributed to leaching of the soluble constituents. These data therefore indicate that the nitrosamines were degraded at 32 °C and 90% RH. Data from this study indicated that nitrite was formed in significant quantities from nitrate under aerobic conditions.

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Characterization of Peanut Proteins during Roasting As Affected by Initial Moisture Content

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Dry peanut kernels (Tainan 9, a Spanish cv.) were moisture-conditioned, and their internal temperature profiles roasted at 150 °C were monitored. Higher initial moisture content resulted in a slower rate of internal temperature increase. Phosphate buffer extracts of proteins from splits roasted to internal temperatures of 80, 100, 120, and 150 °C varied with regard to initial moisture content and tendency to denature. When splits were roasted at temperatures up to 140 °C, the amount of extracted protein declined with an increase in moisture content from 3.2 to 23.2%. Gradient PAGE analysis of proteins extracted from splits and meals subjected to roasting at 150 °C and splits heated in phosphate buffer in a boiling water bath for 5, 15, 25, and 60 min was done. The thermal behavior of arachins and non-arachins varied depending upon the means of heat treatment, extent of heating, and initial moisture content.

Roasting is the most common method of processing peanuts. Moisture measurement of raw peanuts using instruments or by subjective estimation before roasting is usually a prerequisite step in establishing the roasting process, i.e., roasting temperature and time, in order to optimize the roasted peanut quality. The functions of water in this process are multiple, and the reactions involved are complicated. One of the most important effects of water on chemical reactivity in foods is its ability to mobilize and act as a solvent for food components. Chemical reaction rates generally accelerate with increasing moisture content due to increased reactant mobility. However, at elevated moisture content, chemical reactivity may decrease with increasing moisture because of the dilution effect of excessive water on reactant concentrations. A reactant in foods must dissolve in water before the reactivity can be initiated. Consequently, optimal reactivity can be eventually achieved when the reactants are dissolved in a specific amount of water to reach the critical concentration for the reaction.

One of the most important reactions during peanut roasting is the unique change in proteins. The effect of heat on such proteins has been studied. Newell et al. (1967) reported that biochemical reactions between sugars and amino acids produced specific roasted peanut flavor components. The effect of roasting on stability and nitrogen solubility of peanut proteins has been intensively studied (Neucere et al., 1969; Ory et al., 1970; Labib et al., 1977). The effect of oil cooking on changes in the peanut seed polypeptide composition was studied (Basha and Young, 1985). However, attention was directed toward the effect of heat on changes in protein at one or two moisture levels. Knowledge concerning how water functions directly or indirectly to change peanut protein properties is limited. In other foodstuffs and constituents such as myoglobulin, apples, fababean protein, oat globulin, strawberries, and soybean lipoxygenase, the effect of water on thermal be-

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