Chemical Composition of Nonsmoking Tobacco Products

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Samples of commercial smokeless tobacco products were analyzed for tobacco specific *N*-nitrosamines, alkaloids, and other major leaf components. Values for these leaf chemicals were compared for six moist snuff and four loose-leaf chewing tobaccos with one commercial pipe tobacco. Levels of *N*-nitrosonornicotine (NNN) varied from 0.75 to 17.75 ppm, while 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) varied from 0.3 to 7.37 ppm. The nitrosamine levels did not correlate with contents of total alkaloids or nicotine. This seems to indicate that their formation depends more on the manufacturing procedures. Fructose, glucose, and sucrose levels in these products were shown to vary considerably. Evaluation of dark tobaccos used in these products indicated that sugar additives were used in most of these smokeless tobaccos. Since more than 12 million people in the United States used smokeless tobacco products in 1985, our data indicate a need for a modification in these products to lower the levels of health-related nitrosamines and sugars.

While cigarette smoking has declined during the past 20 years, the production and consumption of smokeless tobacco products has increased significantly. Smokeless tobacco products, snuff and chewing tobacco, were used by at least 12 million Americans in 1985. In the 1985 Report of the Surgeon General Advisory Committee on the health consequence of using smokeless tobacco products, Dr. C. Everett Koop concluded that "it is not a safe substitute for smoking cigarettes. It can cause cancer and a number of non-cancerous oral conditions and can lead to nicotine addiction and dependence". Several reports have indicated a statistical association between oral cancer and chewing tobacco or snuff dipping (Winn, 1985; Wynder and Stellman, 1977; Winn et al., 1981; IARC, 1985).

There are several types of chewing tobacco and snuff products on the market. Each product is a mixture of several types of tobacco and additives and may be produced by very different manufacturing processes. Generally, chewing tobacco of the loose-leaf or scrap tobacco variety is made of fermented cigar leaf tobacco with varying amounts of sugars, syrup, licorice, or other flavoring materials. Snuff consists of powdered dark, air-cured and fire-cured tobaccos that may have been sweetened with sugars, molasses, and/or syrup or flavored with wintergreen or mint.

The only well-established carcinogens found in smokeless tobacco products are the tobacco-specific nitrosamines (TSNA), which are mainly *N*-nitrosonornicotine (NNN) and 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Hoffmann and Hecht, 1985). Volatile nitrosamines have also been reported in this type of smokeless product, but at levels of less than 100 ppb (Brunnemann et al., 1985). It is also likely that these smokeless products also contain trace amounts of carcinogenic polynuclear aromatic hydrocarbons (PAH) (Campbell and Lindsey, 1957; Hoffmann et al., 1986) and polonium-210 (Hoffmann et al., 1986), especially those made from fire-cured tobacco. NNN and NNK are formed from the reaction of nitrite with the tobacco alkaloids (Mirvish, 1985; Hoffmann et al., 1981). Tobacco leaves contain 0.5–5.0% of the leaf weight as alkaloids (Sisson and Severson, 1984). The most predominant alkaloid is the habituating agent, nicotine (85–95% of total alkaloids). The other alkaloids are nornicotine, anatabine, and anabasine (Mirvish, 1985). The nitrosation reaction is influenced by the processes involved in the manufacture of the smokeless products (Andersen et al., 1982).

The goal of this study was to compare the major commercial smokeless tobacco products on the basis of chemical composition, including nitrosamines, alkaloids, sugars, and other major leaf constituents.

EXPERIMENTAL SECTION

Smokeless tobacco products were purchased on the open market in 1985. Alkaloids were determined by the method of Severson et al. (1981). Extraction of TSNA was carried out as previously reported (Chamberlain and Arrendale, 1983). The samples were analyzed by gas chromatography (GC) with a Hewlett-Packard Model 5710A gas chromatograph, equipped with a thermionic N–P detector. The standard instrument was modified for glass capillary GC analyses, as previously described (Severson et al., 1980). TSNA analyses were performed in the split mode (100:1) on a 30 m × 0.25 mm (i.d.) capillary column, coated with OV-17. The oven temperature was programmed from 100 to 250 °C at 4 °C/min. The helium flow was 20 cm/s, and the injection port temperature was 280 °C. The thermionic N–P detector was operated under hydrogen and air flow conditions as recommended by the manufacturer (Figure 1).

For the whole-leaf chemical analyses (sugars, chlorogenic acid, solanesol, citric acid, malic acid), 1 mL of a 50:50 *N*,*O*-bis(trimethylsilyl)trifluoroacetamide–*N*,*N*-dimethylformamide solution, containing 0.5 mg/mL of 1,3-dimyristin as internal standard, was added to 25 mg of ground tobacco in a sample vial (Severson et al., 1980). The sample was placed in an ultrasonic bath for 1 h, followed by heating at 76 °C for 45 min, and was then centrifuged 1–2 min. For GC analysis, 2 μL of the supernatant solution was injected on a 45 cm × 3 mm glass column, packed with Chromosorb coated with Dexsil 300 GC (5% on 100/120-mesh Chromosorb). Helium flow rate was 60 mL/min. The column oven was held at 100 °C for 2 min and then programmed to 330 °C at 8 °C/min and held there for 8 min. The injection port temperature was 250 °C, and the flame ionization detector was heated at 350 °C (Severson et al., 1980) (Figure 2).

RESULTS AND DISCUSSION

Analytical data for the selected smokeless tobacco products and one pipe tobacco, tested for comparison, are listed in Table I. There is a wide variation in the quantitative values of all components of the different samples.

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Table I. Chemical Composition of Commercial Tobacco Products

		moist snuff						chewing tobacco			
	pipe tobacco	A	B	C[a]	D[b]	E	F[b]	A	B	C	D
NNN, μg/g	1.80	2.20	17.75	9.25	ND[c]	1.55	4.00	0.75	6.50	1.50	0.65
NNK, μg/g	0.30	7.30	3.20	3.75	ND	2.10	1.10	0.30	0.50	0.55	1.05
nicotine[d]	11.05	3.87	14.61	23.97	29.47	3.25	12.05	7.99	4.73	13.19	5.49
nornicotine		0.01	0.02	0.29	0.20	0.05	0.06	0.07	0.06	0.18	0.03
anabasine			0.04	0.10	0.12		0.06			0.10	
anatabine	0.08	0.02		0.19	0.22	0.06		0.13	0.05	0.31	0.03
total alkaloids	11.14	3.90	14.67	24.54	30.29	3.46	12.16	8.19	4.84	13.78	5.54
malic acid	4.72	2.08	0.45		0.56	5.36	11.45	7.24	1.85	8.73	3.37
citric acid	6.84	8.82	0.70	1.54	2.79	7.70	3.09	4.79	3.68	9.09	5.22
α-glucose	25.73	23.47	0.30			36.67		4.02	17.03	37.15	21.29
β-glucose	35.18	31.14	0.12			35.98		31.19	21.28	47.42	26.67
fructose	53.88	32.63	0.30			53.46		13.27	11.82	52.18	19.09
sucrose	136.58	139.87	0.75			136.84		175.62	187.04	168.09	224.61
chlorogenic acid	0.45	0.22	0.27	3.43	0.22	0.75	0.33	0.43	0.31	0.45	0.26
solanesol	4.01	4.19	6.02	8.50	8.29	2.25	11.30	4.43	1.86	6.13	1.37

[a] Mint flavored. [b] Wintergreen flavored. [c] ND = not determined. [d] Values are in milligrams/gram, except for NNN and NNK.

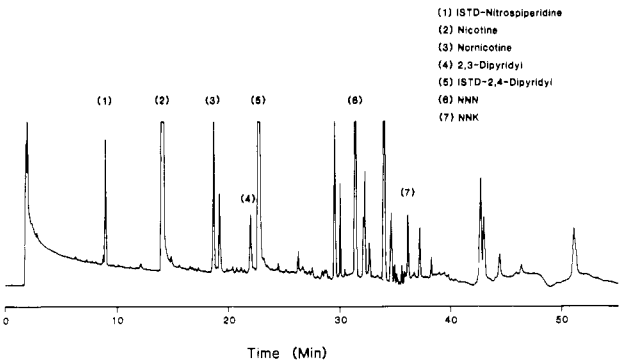

Figure 1. Gas chromatographic nitrosamine analysis of chewing tobacco D.

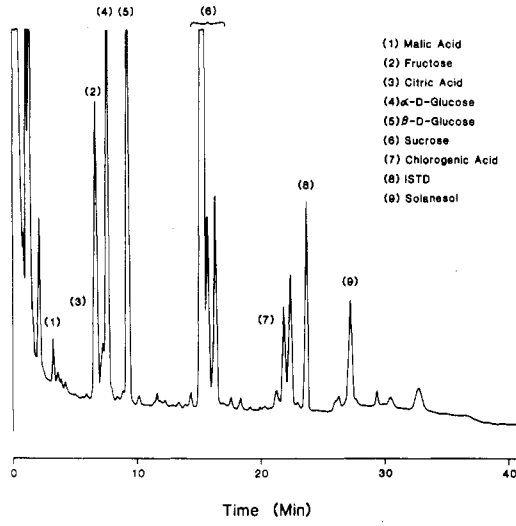

Figure 2. Gas chromatographic leaf analysis of chewing tobacco D.

NNN ranged from 0.65 ppm for a chewing tobacco to 17.75 ppm for a moist snuff sample. In general, the TSNAs were higher for the snuff tobaccos than for the chewing tobaccos. These results confirmed findings by others that the TSNA concentrations may vary greatly, depending on the type of tobacco and processing used (Hoffmann et al., 1986; Brunnemann et al., 1983). The data also support findings that the total alkaloid or nicotine contents are not the determining factors of TSNA concentrations of processed tobaccos (Brunnemann et al., 1983). Only one of the chewing tobaccos (B) had an NNN level higher than the pipe tobacco. Surprisingly, this tobacco had the lowest total alkaloid content of the chewing tobaccos. There were

Table II. Chemical Composition of Various Tobaccos

	flue-cured NC 2326	dark air-cured KY 160	dark air-cured KY 171	dark fire-cured KY 171
NNN, μg/g	1.65	2.16	0.48	0.74
NNK, μg/g	0.49	0.17	0.12	0.50
nicotine[a]	26.6	29.0	44.5	33.7
malic acid	19.1	50.1	88.4	85.8
α-glucose	30.1			0.4
β-glucose	40.8			
fructose	45.7			
sucrose	92.9			
chlorogenic acid	31.8	1.4	0.5	0.8
solanesol	12.2	28.2	21.3	17.7

[a] Values are in milligrams/gram, except for NNN and NNK.

no obvious differences in the rest of the analyzed constituents to suggest a reason for the high NNN value. It was interesting to note a large variation (22–30% by weight) in sugar levels (Table I). The pipe tobacco, chewing tobaccos, and snuff samples A and E had much higher sugar levels than normal tobacco, suggesting some addition (Table II). Apparently, there were no sugars added to the flavored tobaccos C, D, and F or to the one nonflavored snuff (B). There are numerous reports on the relationship of sugars to dental caries; excessive sugar levels in smokeless tobaccos may be a cause of concern. Presently, there are conflicting reports in the literature as to the role played by smokeless tobacco products in the development of dental caries; some reports claim a causative effect (Sitzeo, 1981; Croft, 1981), while others postulate a potentially protective effect (Shannon and Trodahl, 1981). Thus, the role of sugar additives in these products has not been settled. It was also interesting to note that products with low sugar levels (snuff samples B–D and F) also had high levels of solanesol (6.02–11.30 mg/g). Although, solanesol has been shown to be a precursor of carcinogenic PAH in tobacco smoke (Schlotzhauer et al., 1976), its role in smokeless products is unknown and its levels are probably related to the typical tobacco used in these products.

We subsequently compared the levels of TSNA, nicotine, and the other leaf components in a normal flue-cured (NC 2326) and two dark tobaccos of the varieties used in the manufacture of snuff and chewing tobaccos (Table II). The sugar levels in dark tobaccos were very low; consequently, the sugars found in the products manufactured from this type of tobacco were additives. Nicotine contents were slightly higher, and malic acid levels were very high. Solanesol levels in these tobaccos were almost double that of a standard flue-cured tobacco, such as NC 2326 tobacco.

Thus, the use of these high-solanesol tobaccos in pipe smoking products could actually raise PAH levels in smoke and produce a more hazardous smoke (Schlotzhauer et al., 1976). We also examined the same tobacco, KY 171, which was either air-cured or fire-cured and found that the NNN levels were higher in the fire-cured sample. As these values were determined on only one crop, analyses need to be repeated on next year's crop to confirm the significance of this finding. However, compared to the other tobaccos, the TSNA levels in KY 171 appeared to be low. This again pointed out the effect of postharvest treatments or curing on the concentration of TSNA in smokeless tobacco products.

This study confirmed the presence of high levels of tobacco specific nitrosamines in smokeless tobacco products and found that excessive levels of sugars that cause dental caries were added to these products. Again, no relationship was found between TSNA and alkaloids, indicating that most of the nitrosamines are formed during the manufacturing process. The use of fire-cured tobaccos in these products should be discouraged since, during the fire curing, the tobacco may become coated with hazardous smoke constituents such as phenols, catechols, and PAH. Also, the use of high-solanesol tobacco for either pipe or cigarette smoking products should be avoided due to the possible formation of excessive levels of carcinogenic PAH, such as benzo[*a*]pyrene from the solanesol. As more and more young people in the United States are taking up the use of smokeless tobacco as a more benign alternative to cigarette smoking, they should be made aware of these health-related compounds in such products.

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Registry No. NNN, 16543-55-8; NNK, 64091-91-4; nicotine, 54-11-5; nornicotine, 494-97-3; anabasine, 494-52-0; anatabine, 581-49-7; malic acid, 6915-15-7; citric acid, 77-92-9; α-D-glucose, 492-62-6; β-D-glucose, 492-61-5; D-fructose, 57-48-7; sucrose, 57-50-1; chlorogenic acid, 327-97-9; solanesol, 13190-97-1.

LITERATURE CITED

Andersen, R. A.; Kasperbauer, M. J.; Burton, H. R.; Hamilton, J. L.; Yoder, E. E. "Changes in Chemical Composition of Homogenized Leaf-Cured and Air-Cured Burley Tobacco Stored in Controlled Environments". *J. Agric. Food Chem.* **1982**, *30*, 663.

Brunnemann, K. D.; Scott, J. C.; Hoffmann, D. "*N*-Nitrosoproline, an Indicator for N-Nitrosation in Processed Tobacco". *J. Agric. Food Chem.* **1983**, *31*, 905.

Brunnemann, K. D.; Genoble, L.; Hoffmann, D. "*N*-Nitrosamines in Chewing Tobaccos: An International Comparison". *J. Agric. Food Chem.* **1985**, *33*, 1178.

Campbell, J. M.; Lindsey, A. J. "Polycyclic Aromatic Hydrocarbons in Snuff". *Chem. Ind.* **1957**, 951.

Chamberlain, W. J.; Arrendale, R. F. "An Alternate Method for the Analysis of N-Nitrosonornicotine in Tobacco". *J. Agric. Food Chem.* **1983**, *31*, 909.

Croft, L. "Smokeless Tobacco: A Case Report". *Tex. Dent. J.* **1981**, *99*, 15.

Hoffmann, D.; Hecht, S. S. "Nicotine-Derived *N*-Nitrosamines and Tobacco-Related Cancer: Current Status and Future Directives". *Cancer Res.* **1985**, *45*, 935.

Hoffmann, D.; Adams, J. D.; Brunnemann, K. D.; Hecht, S. S. "*N*-Nitroso Compounds". *ACS Symp. Ser.* **1981**, *No. 174*, 247.

Hoffmann, D.; Harley, N. H.; Fisenne, I.; Adams, J. D.; Brunnemann, K. D. "Carcinogenic Agents in Snuff". *JNCI* **1986**, *76*, 435–437.

IARC "Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines". *IARC Monogr.* **1985**, *37*, 37–141.

Mirvish, S. S. "Formation of *N*-Nitroso Compounds: Chemistry, Kinetics and in Vivo Occurrence". *Toxicol. Appl. Pharmacol.* **1985**, *31*, 325.

Schlotzhauer, W. S.; Severson, R. F.; Chortyk, O. T.; Arrendale, R. F.; Higman, H. C. "Pyrolytic Formation of Polynuclear Aromatic Hydrocarbons from Petroleum Ether Extractable Constituents of Flue-Cured Tobacco Leaf". *J. Agric. Food Chem.* **1986**, *24*. 992.

Severson, R. F.; Arrendale, R. F.; Chortyk, O. T. "Simple Conversion of Two Standard Gas Chromatograph to All-Glass Capillary Systems". *HRC CC, J. High Resolut. Chromatogr. Commun.* **1980a**, *3*, 11.

Severson, R. F.; McDuffie, K. L.; Arrendale, R. F.; Chortyk, O. T. "A One-Step Gas Chromatographic Analysis of Major Tobacco Components". *Abstracts of Papers*, Proceedings of 34th Tobacco Chemists' Research Conference, Richmond, VA 1980*b*; 21.

Severson, R. F.; McDuffie, K. L.; Arrendale, R. F.; Gwynn, G. R.; Chaplin, J. F.; Johnson, A. W. "Rapid Method for the Analysis of Tobacco Nicotine Alkaloids". *J. Chromatogr.* **1981**, *211*, 111.

Shannon, I. L.; Trodahl, J. N. "Sugars and Fluoride in Chewing Tobacco and Snuff". *Tex. Dent. J.* **1981**, *96*, 6.

Sisson, V. A.; Severson, R. F. "Alkaloid Composition of the *Nicotiana* Species". *Abstracts of Papers*, Proceedings of 38th Tobacco Chemists' Research Conference, Atlanta, GA, 1984; p 10.

Sitzeo, L. "On Chewing Tobacco". *ADA News* **1981**, *8*, 2.

Winn, D. M. "Tobacco Chewing and Snuff Dipping: An Association with Human Cancer". O'Neil, I. K., Borstel, R. C. Miller, C. T., Long, J., Burtch H., Eds.; IARC, Oxford University: London, 1985; Scientific Publication No. 57.

Winn, D. M.; Blok, W. J.; Shy, C. M.; Pickle, L. W.; Toledo, A.; Fraumeni, J. F., Jr. "Snuff Dipping and Oral Cancer among Women in the Southern United States". *N. Engl. J. Med.* **1981**, *304*, 745.

Wynder, E. L.; Stellman, S. D. "Comparative Epidemology of Tobacco-Related Cancers". *Cancer Res.* **1977**, *37*, 4608.

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