pH Changes in Smokeless Tobaccos Undergoing Nitrosation during Prolonged Storage: Effects of Moisture, Temperature, and Duration

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Three smokeless tobacco research products were each adjusted to two moisture levels before storage for 48 weeks at 24 and 32 °C. The pHs of high water content moist snuff (55.5%) and dry snuff (remoisturized to 51.4%) increased 0.3–2.1 pH units during the storage. In contrast, the pHs of decreased water content moist snuff (21.9%) and dry snuff (12.3%) decreased by 0.2–0.4 unit. Chewing tobacco pHs at high (49.3%) and low (22.3%) moisture levels and at each moisture–temperature treatment decreased during storage. Nitrosated pyridine alkaloids increased only in tobaccos that became more alkaline during storage. Interactions between the effects of moisture and temperature occurred. Heating snuffs at 100 °C for 30 min at zero storage time prevented the increases of pH, nitrosamines, and nitrite observed in non-heat-treated controls during storage. The results support the view that increases of nitrosamines may be mediated by microbial growth.

INTRODUCTION

Nitrosated pyridine alkaloids related to nicotine are often referred to as tobacco-specific nitrosamines and may act as carcinogens (Hecht et al., 1986) or promoters of carcinogenic activity (Johansson, et al., 1991). A previous study of moist snuff and dry snuff research products (Andersen et al., 1991) indicated that these nitrosamines generally accumulated at higher rates during storage at high moisture (ca. 50%) and high temperature (32 °C) compared to low moisture (<22%) and low temperature (24 °C). High moisture compared to high temperature resulted in larger increases of nitrosated alkaloid levels and nitrite in these tobaccos. Neurath et al. (1976) determined that the in vitro nitrosative formation of *N*-nitrosonornicotine (NNN), the principal carcinogenic nitrosated pyridine alkaloid in tobacco and tobacco smoke, was highest in aqueous solutions of nitrite and nornicotine at pH 3.8. At pH 1.5 and lower and at pH 5.2 and higher, almost no nitrosation occurred. Aqueous extracts of air-cured tobaccos characteristically have pH values higher than 5.0.

Ghabrial (1976) determined that air-cured burley tobacco leaf incubated under fermentation conditions (30–40% moisture content) underwent a sharp increase in bacterial counts within 1 week of incubation at 35 °C, after which counts slowly decreased during the next 8 weeks. Interestingly, an increase in pH value of aqueous extracts prepared from the fermenting leaf followed the rise in bacterial counts; higher bacterial counts and pH values were obtained for tobacco samples with 40% moisture than with 30% moisture. Burton et al. (1989) showed that the pH of burley tobacco leaf lamina and midribs increased along with nitrosamine accumulation during a 3-week controlled-environment storage period at relatively high moisture–temperature conditions (32 °C at 83% and 90% relative humidities). In their work, topical treatment of the tobacco with an antibiotic and fungicides during the storage did not appear to inhibit the pH changes and nitrosamine concentrations.

Parsons et al. (1986) found that nitrite was formed during incubations of nitrate-containing burley tobacco. They also showed that bacteria capable of converting nitrate to nitrite occurred on leaves of field- and greenhouse-grown tobacco and that nitrite was produced by nitrate-utilizing bacteria. Studies related to human stomach contents have shown that increased nitrosamine formation is effected by bacteria at the higher pH characteristic of the surgically-operated stomach than at the lower pH values of the nonoperated stomach (Calmels et al., 1985; O'Donnell et al., 1987).

The purpose of this investigation was to determine whether there are pH changes in smokeless tobaccos that are accompanying nitrosation reactions during prolonged storage. Effects of moisture, temperature, and their interactions related to pH changes and accumulations of nitrite and total nitrosated pyridine alkaloids were assessed.

EXPERIMENTAL PROCEDURES

Three reference smokeless tobacco research products, loose-leaf chewing tobacco (1S1), dry snuff (1S2), and moist snuff (1S3), were obtained from the Tobacco and Health Research Institute, University of Kentucky, Lexington, KY. The tobacco-type compositions of these products were previously described (Andersen et al., 1991). Moisture contents in the products as received were 22.3% for 1S1, 12.3% for 1S2, and 55.5% for 1S3. Portions of each product were adjusted to one additional moisture content to provide a high–low range of moisture for the products as previously described (Andersen et al., 1991). The resultant additional moisture levels were 49.3% for 1S1, 51.4% for 1S2, and 21.9% for 1S3. Three replications of 12 treatment combinations were used consisting of three tobacco products, two moisture levels per product, and two temperatures, 24 ± 1 and 32 ± 1 °C.

Approximately 150-g portions of 1-kg lots of each reference product of given moisture content were transferred to 1-qt Mason jars with rubber-sealed screw tops for storage in the dark in temperature-controlled environment chambers. Seals were broken at 2-week intervals throughout storage to equilibrate to atmospheric pressure, and the sample contents were mixed during a 30-s period. Samples (15 g) were removed at the beginning of

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storage (0-time) and after storage durations of 6, 12, 24, and 48 weeks and then frozen at −70 °C. The frozen tobaccos were then freeze-dried, ground to 100–200-mesh size, and equilibrated overnight to ambient moisture and temperature (at about 60% relative humidity and 25 °C) in darkness on a laboratory bench to decrease the tendency for weight fluctuations during subsequent weighings. All samples were stored in sealed plastic containers at −70 °C until analyzed.

For determinations of effects of heat treatment at 0-time on snuffs, 10-g samples of moist snuff and dry snuff at 55.5 and 51.4% moisture, respectively, from the 1-kg lots of the reference products described above were transferred to 1-qt Mason jars. An average of three replications of eight treatment combinations were used, consisting of two tobacco products, either heat treatment or no heat treatment at 0-time, and two storage temperatures. Heat treatment consisted of placement of the sealed Mason jars and contents in an autoclave at 100 °C for 30 min. Storage of samples was for 24 weeks at either 24 or 32 °C. Seals on the Mason jars were not broken periodically during storage; this avoided introduction of microorganisms from ambient air.

Determinations of pH were made on 1-g samples of tobacco that were suspended and mixed in 15–25 mL of water for 15–30 min; initial tests showed that pHs did not change significantly from 15 to at least 30 min. Reference compounds of the nitrosated pyridine alkaloids NNN, 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and *N'*-nitrosoanatabine (NAT) were synthesized as previously described (Andersen et al., 1991). Total pyridine alkaloid nitrosamines were determined from summations of NNN, NNK, and NAT which were each determined by a capillary GC procedure (Andersen et al., 1990). Nitrite N was determined according to a spectrophotometric procedure (Crutchfield and Burton, 1989). Calcium was determined as previously described (Andersen et al., 1989).

Quantitative results for nitrosated alkaloids in tobacco were expressed as micrograms per gram of dry weight normalized during storage on the basis of Ca concentration in the appropriate tobacco and moisture level at the start of storage. This corrected for dry-weight changes during storage. Standard errors of the mean were calculated. ANOVA was also performed on results, and Fisher's least significant difference (LSD) test was used only where the *F* test was significant at the $P = 0.05$ level of probability (Einot and Gabriel, 1975).

RESULTS AND DISCUSSION

The effects of storage durations up to 48 weeks on the pH's of three smokeless tobaccos are illustrated in Figure 1. Effects of moisture, temperature, and their interactions on pH changes and concentrations of total nitrosated pyridine alkaloids in the smokeless tobaccos during storage are presented in Table I. Nitrite N concentrations referred to in our discussions of these results were previously reported by Andersen et al. (1991, Table II).

The pH values of moist snuff stored at the high 55.5% moisture level (which is near normal for this product) gradually increased from an initial value of 6.9 to an average final value of 7.2 at 48-weeks storage duration (Figure 1; Table I). In contrast, moist snuff stored at the low 21.9% moisture level decreased from an initial value of 6.7 to an average value of 6.4 at 48 weeks. These changes are significant on the basis of standard errors of the mean. Increased ratios of acidic glucose metabolites to ammonia may have caused this change, although we have no direct evidence for this. These results of pH changes in moist snuff are consistent with those reported by Ghabrial (1976) and Burton et al. (1989), who found that the pH of air-cured burley leaf increased during exposure to fermentation conditions (high moisture). Ghabrial (1976) also found that bacterial counts on leaf increased sharply during this exposure. Since the compositions of cured burley tobacco leaf and moist snuff generally have the same known precursors of nitrosated pyridine alkaloids and factors that

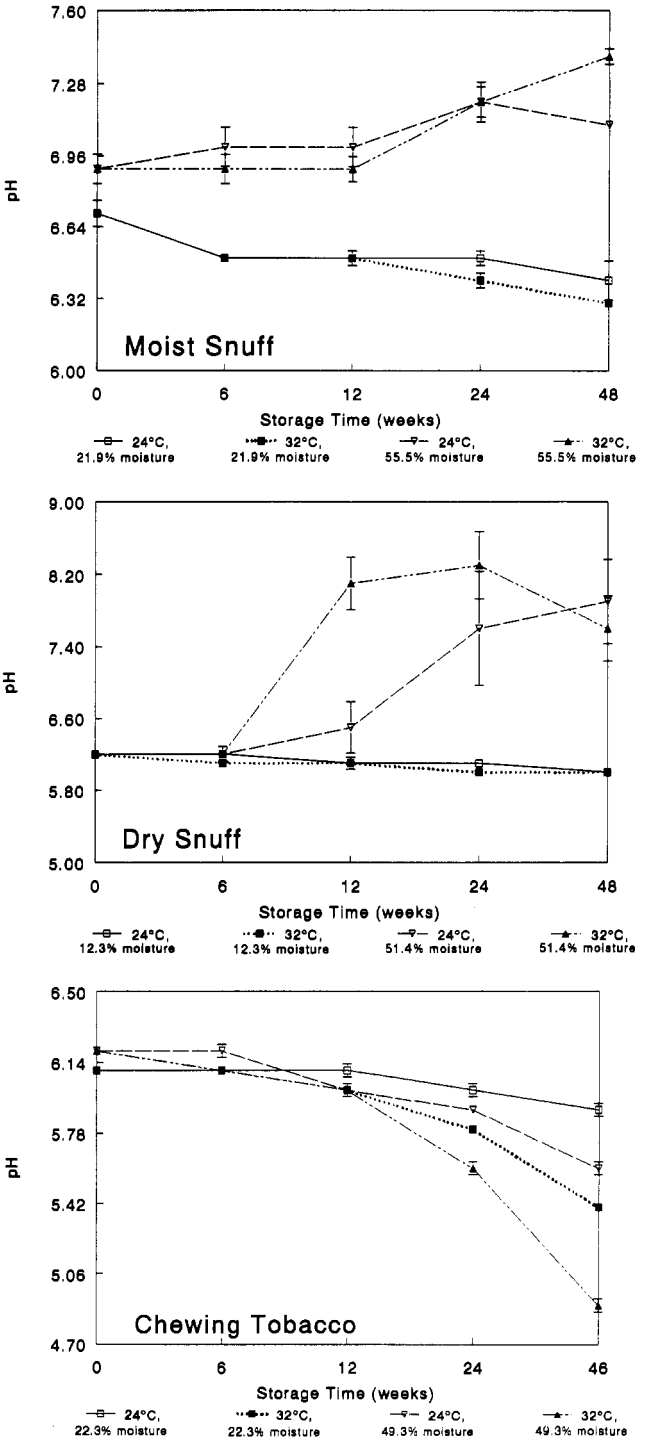

Figure 1. pH (± standard error of the mean) of smokeless tobacco products during storage under different moisture–temperature environments.

affect pH, we believe it is possible that growth of microorganisms in moist snuff at different moisture–temperature combinations varied in the same manner and caused associated changes in pH, nitrite, and total nitrosated pyridine alkaloid concentrations. In a preliminary experiment using four replicates per treatment, 67.3-g samples of moist snuff at 55.5% moisture were stored at 32 °C for 12 weeks in sealed Mason jars with or without the addition of 100 μL of (*E*)-2-hexanal to provide headspace levels of this volatile aldehyde known to have antimicrobial properties (Hamilton-Kemp et al., 1992; Deng et al., 1993). Snuff exposed to (*E*)-2-hexenal accumulated 330 ± 81 μg/g nitrite N (mean ± SD) compared to 589 ± 48 μg/g for controls without exogenous

Table I. Effect of Moisture, Temperature, and Their Interactions on pH Changes and Total Nitrosated Pyridine Alkaloids in Smokeless Tobaccos during Prolonged Storage[a,b]

storage conditions				total nitrosated pyridine alkaloids, μg/g
moisture, %	temp, °C	duration, weeks	pH	
		(A) Moist Snuff		
21.9		0	6.7 A	37.4 A
55.5		0	6.9 A	38.0 A
21.9	24	6	6.5 B	33.9 C
55.5	24	6	7.0 A	142.0 B
21.9	32	6	6.5 B	42.3 C
55.5	32	6	6.9 A	266.1 A
21.9	24	12	6.5 B	38.1 C
55.5	24	12	7.0 A	254.8 B
21.9	32	12	6.5 B	40.7 C
55.5	32	12	6.9 A	413.1 A
21.9	24	24	6.5 B	55.4 C
55.5	24	24	7.2 A	536.2 B
21.9	32	24	6.4 B	46.8 C
55.5	32	24	7.2 A	769.0 A
21.9	24	48	6.4 C	43.0 B
55.5	24	48	7.1 B	728.9 A
21.9	32	48	6.3 C	38.6 B
55.5	32	48	7.4 A	767.6 A
		(B) Dry Snuff		
12.3		0	6.2 A	268.3 A
51.4		0	6.2 A	269.3 A
12.3	24	6	6.2 A	236.9 B
51.4	24	6	6.2 A	239.0 B
12.3	32	6	6.1 A	248.7 B
51.4	32	6	6.2 A	328.8 A
12.3	24	12	6.1 B	266.8 B
51.4	24	12	6.5 B	300.2 B
12.3	32	12	6.1 B	273.6 B
51.4	32	12	8.1 A	545.1 A
12.3	24	24	6.1 B	299.6 C
51.4	24	24	7.6 A	404.2 B
12.3	32	24	6.0 B	299.4 C
51.4	32	24	8.3 A	534.6 A
12.3	24	48	6.0 B	279.1 C
51.4	24	48	7.9 A	899.2 A
12.3	32	48	5.9 B	279.4 C
51.4	32	48	7.6 A	424.4 B
		(C) Chewing Tobacco		
22.3		0	6.1 A	8.6 A
49.3		0	6.2 A	7.5 A
22.3	24	6	6.1 B	10.4 A
49.3	24	6	6.2 A	10.0 A
22.3	32	6	6.1 B	8.6 B
49.3	32	6	6.1 B	8.9 B
22.3	24	12	6.1 A	7.7 A
49.3	24	12	6.0 B	7.0 A
22.3	32	12	6.0 B	8.0 A
49.3	32	12	6.0 B	8.5 A
22.3	24	24	6.1 A	11.0 A
49.3	24	24	5.9 B	11.2 A
22.3	32	24	5.8 C	9.8 A
49.3	32	24	5.6 D	10.0 A
22.3	24	48	5.9 A	11.8 A
49.3	24	48	5.6 B	9.5 B
22.3	32	48	5.4 C	10.2 AB
49.3	32	48	4.9 D	9.5 B

[a] Mean values are for three replicated samples, and values for nitrosated alkaloids are corrected for Ca content departures from those at zero-time storage. [b] Mean values in a vertical column subset for a tobacco product and storage duration followed by a different corresponding letter are significantly different at $P = 0.05$.

(*E*)-2-hexenal. These results provided evidence for involvement of microorganisms in the increased nitrite N levels in tobacco serving as a putative precursor of nitrosated pyridine alkaloids (Andersen and Kasperbauer, 1984).

Moisture–temperature interaction effects for high water content moist snuff stored for 48 weeks resulted in a higher pH value for high-temperature storage (32 °C) than for low-temperature storage (24 °C); interaction effects for low water content moist snuff resulted in lower pH after storage at 32 °C than at 24 °C (Table I). Total nitrosated pyridine alkaloid concentration changes, as well as pH changes, were of greater magnitude in high moisture content moist snuff than in low moisture content moist snuff. There were generally no significant changes in pH and nitrosated alkaloids in low moisture content moist snuff. Total nitrosated pyridine alkaloids in high moisture content moist snuff during storage were at their highest concentrations at 24–48 weeks, but large increases were evident as early as 6 weeks. Corresponding maximal pH values in high moisture moist snuff were evident at 24- and 48-week storage durations. On the basis of the times of change, pH shifts to more alkaline conditions were probably not a causative factor in the putative increased bacterial growth and reduction of nitrate to nitrite. Increased nitrite has been postulated to lead to increased total nitrosated pyridine alkaloid accumulations in tobaccos (Andersen and Kasperbauer, 1984).

Dry snuff pH values at the high (51.4%) moisture level increased from 6.2 at 0-time storage to an average final value of 8.0 at 48-weeks duration (Figure 1; Table I). In contrast, dry snuff stored at the low (12.3%) moisture content (which is nearly normal for this product) decreased from 6.2 to an average of 6.0 at 48 weeks. These trends were similar to those observed for moist snuff pH changes and are consistent with the findings of Ghabrial (1976) and Burton et al. (1989) related to pH changes of air-cured leaf. Moisture–temperature interactions were noted for high-moisture dry snuff for each storage duration after 6 weeks, resulting in generally higher pHs for 32 °C storage at 12 and 24 weeks compared to 24 °C. Total nitrosated pyridine alkaloid concentrations, as well as pH changes, were generally of greater magnitude in high moisture content dry snuff than in low moisture content dry snuff. Total nitrosated pyridine alkaloids in dry snuff were at their highest concentrations from 12 to 24 weeks, and one large increase occurred as early as 6 weeks. These observations of pH changes and the time sequence of increased accumulations of total nitrosated pyridine alkaloids and nitrite as previously reported (Andersen et al., 1991) do not support a view that preconditions of more alkaline pH levels in smokeless tobaccos caused increases in bacterial growth and reduction of nitrate to nitrite with concomitant increases of nitrite and total nitrosated pyridine alkaloids. Dry snuff concentrations of total nitrosated pyridine alkaloids prior to storage were nearly 1 order of magnitude higher than those in moist snuff. Presumably these differences were caused by variations in postharvest treatments and handling of the tobacco raw materials used in the snuffs prior to our controlled-storage treatments. Trends of results of dry snuff pH changes and accumulations of total nitrosated pyridine alkaloids, as well as previously reported nitrite (Andersen et al., 1991), during prolonged storage are similar to those for moist snuff.

The pH values of chewing tobacco at both the 49.3% moisture level and the 22.3% level (the latter content being nearly normal) and for each moisture–temperature treatment decreased with increased storage time. The greatest

Table II. Effect of Heat Treatment at Zero-Time Storage on pH Changes, Total Nitrosated Pyridine Alkaloids, and Nitrite in Snuff Tobaccos after Prolonged Storage[a,b]

heat treatment	storage duration, weeks	storage temp, °C	pH	total nitrosated pyridine alkaloids, μg/g	nitrite N, μg/g
		(A) Moist Snuff at 55.5% Moisture			
no	0	na[c]	6.9 A	38.0 A	12.8 A
yes	24	24	6.8 A	39.9 A	5.8 A
no	24	24	7.2 B	532.9 B	1203 B
no	0	na	6.9 A	38.0 A	12.8 A
yes	24	32	6.8 A	39.5 A	6.4 A
no	24	32	7.2 B	765.5 B	1580 B
		(B) Dry Snuff at 51.4% Moisture			
no	0	na	6.2 A	269.3 A	14.8 A
yes	24	24	6.4 A	231.5 A	2.7 A
no	24	24	6.9 B	393.0 B	121.0 B
no	o	na	6.2 A	269.3 A	14.8 B
yes	24	32	6.3 A	236.2 A	3.2 A
no	24	32	8.3 B	531.8 B	32.7 C

[a] Mean values given for pH and concentrations of chemical components are corrected for Ca content departures from those at 0-time storage. [b] Means in a vertical column of a heat treatment–no heat treatment–storage duration subset for a given tobacco product and storage temperature followed by a different letter are significantly different at $P = 0.05$. [c] na, not applicable.

magnitude of pH change was that for high-moisture chewing tobacco stored at 32 °C, which decreased from 6.2 at 0-time to 4.9 at 48 weeks. The least significant change in pH occurred for the low-moisture–low-temperature storage treatment, which decreased only 0.2 pH unit from 0-time to 48 weeks. The sugar content of chewing tobacco (ca. 30%) may inhibit bacterial growth or result in bacterially mediated production of acid metabolites of glucose that lower pH levels as a function of storage time. High concentrations of sugars in natural products can act as effective preservatives by making moisture unavailable to microorganisms (Frazier, 1958). Moisture–temperature interaction effects were observed for high water content chewing tobacco stored for 24 and 48 weeks and for low water content chewing tobacco at these same storage durations. Nitrite nitrogen and total nitrosated pyridine alkaloid levels generally either were not significantly different or changed only slightly among the moisture–temperature treatments at a given storage duration or as a function of duration.

The effects of a 0-storage-time 30-min heat treatment at 100 °C on changes in pH, total nitrosated pyridine alkaloids, and nitrite N in high moisture content moist snuff and dry snuff after 24 weeks of storage, compared to unstored controls of the snuffs, are summarized in Table II. Significant increases in pH, total nitrosamines, and nitrite were found for both non-heat-treated snuffs after storage. In nearly every case, however, there was no significant change in pH, total nitrosamines, and nitrite in heat-treated snuffs after storage. The increases tended to be larger for 32 °C than for 24 °C storage. Increases of pH in non-heat-treated dry snuff were larger than in non-heat-treated moist snuff. On the other hand, increases of total nitrosated pyridine alkaloids and nitrite were larger in non-heat-treated moist snuff than in non-heat-treated dry snuff. The observed effects of the heat treatment of the snuffs carried out in an autoclave may be related to the bactericidal conditions of this treatment.

Although direct effects caused by endogenous tobacco enzymes were not ruled out, our current results on pH change and total nitrosated pyridine alkaloid accumulations and past results on nitrite during the prolonged storage of smokeless tobacco products support the view that increases of nitrosamines are mediated by bacterial growth at nearly neutral pH. Results of our current research on snuffs showing effects of 0-storage-time heat treatment on changes in pH, nitrosamines, and nitrite also support this concept. The bacteria are thought to reduce ubiquitous nitrate in tobacco to nitrite, which then reacts with secondary or tertiary amine pyridine alkaloids to yield nitrosated derivatives. Previous work that supports this hypothesis includes investigations on tobacco leaf (Ghabrial, 1976; Parsons et al., 1986; Burton et al., 1989) and on bacterial systems (Calmels et al., 1988).

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LITERATURE CITED

Andersen, R. A.; Kasperbauer, M. J. Post-Harvest Treatment and the Accumulation of Nitrite and N'-Nitrosonornicotine in Burley Tobacco. In *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer*; O'Neill, I. K., Von Borstel, R. C., Miller, C. T., Long, J., Bartsch, H., Eds.; Scientific Publication 57; International Agency for Research on Cancer (IARC): Lyon, France, 1984; pp 877–893.

Andersen, R. A.; Fleming, P. D.; Burton, H. R.; Hamilton-Kemp, T. R.; Sutton, T. G. N'-Acyl and N'-Nitroso Pyridine Alkaloids in Alkaloid Lines of Burley Tobacco during Growth and Air Curing. *J. Agric. Food Chem.* **1989**, *37*, 44–50.

Andersen, R. A.; Fleming, P. D.; Burton, H. R.; Hamilton-Kemp, T. R.; Hildebrand, D. F.; Sutton, T. G. Levels of Alkaloids and their Derivatives in Air- and Fire-Cured KY 171 Dark Tobacco During Prolonged Storage: Effects of Temperature and Moisture. *Tob. Sci.* **1990**, *34*, 50–56.

Andersen, R. A.; Fleming, P. D.; Burton, H. R.; Hamilton-Kemp, T. R.; Sutton, T. G. Nitrosated, Acylated, and Oxidized Pyridine Alkaloids during Storage of Smokeless Tobaccos: Effects of Moisture, Temperature, and Their Interactions. *J. Agric. Food Chem.* **1991**, *39*, 1280–1287.

Burton, H. R.; Bush, L. P.; Djordjevic, M. V. Influence of Temperature and Humidity on the Accumulation of Tobacco-Specific Nitrosamines in Stored Burley Tobacco. *J. Agric. Food Chem.* **1989**, *37*, 1372–1377.

Calmels, S.; Ohshima, H.; Vincent, P.; Gounot, A.-M.; Bartsch, H. Screening of Microorganisms for Nitrosation Catalysis at pH 7 and Kinetic Studies on Nitrosamine Formation from Secondary Amines by *E. coli* Strains. *Carcinogenesis* **1985**, *6*, 911–915.

Calmels, S.; Ohshima, H.; Bartsch, H. Nitrosamine Formation by Denitrifying and Non-Denitrifying Bacteria: Implication of Nitrite Reductase and Nitrate Reductase in Nitrosation Catalysis. *J. Gen. Microbiol.* **1988**, *134*, 221–226.

Crutchfield, J.; Burton, H. R. Improved Method for the Quantification of Nitrite in Plant Materials. *Anal. Lett.* **1989**, *22*, 555–571.

Deng, W.; Hamilton-Kemp, T. R.; Nielsen, M. T.; Andersen, R. A.; Collins, G. B.; Hildebrand, D. F. Effects of Six-Carbon Aldehydes and Alcohols on Bacterial Proliferation. *J. Agric. Food Chem.* **1993**, *41*, 506–510.

Einot, I.; Gabriel, K. R. A Study of the Powers of General Methods of Multiple Comparisons. *J. Am. Stat. Assoc.* **1975**, *70*, 574–583.

Frazier, W. C. Preservation by Preservatives. In *Food Microbiology*; McGraw-Hill: New York, 1958.

Ghabrial, S. A. Studies on the Microflora of Air-Cured Burley Tobacco. *Tob. Sci.* **1976**, *20*, 95–97.

Hamilton-Kemp, T. R.; McCracken, C. T.; Loughrin, J. H.; Andersen, R. A.; Hildebrand, D. F. Effects of Some Natural Volatile Compounds on the Pathogenic Fungi *Alternaria alternata* and *Botrytis cinerea*. *J. Chem. Ecol.* **1992**, *18*, 1083–1091.

Hecht, S. S.; Rivenson, A.; Braley, J.; DiBello, J.; Adams, J. D.; Hoffmann, D. Induction of Oral Cavity Tumors in F344 Rats

by Tobacco-Specific Nitrosamines and Snuff. *Cancer Res.* **1986**, *46*, 4162–4166.

Johansson, S. L.; Sadi, J.; Osterdahl, B.-G.; Smith, R. A. Promoting Effect of Snuff in Rats Initiated by 4-Nitroquinoline-N-Oxide or 7,12-Dimethylbenz(a)anthracene. *Cancer Res.* **1991**, *51*, 4388–4394.

Neurath, G. B.; Dunger, M.; Pein, F. G. Nitrosation of Nornicotine and Nicotine in Gaseous Mixtures and Aqueous Solutions. In *Environmental N-Nitroso Compounds Analysis and Formation*; Walker, E. A., Bogorski, P., Griciute, L., Eds.; Scientific Publication 14; International Agency for Research on Cancer (IARC): Lyon, France, 1976; pp 227–236.

O'Donnell, C. M.; Edwards, C.; Corcoran, G. D.; Ware, J.; Edwards, P. R. In-Vitro Production of Nitrosamines by Bacteria Isolated from the Operated Stomach. In *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms*; Bartsch, H., O'Neill, I. K., Schulte-Hermann, R., Eds.; Scientific Publication 84; International Agency for Research on Cancer (IARC): Lyon, France, 1987; pp 400–403.

Parsons, L. L.; Smith, M. S.; Hamilton, J. L.; MacKown, C. T. Nitrate Reduction During Curing and Processing of Burley Tobacco. *Tob. Sci.* **1986**, *30*, 100–103.

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