

Flue Cured Tobacco

Flue Cured Tobacco : unburned chemistry studies

Amin, A. N. M. (1979). Dynamic transformations of chemical constituents during flue-curing of *Nicotiana tabacum* L.: I. Metabolism of carbohydrates and polyphenols. II. Metabolism of nitrogenous and related constituents. III. Biochemistry and metabolism of plastid pigments. IV. Chemistry and metabolism of the essential oil: 129 pp.

Andersen, R. A., T. C. Tso, et al. (1972). "Variation of soluble phenolic compounds in flue-cured tobacco cultivars with stalk position." Agron. J. **64**(4): 417-420.

Phenolic levels in field-grown flue-cured leaves of Coker 139, NC 95, SC 58, and LN 38 were compared within each cultivar according to leaf positions on the stalks and among the 4 cultivars. Each of the cultivars showed a significant but generally nonlinear increase in levels of total phenolics (~2-fold for each) and sums of chlorogenic acids and rutin (2 to 9-fold depending on cultivar) with 8 ascending leaf positions on the stalk from lower to upper leaves. However, mean values of total phenolic reducing power equiv. to a chlorogenic acid std. and averaged over all stalk positions ranged from 7.17% in Coker 139 to 7.92% in NC 95; these values were not significantly different among the cultivars. Likewise, means of the sums of chlorogenic acids and rutin averaged over all leaf positions on the stalk ranged from 1.20% in Coker 139 to 1.59% in NC 95, and the 4 cultivars were not significantly different. Total sol. phenolic compds. were estd. by their bonding to insol. polyvinylpyrrolidinone at pH 3.5. Chlorogenic acid isomers and rutin were detd. by electron capture gas chromatog. anal. based upon the amt. of sol. caffeic acid and quercetin moieties found. Leaf phenolic compds. affect color and quality and may contribute to the tumor-promoting weak acid fraction of tobacco (*Nicotiana tabacum*) smoke condensate. Therefore, leaves of these cultivars from different positions on the stalk will be used for cigarette manufacture to correlate the phenolic-stalk position findings with smoke characteristics and compn. [on SciFinder(R)]

Anderson, R. C. and A. G. Kelly (1988). "Identification of a low flavor threshold carboxylic acid constituent, 4-ethyloctanoic acid, in flue-cured Virginia tobacco." J. Agric. Food Chem. **36**(2): 353-354.

4-Ethyloctanoic acid has been identified as a minor constituent of a flavorful acidic dichloromethane-sol. fraction of an aq. BuOH ext. of flue-cured Virginia tobacco. The presence of this type of potent acidic flavorant is proposed to account for the dominant flavor quality exhibited by this tobacco fraction. Subfractionation of a conc. of some flavor principles of an aq. BuOH ext. of flue-cured Virginia tobacco resulted in identification of some weakly flavorful acidic constituents new to tobacco (Anderson, R. C. et al., 1983). It was subsequently recognized that the major characteristic flavor effect of this conc., however, was due to compd.(s) still unknown.

Further investigations have now revealed the presence in this tobacco fraction of the aliph. carboxylic acid with the lowest odor threshold yet obsd. for this class of compd., viz. 4-ethyloctanoic acid (Boelens, H. et al., 1983). It has also been obsd. that the smoke flavor quality this acid produces when added to a cigarette has the same main character as that produced by the acidic tobacco fraction under investigation. [on SciFinder(R)]

Anderson, R. C., A. G. Kelly, et al. (1983). "Two new acidic constituents of flue-cured Virginia tobacco." J. Agric. Food Chem. **31**(2): 458-459.

The aq. BuOH sol. portion of flue-cured Virginia tobacco was fractionated to produce a flavorful acidic, CH₂Cl₂-sol. fraction. High-performance liq. chromatog. of this fraction allowed the isolation of 2 compds. new to tobacco, i.e., (4-hydroxy-3-methoxyphenyl)-2-ethanol and 2-formyl-5-(ethoxymethyl)pyrrole-1-acetic acid (I). [on SciFinder(R)]

Bolt, A. J. N. (1972). "1'-Hexanoylnornicotine and 1'-octanoylnornicotine from tobacco." Phytochemistry **11**(7): 2341-2343.

Two minor alkaloids, isolated from flue-cured and air-cured tobacco (*Nicotiana tabacum*), were 1'-hexanoylnornicotine (I, R = n-C₅H₁₁) and 1'-octanoylnornicotine (I, R = n-C₇H₁₅). [on SciFinder(R)]

Bowman, D. R., T. C. Tso, et al. (1973). "Leaf characteristics of four flue-cured varieties according to stalk position. III. Starch, cellulose, lignin, wax, crude ash, and alkalinity of water soluble ash." Tob. Sci. **17**: 34-35.

The study was undertaken to obtain information on 6 chem. constituents of flue-cured tobacco leaf from 4 cultivars by stalk position. The cultivars were selected according to their different alkaloid contents; LN38(0.24%), Coker 139(1.24%), NC95(2.74%), and SC58(3.55%). Starch, cellulose, lignin, wax, crude ash, and alky. of the H₂O-sol. ash were detd. Anal. data are tabulated and show ranges of constituents depending on stalk position and cultivar. [on SciFinder(R)]

Burdick, D. and H. R. Burton (1969). "Thermal decomposition of tobacco. II. Thermogravimetric and differential thermal analyses of pigments from tobacco leaf and smoke condensate." Tob. Sci. **13**: 16-18.

Thermogravimetric and differential thermal analyses were made of the alc. sol. and alc. insol. fractions of leaf pigments from flue-cured tobacco (CA 66: 44313w), a mixt. of these pigments, and the smoke pigment from com. cigarettes (CA 67: 88420d). The decompn. studies made in air and in He reveal definite differences among the pigments. The alc. sol. fraction loses a

higher percent of its wt. at a higher temp. and contains a higher proportion of a more readily oxidizable component than the insol. fraction. In this respect the alc. sol. fraction is more similar to the smoke pigment. The data for the latter suggest that it is less complex and undergoes oxidative degradation to a greater extent than the leaf pigment. In He the smoke pigment is thermally more stable, an observation which supports the phys. conditions present during smoking. [on SciFinder(R)]

Cai, K., Z. Xiang, et al. (2012). "Determination of eight tobacco alkaloids in flue-cured tobacco samples by gas chromatography with nitrogen chemiluminescence detection (NCD)." Anal. Methods **4**(7): 2095-2100.

A reliable, sensitive and rapid method for detn. of eight alkaloids in flue-cured tobacco by dual-column-dual-detector gas chromatog. with nitrogen chemiluminescence detection (GC-NCD) has been established. At first, the extrn. effects of several factors such as solid to liq. ratio, extrn. time, sodium hydroxide soln. vol. and sodium hydroxide soln. mass fraction on the extrn. efficiency were simultaneously investigated using an orthogonal array design (OAD). Then, the NCD was compared to other detectors and showed some unique advantages. The anal. method met the strict quant. requirement by optimizing chromatog. conditions and using dual-column confirmation. Finally, the performance of the proposed method was tested for the extrn. and detn. of the eight alkaloids in flue-cured tobacco and satisfactory results were achieved. [on SciFinder(R)]

Chamberlain, W. J. and R. F. Arrendale (1983). "An alternate method for the analysis of N-nitrosornicotine in tobacco." J. Agric. Food Chem. **31**(4): 909-911.

A method is described for the anal. of N-nitrosornicotine (I) in tobacco. This method was applied to a no. of flue-cured tobacco samples, differing in nicotine and nornicotine contents, to det. whether or not there was a direct correlation between nicotine and nornicotine levels and I level in tobacco leaf. There appears to be no correlation between the alkaloid content of tobacco leaf and the level of I. [on SciFinder(R)]

Chamberlain, W. J. and O. T. Chortyk (1992). "Effects of curing and fertilization on nitrosamine formation in Bright and Burley tobacco." Beitr. Tabakforsch. Int. **15**(2): 87-92.

A Bright and a Burley tobacco were grown at four fertilization rates and each tobacco was then both flue-cured and air-cured. Levels of alkaloids and nitrosamines increased with increasing fertilization levels. Levels of alkaloids, N-nitrosornicotine (NNN), and other tobacco-specific nitrosamines (TSNA) were consistently higher in the Burley tobacco than in the Bright tobacco, regardless of curing method. In comparing the effects of curing, NNN and total TSNA levels were higher in the midrib than in the lamina of the air-cured samples, while just the opposite was found for the flue-cured samples. Flue-curing Bright tobacco produced three times the level

of TSNA vs air-curing the same tobacco. On the other hand, flue-curing Burley tobacco reduced the alkaloids, but greatly increased the TSNA in the lamina. As midribs from the air-cured Burley leaves had three times the TSNA concn. of the lamina, the use of air-cured midribs in tobacco products should be avoided. Thus, lower fertilization levels and careful manipulations of curing parameters could lower nitrosamine levels in cured tobacco. [on SciFinder(R)]

Chaplin, J. F. and L. G. Burk (1984). "Agronomic, chemical, and smoke characteristics of flue-cured tobacco lines with different levels of total alkaloids." Agron. J. **76**(1): 133-136.

Expts. were conducted at 2 locations to develop lines of flue-cured tobacco with different levels of total alkaloids and to evaluate these lines for agronomic, chem., and smoke characteristics. Three flue-cured cultivars with different total alkaloid levels were chosen as recurrent parents. The parents and alkaloid levels were: Coker 139 (1.88%), NC 95 (3.28%), and SC 58 (4.51%). Each cultivar was crossed with a low-alkaloid line (0.20%). After the initial cross, backcrosses were made in each F₂ population between a low-alkaloid plant and its resp. recurrent parent. BC₅F₂ generation plants with different levels of total alkaloids were selected and advanced to the BC₅F₇ to obtain stable lines. Also dihaploids were developed from BC₅F₁ plants and lines with different levels of total alkaloids were selected. Both the BC₅F₇ and dihaploid populations were evaluated. The line with the highest yield and grade index at each alkaloid level was further evaluated for total alkaloids, reducing sugars, yield, grade index, days to flower, plant height, and no. of leaves per plant. Lines with the following levels of total alkaloids were evaluated: NC 95 family, 0.34, 1.08, 2.05, 3.11, and 3.55%; SC 58 family, 0.38, 1.59, 1.90, 2.82, 4.18, and 4.82%; and the Coker 139 family, 0.20, 0.93, 1.66, 1.84, and 2.17%. The 1.08% line in the NC 95 and 1.59% line in the SC 58 families were dihaploids. None of the lines converted appreciable amts. of nicotine to nor nicotine. Therefore, the total alkaloid levels reflected predominantly nicotine. In most families, the high-alkaloid lines gave the highest grade index. Days to flower, plant height, and no. of leaves per plant were not assocd. with total alkaloid levels in cured leaf. Cigarette smoke from the different tobacco lines were evaluated by 2 smoke panels. The results from Panel A indicated that it was difficult to assoc. any of the taste parameters with levels of total alkaloids in the leaf. The results from Panel B indicated that an increase in total alkaloids in the leaf was accompanied by an increase in flavor of the smoke. [on SciFinder(R)]

Cheng, A. L. S., T. C. Tso, et al. (1971). "Leaf characteristics of four flue-cured tobacco varieties according to stalk positions. I. Phytosterols and petroleum ether extracts." Crop Sci. **11**(4): 580-582.

The phytosterol content and petroleum ether (PE) exts. of leaves varied with the stalk positions of the leaves in 3 flue-cured *Nicotiana tabacum* (tobacco) varieties (SC-58, NC-95, and Coker-139) and one breeding line (LN-38). The varieties differed widely in their alkaloid contents. The general stalk distribution patterns of phytosterol content and PE exts. were about the same among the different varieties except LN-38. Of the 4 phytosterol fractions detd., cholesterol and

campesterol were fairly const. in leaves at different stalk positions but β -sitosterol increased and stigmasterol decreased as position of the leaves went from bottom to top of the stalk. [on SciFinder(R)]

Christakopoulos, A., K. Feldhusen, et al. (1992). "Determination of natural levels of coumarin in different types of tobacco using a mass fragmentographic method." *J. Agric. Food Chem.* **40**(8): 1358-1361.

An accurate anal. method for qual. and quant. detn. of endogenous coumarin in tobacco was developed. Coumarin is extd. from the tobacco, and the ext. obtained is purified by HPLC or TLC. The quantification is performed by gas chromatog.-mass spectrometry using a selected ion monitoring technique and ^{13}C -labeled coumarin as internal std. The detection limit of the method is 0.050 μg of coumarin/g of tobacco. Coumarin was estd. in a no. of different tobacco types. The concns. were generally below 1 $\mu\text{g/g}$ of tobacco. In fire-cured tobacco, however, the concn. varied between 1.1 and 4.7 $\mu\text{g/g}$. The higher concns. in this tobacco may be explained by the presence of coumarin in the hickory smoke, which is condensed onto the leaf during the fire-curing process. [on SciFinder(R)]

Chu, D., Y. Xie, et al. (2010). "Major carotenoids in Tobacco laminas: identification and quantification by HPLC with photodiode array detection." *Asian J. Chem.* **22**(4): 2635-2647.

The carotenoid compns. in various tobacco laminas are detd. by high-performance liq. chromatog. (HPLC) with the photodiode array detection (DAD). The carotenoids are extd. from powd. tobacco laminas by cold acetone contg. 0.1 % butylated hydroxytoluene (BHT). A mobile phase of CH_3CN - H_2O (90:10, vol./vol.) (A) and $\text{CH}_3\text{COOC}_2\text{H}_5$ (100 %) (B) with following gradient elution is developed: 100 % A in the beginning, maintained for 20 min, decrease to 50 % A in 50 min, maintain for 5 min and return to 100 % A in 1 h. A total of 11 carotenoids including some isomers in tobacco laminas are resolved within 55 min by using a ZorBAX SB-C18 column with the flow-rate at 1.0 mL/min and detection at 445 nm. The limits of detection (LODs) of carotenoids varies from 6.0 to 14.0 ng/mL. The relative std. deviations (RSD) are from 2.69 to 3.63 % and the recovery ranges from 89.6 to 96.3 %. The method is successfully applied for the quantification of carotenoids in green, fresh flue-cured and aging tobacco laminas and the anal. results show that total carotenoids in tobacco laminas range from 49.66 to 820.1 $\mu\text{g/g}$. Lutein and β -carotene are the most representative carotenoids in flue-cured and aging tobacco laminas because other carotenoids degrade greatly during flue-curing and aging periods. Furthermore, it worth to mention that zeaxanthin is not found in green tobacco laminas while presented in fresh flue-cured and aging tobacco laminas, luteoxanthin as a new carotenoid is first tentatively identified in the present work and α -carotene is not found in any significant amt. in all those tobacco laminas. [on SciFinder(R)]

Chu, H., T. C. Tso, et al. (1972). "Higher fatty acids of flue-cured tobacco leaves varying in stalk position." Agron. J. **64**(3): 280-282.

Cured leaves from 8 stalk positions of 3 varieties and 1 breeding line of flue-cured tobacco (*Nicotiana tabacum* L.) were analyzed for fatty acid content and lipid residues. The higher fatty acids with 14 to 18 carbons were detd. The varieties analyzed were Coker 139, SC-58, NC-95, and a breeding line LN-38. These varieties differed in alkaloid content and other phys. and chem. characteristics. Leaves from SC-58 and LN-38 had the highest lipid residue. For all varieties lipid residues were lowest in leaves from the bottom stalk position and next lowest in leaves at the top position. LN-38 had the highest total fatty acid content of any of the varieties. All varieties except Coker 139 yielded the highest total fatty acid content in leaves at the 2nd stalk position. Also, the proportion of C18:3 acid was highest at position 2 in all varieties and decreased toward the top of the plant. [on SciFinder(R)]

Clark, T. J. and J. E. Bunch (1997). "Derivatization solid-phase microextraction gas chromatographic-mass spectrometric determination of organic acids in tobacco." J. Chromatogr. Sci. **35**(5): 209-212.

Isovaleric, valeric, hexanoic, benzoic, phenylacetic, 3-methylvaleric, heptanoic, octanoic, and nonanoic acids were converted to their Me esters and quantitated for flue-cured tobacco grades of increasing stalk position using derivatization headspace solid-phase microextrn. (SPME) with selected ion monitoring mode mass spectrometry. Qual. anal. of the headspace of tobacco derivatized with methanolic HCl using a 65- μ m Carbowax-divinylbenzene SPME fiber indicates selectivity for relatively nonpolar volatile and semivolatile org. acid Me esters. By contrast, direct exposure of an 85- μ m polyacrylate SPME fiber to derivs. followed by GC-MS anal. provides the capability to evaluate significantly more polar org. acid Me esters such as malic acid dimethylester and citric acid trimethylester. [on SciFinder(R)]

Clark, T. J. and J. E. Bunch (1997). "Determination of volatile acids in tobacco, tea, and coffee using derivatization-purge and trap gas chromatography-selected ion monitoring mass spectrometry." J. Chromatogr. Sci. **35**(5): 206-208.

Short-chain acids from agricultural products are simultaneously extd. and derivatized using methanolic hydrochloric acid. Acetic, isobutyric, butyric, isovaleric, valeric, hexanoic, 3-methylvaleric, heptanoic, and nonanoic acids are quantitated using purge and trap gas chromatog. with selected ion monitoring mode mass spectrometry. Flue-cured, burley, and oriental tobacco varieties are compared based on their volatile org. acid content. Com. coffee, tea, and three cigarette brands are analyzed, and their differences are evaluated to demonstrate the utility of this technique for natural product anal. [on SciFinder(R)]

Cook, C. E., M. E. Twine, et al. (1969). "An examination of the hexane extract of flue-cured tobacco involving gel-permeation chromatography." Phytochemistry **8**(6): 1025-1033.

Techniques have been investigated for sepg. the hexane ext. of flue-cured tobacco into classes based on polarity and mol. size. The method developed, which depends upon countercurrent distribution for polarity sepn. and gel permeation chromatog. for mol. size sepn., is reproducible, nondestructive, and permits quant. recovery. Its applicability has been demonstrated by the isolation and identification of several compds. and classes of compds. by following it with conventional chromatographic techniques (column, thin-layer, and gas-liquid chromatog.). Among the substances isolated were glycerides, solanesol esters, sterol esters (including cholesterol esters), solanochromene (plastochromenol- 8), solanesol, α -tocopherol, β -amyrin, and sterols (including cholesterol). Mol. wt. detns. indicated that 70% of the nonbasic hexane ext. was below 800 in mol. wt. [on SciFinder(R)]

Court, W. A. (1982). "Factors affecting the concentration of the divatrienediols of flue-cured tobacco." Tob. Int. **184**(7): 94-97.

Field expts. were conducted to det. the influences of the stage of plant development, stalk position, plant spacing, topping, and curing on the concn. of divatrienediols in flue-cure tobacco. The concn. of these diterpenes decreased in all stalk positions prior to topping. After topping the divatrienediol concn. increased with age in the middle and upper stalk positions. The divatrienediols increased with ascending stalk position. Divatrtienediol concns. decreased with redns. in plant population, lower topping height, and during curing. Typically, the concn. of these diterpenes in cured tissue was less than one-half that in green tissue. [on SciFinder(R)]

Court, W. A., M. R. Binns, et al. (1983). "Examination of the influence of curing and stalk position on the phenolic constituents of flue-cured tobacco." Tob. Int. **185**(9): 25-29.

Chlorogenic acid, neochlorogenic acid, 4-O-caffeoylquinic acid, rutin, scopolin, and scopoletin in ripe green and cured leaves of flue-cured tobacco from different stalk positions were detd. by high performance liq. chromatog. Chlorogenic acid, rutin, and scopolin concns. were higher in the leaves from the upper stalk positions. Neochlorogenic acid and 4-O-caffeoylquinic acid did not vary greatly with stalk position. Scopoletin concns. appeared to be unrelated to stalk position. Except for the leaves from the lower stalk positions increases were obsd. during curing for each of the isomers of chlorogenic acid, although the extent of these changes varied somewhat with individual years. The changes found for rutin after curing were similar to those of chlorogenic acid. An approx. prediction equation, for each stalk position, is suggested for the changes in rutin and chlorogenic acid as a result of curing. Scopolin and scopoletin were higher in the cured tissue. The percentage increase of scopoletin after curing was the largest of any of the phenolic constituents examd. [on SciFinder(R)]

Court, W. A. and J. M. Elliot (1978). "Influence of nitrogen, phosphorus, potassium, and magnesium on the phenolic constituents of flue-cured tobacco." Can. J. Plant Sci. **58**(2): 543-548.

A field expt. was conducted in 1974 and 1975 on Fox loamy sand to study the effects of different rates (0, 22.4, 44.8, and 67.2 kg/ha of N fertilization on selected chem. constituents of flue-cured tobacco (*Nicotiana tabacum* cv. Delhi 34). N fertilization increased the levels of total alkaloids, total N, and scopoletin [92-61-5] in cured leaf. Chlorogenic acid [327-97-9], neo-chlorogenic acid [906-33-2], 4-caffeoylquinic acid [905-99-7], rutin [153-18-4], and scopolin [531-44-2] were found to be inversely related to the rate of N. Phenolic constituents and inorg. nutrients in the leaves and inorg. nutrients in the soil (Fox loamy sand) were studied in 1975 and 1976 in another field expt. Treatments included selected omissions of P, K, or Mg from field plots grown continuously with tobacco since 1967. Marked nutrient deficiency symptoms were obsd. only in plots in which K had been omitted. Tobacco from these plots was generally lower in phenolic constituents. [on SciFinder(R)]

Court, W. A., J. M. Elliot, et al. (1984). "Influence of applied nitrogen fertilization on certain lipids, terpenes, and other characteristics of flue-cured tobacco." Tob. Int. **186**(13): 91-94.

In a field expt. conducted in 1980 and 1981 on Fox loamy sand in Ontario to study the effects of different rates (16.8, 33.6 and 50.4 kg/ha) of N fertilization on individual constituents of flue-cured tobacco (*Nicotiana tabacum* cv. Nordel), fertilization increased yield, total alkaloids, hexane exts., cytoplasmic lipids, individual pigments, neophytadiene and solanesol. Increasing the rate of N fertilization decreased grade index, reducing sugars, and divatrienediols. N fertilization had no influence on maturity index, surface waxes, hydrocarbons, and total phytosterols. [on SciFinder(R)]

Court, W. A. and J. G. Hendel (1982). "Determination of flue-cured tobacco pigments by high-performance liquid chromatography." Tob. Int. **184**(18): 68-70.

Pigments were quantitated in flue-cured tobacco by reversed-phase high-performance liq. chromatog. (HPLC) with UV detection, following sample homogenization in 70% aq. MeOH and passing through a C18 Sep-Pak cartridge. HPLC was carried out on a µBondapak C18 or a Partisil PXS 50DS column equipped with a C18/Corasil Bondapak precolumn. Gradient elution was carried out with 90% aq. MeOH and EtOAc. Pigments were identified by comparison of retention times with ref. samples. Zeaxanthin coeluted with lutein and attempts to resolve these 2 pigments were unsuccessful. The method is simple and reproducible, and the results were in good agreement with those obtained by spectrophotometry. [on SciFinder(R)]

Court, W. A. and J. G. Hendel (1985). "Phenolic constituents of flue-cured tobacco at different stages of plant growth." Tob. Int. **187**(12): 32-35.

Chlorogenic acid, its isomers (4- and 5-O-caffeoylquinic acid), rutin, kaempferol-3-rhamnoglucoside, scopoletin, and scopolin were detd. in the leaves of flue-cured tobacco (Nicotiana tabacum) collected at intervals from early in July through harvest. Quant. differences were found among the 4 cultivars studied but the changes during growth were very similar for each cultivar and for each of the 4 yr studied. Chlorogenic acid and scopolin increased with plant growth in the leaves of each stalk position examd. Rutin, 5-O-caffeoylquinic acid, and kaempferol-3-rhamnoglucoside did not change appreciably, although there was a tendency toward lower levels with the later sampling dates. The 4-O-caffeoylquinic acid content progressively declined throughout the study in the leaves of each stalk position. Scopoletin was below detectable levels in most of the early sampling periods but was present in mature leaves in the sampling period. [on SciFinder(R)]

Court, W. A. and J. G. Hendel (1986). "Capillary gas chromatography of nonvolatile organic acids, fatty acids, and certain carbohydrates in flue-cured tobacco." Tob. Int. **188**(11): 58-61.

An improved method is described for the anal. of the major nonvolatile org. and fatty acids of flue-cured tobacco (Nicotiana tabacum). The tobacco acids were analyzed by capillary gas chromatog. as their Me esters on a fused silica capillary column bonded with a cyanopropylsiloxane stationary phase. Fructose, glucose, sucrose and inositol and several tobacco acids including citric, malic, caffeic and quinic acids could also be detd. on a aq. ext. of tobacco. These were quantitated by capillary gas chromatog. as their trimethylsilyl derivs. on a fused silica capillary column bonded with a dimethylpolysiloxane stationary phase. Oxalic, succinic and malonic acids could not be detd. by this procedure. The extrn. of tobacco with water removed only traces of fatty acids. [on SciFinder(R)]

Dickerson, J. P., D. L. Roberts, et al. (1976). "Flue-cured tobacco flavor. II. Constituents arising from amino acid-sugar reactions." Tobacco **178**(9): 71-72, 75-77.

A novel series of N-substituted-2-formylpyrroles, not previously isolated from natural products, was isolated from flue-cured leaf. Two of the pyrrole derivs., 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone and 2-(2-formylpyrrol-1-yl)-4-methylvaleric acid, were reported as products of model amino acid-sugar reaction. The remaining 3 pyrrole-aldehydes, 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) phenylpropionic acid lactone, 2-(2-formylpyrrol-1-yl)-3-methylbutyric acid, and 2-(2-formylpyrrol-1-yl)-3-methylvaleric acid, have not been reported in the literature. A procedure for synthesizing the 2-(2-formylpyrrol-1-yl) alkyl acids from pyrrole-2-carboxaldehyde and α -bromoesters was developed. The heterocyclic compds. isolated in this study are believed to arise from the decompn. of Amadori compds. during the curing and aging of flue-cured tobacco. It is proposed that part of the contribution of amino acid-sugar reactions to desirable flue-cured flavor in tobacco smoke is due to the formation of these O and N heterocycles. [on SciFinder(R)]

Djordjevic, M. V., L. P. Bush, et al. (1990). "Accumulation and distribution of acylated nornicotine derivatives in flue-cured tobacco alkaloid isolines." J. Agric. Food Chem. **38**(2): 347-350.

Acylornicotine derivs. were analyzed and quantified in several alkaloid isolines of NC 95 flue-cured tobacco during growth and curing. N'-Formyl-, N'-acetyl-, N'-butanoyl-, N'-hexanoyl-, N'-octanoyl-, and N'-(hydroxyoctanoyl)nornicotines were detd. by extrn. and chromatog. procedures frequently used for tobacco-specific nitrosamines. Detection was with a thermionic N-P detector. Formyl-, (hydroxyoctanoyl)-, and octanoylnornicotine were present in greatest amts. in these flue-cured tobaccos. Mature green lamina of NC 95 contained 39-92 $\mu\text{g g}^{-1}$ of acylornicotines, and the amt. increased during curing (56-328 $\mu\text{g g}^{-1}$). Cured lamina from the alkaloid isolate tobaccos contained from 41 to 1182 $\mu\text{g g}^{-1}$ of N'-acylnornicotines. The highest amts. of acylated nornicotine derivs. were in the lamina from the upper stalk positions. The accumulation of acylornicotines in the different alkaloid isolines was correlated pos. with the alkaloid precursor nornicotine. Correlation coeffs. of the individual acylornicotines with N'-nitrosonornicotine were highly significant in cured lamina from the third priming of the alkaloid isolate tobaccos. [on SciFinder(R)]

Djordjevic, M. V., S. L. Gay, et al. (1989). "Tobacco-specific nitrosamine accumulation and distribution in flue-cured tobacco alkaloid isolines." J. Agric. Food Chem. **37**(3): 752-756.

Changes in 3 tobacco-specific nitrosamines (TSNA) and their alkaloid precursors during growth and curing were studied in 7 NC 95 flue-cured isolines with different alkaloid levels. N'-Nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) were sepd. by gas chromatog. and measured with a thermionic N-P detector. Mature green leaves contained small amts. (0.6-1.5 $\mu\text{g g}^{-1}$) of TSNA that increased (0.9-17.8 $\mu\text{g g}^{-1}$) during curing. Leaves from higher stalk positions and leaves with increased time in the curing process had greater amts. of TSNA. Among the alkaloid isolines, highest significant correlation coeffs. between a TSNA and the related alkaloid precursor were 0.95 and 0.76 for the correlations between nornicotine with NNN and anatabine with NAT, resp. The correlation between nicotine and NNN ($r = 0.40$) was not significant. [on SciFinder(R)]

Dominguez, L. M. and R. S. Dunn (1987). "Analysis of OPA-derivatized amino sugars in tobacco by high-performance liquid chromatography with fluorimetric detection." J Chromatogr Sci **25**(10): 468-471.

An analytical method is developed to quantitatively determine glucosamine, galactosamine, and mannosamine in dried-and-ground burley and flue-cured tobaccos. Extraction is shown to be quantitative in the range of 0.01 to 2.0% (w/w). The extraction procedure consists of shaking one g of sample with 50 mL of deionized water adjusted to pH 7 for 30 min. This extract is filtered directly into an autosampler vial. An autosampler is programmed to withdraw two

different aliquots: one with o-phthalaldehyde (OPA) derivatizing solution and the second one from the tobacco extract. The derivatization reaction occurs in the tubing connecting the autosampler and the chromatographic column. The OPA derivatives of these aminosugars are then detected with a fluorimetric detector, and their simultaneous analysis is performed with an external standard. This method is shown to be selective, accurate, and precise.

Ellington, J. J., P. G. Fisher, et al. (1976). "GC-MS analysis of fatty acids from flue-cured tobacco." J Chromatogr Sci **14**(12): 570-571.

Fatty acids obtained by saponification of a hexane-soluble fraction of flue-cured tobacco were converted to their methyl esters. The esters were purified by TLC and identified by GC-MS. Qualitative and quantitative results are reported for acids of chain lengths C12-C34.

Ellington, J. J., P. F. Schlotzhauer, et al. (1978). "Lipid distribution in flue-cured tobacco plants." J. Agric. Food Chem. **26**(2): 407-410.

Various plant parts of 3 varieties of flue-cured tobacco were analyzed for hexane extractables, total solanesol [13190-97-1], neophytadiene [504-96-1], hydrocarbon waxes, total major fatty acids, and total sterols. The total lipid content was highest in strip, followed by whole leaf, stem, and stalk in decreasing order. Solanesol, the major lipid, was present only in whole leaf and strip. Concns. of the other lipids generally followed the same decreasing order as seen for the total lipids. [on SciFinder(R)]

Ellington, J. J., P. F. Schlotzhauer, et al. (1978). "Quantitation of fatty acids from flue-cured tobacco by combined preparative thin layer chromatography-gas chromatography." J. Am. Oil Chem. Soc. **55**(7): 572-573.

Flue-cured tobacco was subjected to alk. hydrolysis and acidification; the fatty acids and nonsaponifiables were extd. into hexane. Treatment of this ext. with diazomethane yielded fatty acid Me esters. The Me esters were sepd. from interfering hydrocarbons and sterols by preparative thin-layer chromatog. After addn. of an internal std., the esters were quantitated by gas chromatog. on the column packing, Silax 1/C. Quantitation of the C14-C32 fatty acid esters was possible by means of temp. programming. [on SciFinder(R)]

Elliot, J. M. (1975). "Production factors affecting chemical properties of the flue-cured leaf. III. Nutrition." Tobacco **177**(4): 22, 24, 26, 28, 31-22, 35.

A review with 49 refs. discussing the role essential elements, supplied via the soil and fertilizer, play on the quality factors of flue-cured tobacco, including yield, chem. compn., burn, and com. value. Elements discussed are N, P, K, Ca, Mg, S, B, Cl, Cu, Fe, Mn, Mo, and Zn. [on

SciFinder(R)]

Elliot, J. M. and W. A. Court (1978). "The effects of applied nitrogen on certain properties of flue-cured tobacco and smoke characteristics of cigarettes." Tob. Sci. **22**(51-58): 54-58.

Four rates of fertilizer N, 0, 20, 40, and 60 lb/acre, were applied to Fox loamy sand in a 3-yr expt. in Ontario to det. the effects on certain agronomic, chem., phys., and smoke characteristics of flue-cured tobacco. Increasing the rate of applied N increased the yield, total alkaloids, total N, filling values, and petroleum ether ext. of cured leaves, the ash and ash constituents (except in the lower leaves), and increased the total alkaloids and pH of the smoke, but had no apparent effect on dry particulate matter, CO, or CO₂. Increasing the rate of N decreased maturity, reducing sugars, and lamina wt. (except in the top leaves). [on SciFinder(R)]

Fenner, R. A., J. Lyons-Hart, et al. (1988). "Fourier transform infrared evolved gas analysis (FT-IR-EGA) to the study of tobacco curing." Beitr. Tabakforsch. Int. **14**(2): 85-91.

FT-IR evolved gas anal., a nonisothermal pyrolysis technique, is used to probe the changes in tobacco leaf compn. which occur during flue and air curing. A unique perspective of major enzymic processes and their dependencies on curing method is obtained through simultaneous detection of multiple pyrolytic products and three-dimensional presentation of the product evolution profiles. The conversion of starch to sugars, proteins to amino acids, and the relationships between these processes are explored. [on SciFinder(R)]

Gaines, T. P., M. G. Stephenson, et al. (1976). "Effects of nutrient deficiencies on chemical constituents of flue-cured tobacco." Tob. Sci. **20**: 101-103.

The effects of nutrient deficiencies on chem. constituents of flue-cured tobacco (*Nicotiana tabacum* cv. Hicks Broadleaf) was studied using hydroponically grown plants in the greenhouse. Plants grown under K deficiency and those grown in a complete nutrient medium had the highest N content at 4.79 and 4.70%, resp. N and Ca deficient plants had the lowest N content at 2.10% and 3.15%, resp. The complete or control media gave the highest amt. of total alkaloids, followed by B and Cl deficiencies. N, P, Mg, Zn, and S deficiencies induced the lowest amt. of total alkaloid formation. The treatment yielding the lowest reducing sugar contents were B, S, and K deficiencies and complete medium; treatments resulting in the highest levels of reducing sugars were Ca and N deficiencies. S, N, and Ca deficiencies produced the highest starch [9005-25-8] concn., while K, B, Mg, Cl, and P produced the lowest. A neg. correlation was found between total N and reducing sugars ($P = 0.02$) and between total N and starch ($P = 0.07$). [on SciFinder(R)]

Gong, C.-r., A.-h. Wang, et al. (2006). "Changes of polyphenols in tobacco leaves during the flue-curing process and correlation analysis on some chemical components." Agric. Sci. China **5**(12): 928-932.

The changes of polyphenols in tobacco leaves during the flue-curing process and correlation anal. on some chem. components were studied. Leaf samples were taken from different tobacco-producing regions in Henan Province, China. The results indicated that the content of total phenols increased during the first 24 h of curing, and then decreased. It reached the lowest value at 72 h of curing and increased rapidly after that. The content of chlorogenic acid also increased during 0-24 h of curing. But the lowest point occurred at 60 or 72 h of curing and then it increased till the end of the curing process. The content of rutin generally increased with curing, and showed little fluctuations. The changes of PPO and POD activity were the opposite. Rutin was found to have a highly significant pos. correlation with total sugar ($r = 0.822^{**}$), but a highly significant neg. correlation with starch, nicotine, and protein. [on SciFinder(R)]

Gopalam, A. and N. C. Gopalachari (1976). "Studies in leaf pigments of flue-cured tobacco. Part I. Their variation in diffeent varieties and stalk positions." Tob. Res. **2**(1): 21-30.

Six varieties and mutants (VE-1, CTRI Spl., GSH3, HE-2, MDS-13, HR 62-7) exhibiting different degrees of greenness in the leaves were used in this study. The leaves were harvested at the correct stage of maturity and total chlorophyll and total carotenoids in the different primings were detd. The total chlorophyll on the green wt. as well as on a leaf area basis generally increased with increasing greenness of the leaf. Lowest chlorophyll content was found in the middle leaves and the highest in the top-most leaves. This tallies with the observations that the bright grades are obtained from the middle priming and green grades from the top leaves. The trends in total carotenoids were similar to the trends in total chlorophyll, i.e., light yellow varieties contained lower amts. of carotenoids than the green varieties. [on SciFinder(R)]

Gopalam, A. and N. C. Gopalachari (1976). "Studies in leaf pigments of flue-cured tobacco. Part II. Variation in important chemical constituents in the different varieties and stalk positions." Tob. Res. **2**(1): 31-37.

The leaves from bottom, middle, and top positions in 6 varieties (4 of them mutants) exhibiting different degrees of greenness (VE-1, CTRI Spl., GSH3, HE-2 MDS-13, and HR 62-7) and the light yellow variety CTRI Spl. contained higher amts. of starch than others. Leaves from the top-most position contained the highest amt. of starch. Lowest amts. of proteins were found in HR 62-7 and the highest in VE-1. Middle leaves contained the lowest amt. of protein and top leaves the highest. There were no significant differences in the ash contents of different varieties. Bottom leaves contained the highest amt. of ash. There was a decreasing gradient in the org. acids from bottom to top positions. Cellulose content of the different varieties did not vary significantly. [on SciFinder(R)]

Gopalam, A. and N. C. Gopalachari (1979). "Biochemical changes in leaf pigments and chemical constituents during flue-curing of tobacco." Tob. Res. **5**(2): 117-124.

In a study of changes in constituents of 2 tobacco varieties (Kanakaprabha and CTRI Special) during curing stages, there was little difference between the 2 varieties in chlorophylls a and b, carotenes, and xanthophylls throughout the various stages studied. From the beginning of curing to the end of yellowing the depletion of pigments was rapid; in the later stages this depletion was slow. The contents of starch and total and reducing sugars were higher in Kanakaprabha than in CTRI Special, whereas sucrose showed a reverse trend. By the end of the curing process there was an ~20-fold increase in sucrose concn. Total N and protein N varied little between the varieties. Nicotine in Kanakaprabha leaves was significantly higher than in CTRI Special. Both N fractions decreased during the curing process. There were higher amts. of chlorogenic acid and rutin in Kanakaprabha than in CTRI Special. During curing rutin decreased steadily in both varieties, whereas chlorogenic acid remained const. [on SciFinder(R)]

Gordon, B. M., M. S. Uhrig, et al. (1988). "Analysis of flue-cured tobacco essential oil by hyphenated analytical techniques." J. Chromatogr. Sci. **26**(4): 174-180.

The major components of an alkaloid-free, flue-cured, tobacco essential oil sample were isolated and identified by an automated multidimensional gas chromatograph/mass spectrometer/flame ionization detector and a multidimensional gas chromatograph/matrix isolation/Fourier transform IR spectrometer. A total of 306 compds. was identified in the essential oil, of which 80 were found as tobacco constituents for the first time. [on SciFinder(R)]

Hammond, D. and R. J. O'Connor (2008). "Constituents in tobacco and smoke emissions from Canadian cigarettes." Tob Control **17 Suppl 1**: i24-31.

BACKGROUND: There is relatively little information available about the chemical constituents of tobacco and individual toxic emissions from cigarettes and other tobacco products. OBJECTIVE: To characterise 21 constituents in whole tobacco and 41 constituents in the smoke emissions of Canadian cigarettes, as well as to compare differences between domestic and imported brands. METHODS: All data were released as part of Canada's Tobacco Reporting Regulations. Data are reported for 247 brands tested in 2004. RESULTS: The results indicate significant differences in the constituent levels of domestic and imported cigarette tobacco. Levels of ammonia compounds were significantly higher in imported "US blended" tobacco compared to domestically manufactured brands. Toxic emissions for tobacco-specific nitrosamines were significantly higher for imported cigarettes under both the ISO and Canadian Intense testing methods; however domestic cigarettes had higher levels of other toxic constituents, including benzo[a]pyrene. The findings also highlight the extent to which nicotine, heavy metals and tobacco-specific nitrosamines are "transferred" from the whole tobacco to the smoke. CONCLUSIONS: The findings illustrate important differences between domestically

manufactured Virginia flue-cured cigarettes and imported US blended cigarettes. Although the findings suggest that domestic cigarettes had lower levels of constituents such as ammonia, which are associated with increased "additives", Canadian cigarettes were by no means "additive-free." Overall, these findings provide important benchmarks for making historical and international comparisons across brands on key constituents.

Hoffmann, D. and H. Woziwodzki (1968). "Chemical studies on tobacco smoke. IV. Quantitative determination of free nonvolatile fatty acids in tobacco and tobacco smoke." Beitr. Tabakforsch. **4**(4): 167-175.

The title method involved the addn. of small amts. of stearic-1-¹⁴C acid and oleic-9,10-³H acid to 5-10 g. of tobacco, followed by extrn. with anhyd. C₆H₆, washing with C₆H₆, and evapg. in vacuo. The residue was dissolved in Et₂O, shaken with 2N KOH, followed by H₂O. The combined aq. layers were acidified with 5N HCl and the free acids were extd. with Et₂O. Fifty mg. of the acids was mixed with 2 ml. MeOH, 0.2 ml. 5N HCl, and 5 ml. 2,2-dimethoxypropane, followed by dissolving in toluene and evapn. to dryness, with the residue being eluted through a Florisil column with hexane-C₆H₆ (1:1) followed by C₆H₆. After evapn. of the solvents and dissolving in toluene, ¹⁴C and ³H were measured. Also, a sample was analyzed on a gas-liq. chromatograph using a 12% diethylene glycol succinate on Gas Chrom P column with temp. programming: 140° for 10 min. followed by 4°/min. to 200°. The carrier gas was He at 55 ml./min. The major free fatty acids in tobacco were in order of decreasing concn., linolenic, palmitic, linoleic, stearic, oleic, myristic, and palmitoleic acids. Further identified were C₁₂, C₁₄, C₁₅, and C₂₀₋₂₆ satd. acids. The concns. of the acids in tobacco were between 0.6% and 0.09%. Higher concns. of these components were found in flue-cured and sun-cured tobaccos compared with air-cured tobacco. Coumarin was identified in the concd. Me esters of Turkish and Bright tobaccos as well as of blended cigarette tobacco. The concn. of the 5 major fatty acids in cigarette smoke was at least 4 higher in the particulate matter than in the original tobacco. [on SciFinder(R)]

Irvine, W. J., B. H. Woallen, et al. (1972). "Bombiprenone from *Nicotiana tabacum*." Phytochemistry **11**(1): 467-469.

Bombiprenone(6,10,14,18,22,26,30,34-octamethyl-5,9,13,17,21,25,29,33-pentatriacontaoctaen-2-one) was isolated from flue-cured tobacco (*N. tabacum*) in yields of 0.1-0.6% dry wt. Plastoquinone-A was converted to bombiprenone by aerobic photooxidn., most probably via a hydroperoxide. [on SciFinder(R)]

Ishiguro, S., S. Sato, et al. (1976). "Studies on compositions of smoke components of lamina and midrib cigarettes. Part I. Comparison of phenols in smokes of lamina and midrib of flue-cured tobacco." Agric. Biol. Chem. **40**(5): 977-982.

To reveal the difference of the main stream smoke compn. between lamina and midrib of flue-cured tobacco, the yield of neutral, basic, acidic, and phenolic fractions of the smoke condensates of lamina and midrib cigarets were compared. The compn. of the phenolic fractions were also compared by glass capillary gas chromatog. Neutral and basic fractions were dominant in lamina smoke condensate, whereas an Et₂O-insol. fraction was dominant and formed ~50% of the midrib smoke condensate. For the semiquant. anal. of phenols with gas chromatog. mono- and dihydroxybenzenes were extd. from the smoke condensates and loss of these compds. by oxidn. was prevented by addn. of DL-ascorbic acid. After trimethylsilylation they were simultaneously examd. with gas chromatog. Apparently, 4-Me, 4-Et, and 4-vinylcatechol in lamina smoke were much more rich than those in midrib smoke, whereas coniferyl alc. was more concd. in midrib smoke. [on SciFinder(R)]

Ishiguro, S., S. Yano, et al. (1976). "Studies on smoke components of lamina and midrib cigarets. Part II. Comparisons of acids in smoke of lamina and midrib of flue-cured tobacco leaves." Agric. Biol. Chem. **40**(10): 2005-2011.

Acids of lamina and midrib cigarette smoke were converted into trimethylsilyl derivs. and analyzed with glass column gas chromatog. The concns. of org. acids were higher for lamina cigarette smoke than for midrib cigarette smoke. Large concn. differences were obsd. in formic, acetic, propionic, lactic, glycolic, furoic, benzoic, phenylacetic, fumaric, and m-hydroxybenzoic acids. Succinic and methylsuccinic acids were similar in lamina smoke and in midrib smoke. A large amt. of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one(aminocarbonyl reaction product) was identified for the first time in lamina smoke. [on SciFinder(R)]

Ito, K., Y. Tanabe, et al. (2000). "Glycosidic fraction of flue-cured tobacco leaves: its separation and component analysis." Biosci., Biotechnol., Biochem. **64**(3): 584-587.

The fraction contg. glycosidic components was sepd. from flue-cured tobacco (*Nicotiana tabacum* L.) leaves by a facile method. Some components of the fraction were isolated and elucidated to be syringin, coniferin, cichoriin, benzyl- β -D-glucoside, Blumenol A- β -D-glucoside, and 5,6-epoxy-5,6-dihydro-3-hydroxy- β -ionyl- β -D-glucoside. Syringin and coniferin were detected in the *Nicotiana* species for the first time. [on SciFinder(R)]

Janardhan, K. V., N. Janakiraman, et al. (1990). "Variability analysis of major mineral nutrients in cured leaf of flue-cured Virginia tobacco." Tob. Res. **16**(2): 129-130.

Considerable variability was noticed in the three major nutrients of cured leaf. However among the genotypes 55% had nitrogen in the range of 2.0 to 2.5%, 65% had phosphorus in the range of 0.5 to 0.7% and 75% had potassium in the range of 2.0 to 3.0. The heritability ests. (broad sense) were moderate to high for N (60.6%), K (75.7%) and P (94.9%). However, genetic advance (percent of mean of population) was of lower order -18.7, 28.5 and 26.0% for N, P and

K, resp. [on SciFinder(R)]

Johnson, W. H. (1975). "Production factors affecting chemical properties of the flue-cured leaf. VI. Curing." Tobacco **177**(11): 12, 15, 17-19.

A review with 19 refs. [on SciFinder(R)]

Khalil, A. A., S. Steyn, et al. (2000). "Isolation and Characterization of a Monoamine Oxidase Inhibitor from Tobacco Leaves." Chem. Res. Toxicol. **13**(1): 31-35.

Recent positron emission tomog. imaging studies have demonstrated a significant decrease in both monoamine oxidase A and B (MAO-A and MAO-B) activities in the brains of smokers. Normal levels of activity are obsd. in former smokers, suggesting the presence of one or more compds. in tobacco smoke that may inhibit these enzymes. In this paper, we report the results of efforts to identify compds. present in flue-cured tobacco leaves that inhibit MAO. The isolation procedure was guided by estimating the inhibitory properties of tobacco leaf extracts on the liver mitochondrial MAO-B-catalyzed oxidation of 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine to the corresponding dihydropyridinium metabolite. Fractionation of exts. from flue-cured tobacco leaves led to the isolation of a competitive inhibitor of human MAO-A ($K_i = 3 \mu\text{M}$) and MAO-B ($K_i = 6 \mu\text{M}$), the structure of which could be assigned by classical spectroscopic anal. and confirmed by synthesis. This information may help to provide insights into some aspects of the pharmacol. and toxicol. of tobacco products. [on SciFinder(R)]

Klus, H. and H. Kuhn (1977). "A study of the optical activity of smoke nicotines." Fachliche Mitt. Oesterr. Tabakregie **17**: 331-336.

Optical rotation values of tobacco and smoke S-(-)-nicotine of flue-cured, dark, burley, and oriental tobaccos ranged -134- -137 (av. -135.1) and -119- -133 [α]_{D20}, resp.; an av. racemization of 6.3, 2.7, 11.7, and 9.4% for the nicotine of the 4 tobacco types was found. The smoke nicotine content was highest in burley (5.01) and lowest in oriental (1.78 mg/cigarette) tobacco. [on SciFinder(R)]

Kodama, H., T. Fujimori, et al. (1981). "Non-volatile constituents in tobacco. Part I. Isolation of a new terpene glucoside, 3-hydroxy-5,6-epoxy- β -ionyl- β -D-glucopyranoside from flue-cured tobacco." Agric. Biol. Chem. **45**(4): 941-944.

A C13-nor-carotenoid glucoside isolated from flue-cured tobacco was identified as 3-hydroxy-5,6-epoxy- β -ionyl- β -D-glucopyranoside. 3-Hydroxy-5,6-epoxy- β -ionol obtained by enzymic hydrolysis of the glucoside had the same relative structure as the naturally occurring trans-epoxide. Blumenol A-B-D glucopyranoside was also obtained. [on SciFinder(R)]

Kodama, H., T. Fujimori, et al. (1982). "Non-volatile constituents in tobacco. Part II. Isolation of a new terpene glucoside, loliolide- β -D-glucopyranoside from flue-cured tobacco." Agric. Biol. Chem. **46**(5): 1409-1411.

A C11-nor-carotenoid glucoside isolated from flue-cured tobacco was identified as loliolide- β -D-glucopyranoside from the spectral data and synthesis. [on SciFinder(R)]

Kodama, H., T. Fujimori, et al. (1984). "Nonvolatile constituents in tobacco. Part 3. A nor-sesquiterpene glycoside, rishitin- β -sophoroside, from tobacco." Phytochemistry **23**(3): 690-692.

Rishitin β -sophoroside (I) was isolated from flue-cured tobacco and its structure was detd. by chem. and spectral methods. [on SciFinder(R)]

Leffingwell, J. C. (1999). "Leaf chemistry: basic chemical constituents of tobacco leaf and differences among tobacco types." Tob. Prod., Chem. Technol.: 265-284.

A review with 59 refs. concerning the known compn. and chem. of tobacco types that impact tobacco quality and differentiate tobacco types. Emphasis is given on major tobacco types utilized com., i.e., Virginia (flue-cured), air-cured (Burley and cigar) and Oriental. [on SciFinder(R)]

Legg, P. D. and J. F. Chaplin (1981). "Influence of chlorophyll content on smoke constituents in burley and flue-cured tobacco." Crop Sci. **21**(5): 692-695.

A backcrossing program was used to obtain near isogenic lines for all genetic factors except alleles at the 2 loci controlling chlorophyll level. Comparisons among std. cultivars indicated that burley cultivars were generally higher in tar/puff and nicotine/cigarette puff and lower in the no. of puffs/cigarette than flue-cured cultivars. The chlorophyll content influenced the level of 9 of the 10 cigarette characteristics and accounted for the difference between burley and flue-cured cultivars for no. of puffs/cigarette. Diversity between the 2 classes of cultivars for tar/puff and nicotine/puff was not totally due to chlorophyll differences. Interclass crosses might be useful in breeding improved cultivars for these components. [on SciFinder(R)]

Liu, B., C. Chen, et al. (2008). "Enantiomeric analysis of anatabine, nornicotine and anabasine in commercial tobacco by multi-dimensional gas chromatography and mass spectrometry." J. Chromatogr., B Anal. Technol. Biomed. Life Sci. **865**(1-2): 13-17.

A fully automated multi-dimensional gas chromatog. (MDGC) system with a megabore precolumn and cyclodextrin-based anal. column was developed to analyze the enantiomeric compns. of anatabine, nornicotine and anabasine in com. tobacco. The enantiomer abundances of anatabine and nornicotine varied among different tobacco. S-(-)-anatabine, as a proportion of

total anatabine, was 86.6% for flue-cured, 86.0% for burley and 77.5% for oriental tobacco. S-(-)-nornicotine, as a proportion of total nornicotine, was 90.8% in oriental tobacco and higher than in burley (69.4%) and flue-cured (58.7%) tobacco. S-(-)-anabasine, as a proportion of total anabasine, was relatively const. for flue-cured (60.1%), burley (65.1%) and oriental (61.7%) tobacco. A simple solvent extn. with dichloromethane followed by derivatization with trifluoroacetic anhydride gave relative std. deviations of less than 1.5% for the detn. of the S-(-)-isomers of all three alkaloids. The study also indicated that, a higher proportion of S-(-)-nornicotine is related to the more active nicotine demethylation in the leaf. [on SciFinder(R)]

Liu, W.-H., G.-P. Yong, et al. (2008). "Free and Conjugated Phytosterols in Cured Tobacco Leaves: Influence of Genotype, Growing Region, and Stalk Position." J. Agric. Food Chem. **56**(1): 185-189.

Although phytosterols in tobacco leaves have specific effects on tobacco quality, there is little research on the distribution of free and conjugated phytosterols in various tobacco leaves. In this paper, we explored the content and compn. of phytosterols in representative cured tobacco leaves by gas chromatog. of TMS-ether derivs. We found that phytosterol contents in tobacco leaves ranged from 1.0 to 2.5 mg/g of dried leaf tissue, depending on different types of tobacco leaves. The majority of phytosterols (75-85%) were conjugated as ester and glycosides, with only about 15-25% existing in the free form. Furthermore, the genetic variability gives rise to the significant differences among different tobacco types with phytosterol levels: the contents of phytosterols in tobacco leaves decreased in the order of flue-cured tobacco, Oriental tobacco, Burley tobacco, cigar tobacco, and Maryland tobacco. At the same time, the tobacco curing process leads to a difference in phytosterol existing-form distribution in some variation laws. [on SciFinder(R)]

Lloyd, R. A., C. W. Miller, et al. (1976). "Flue-cured tobacco flavor. I. Essence and essential oil components." Tob. Sci. **20**: 125-133.

In studies of tobacco ext. and essential oil, a total of 323 compds. was identified. Of these, 275 have not been previously reported as flue-cured tobacco constituents, and 132 were new to all tobacco types. Fifteen of the compds. are previously unreported in the literature. Many of the compds. evaluated organoleptically exhibited desirable flavor properties. [on SciFinder(R)]

Lugon-Moulin, N., F. Martin, et al. (2006). "Cadmium concentration in tobacco (*Nicotiana tabacum* L.) from different countries and its relationship with other elements." Chemosphere **63**(7): 1074-1086.

Cadmium accumulation in crop plants, such as tobacco (*Nicotiana tabacum* L.), can lead to human exposure to this carcinogenic metal. To better define actual Cd distribution in cured or

processed tobacco leaves from chief tobacco-producing regions, we analyzed 755 leaf samples of three major types (Flue-cured, Burley, and Oriental) obtained from 13 countries during 2001-2003. This survey may help identify regions with low- or high-Cd concentrations in tobacco to obtain insight into the cause of the concentration extremes and to assist in defining strategies to reduce Cd in tobacco. Cadmium concentrations in the samples ranged from 0 to 6.78 microg(-1), as determined by ICP-MS. Significant differences were found among types and among countries, but significant interactions between type and country were found. Variations in Cd concentrations were also found in all countries. Our results suggest an important contribution of the field (e.g., bioavailable Cd in soil, other soil characteristics) to the Cd concentration in tobacco. Finally, the correlation between the concentration of Cd and that of other elements differed among the types, which could be effectively discriminated based on the concentrations of 20 elements.

Matsukura, M., K. Takahashi, et al. (1983). "Composition of semivolatiles from roasted tobacco." Agric. Biol. Chem. **47**(10): 2281-2285.

To examine the applicability of the exhaust gas components from the tobacco-roasting process as cigarette flavor ingredients, a preliminary study on the compn. of the semivolatiles included in the volatiles from roasted tobacco was conducted. A relatively large quantity of sugar pyrolyzates (furans, volatile ketones, and lactones) in addn. to lower fatty acids and the components of essential oils were found in the condensate of the volatiles from roasted flue-cured tobacco. The condensate from roasted burley tobacco contained nicotine and neophytadiene as major components, and a no. of other components of essential oils were also fouund. The above mentioned compds. in the condensate from flue-cured tobacco were assumed to contribute to its burnt-sugar-like aroma. A sweet note of the condensate from burley tobacco might be attributable to the high concn. of the components of essential oils. [on SciFinder(R)]

Matsushima, S., S. Ishiguro, et al. (1979). "Composition studies on some varieties of tobacco and their smoke. I. Major components in smoke condensate." Beitr. Tabakforsch. Int. **10**(1): 31-38.

The relative proportions of the major smoke components and N-contg. components in the semi-volatile phase and the non-volatile phase from some varieties of tobacco, i.e. flue-cured, Burley, and Turkish tobacco, were detd. In flue-cured tobacco smoke, 2-furfural, 5-methyl-furfural, 5-hydroxymethylfurfural, and other carbohydrate pyrolyzates, catechols, and org. acids were present in higher concns. than in the smoke from the other tobaccos. In Burley tobacco smoke, myosmine was present in a much higher concn. than in the smoke from the other tobaccos. In Turkish tobacco smoke, glycerol and β -methylvaleric acid were both present in much higher concns. [on SciFinder(R)]

Matsushima, S., T. Ohsumi, et al. (1983). "Composition of trace alkaloids in tobacco leaf lamina." Agric. Biol. Chem. **47**(3): 507-510.

Changes were investigated in trace alkaloid compn. of MC-1 (bright) and Burley-21 leaf lamina during curing. Seventeen alkaloids were identified. The total level of trace alkaloids decreased by ~60% in both MC-1 (flue-cured) and Burley-21 (air-cured). Although the level of most alkaloids also decreased, 2,3'-bipyridyl and 4 nor nicotine derivs., i.e. N'-hexanoyl-, N'-octanoyl-, 1'-(6-hydroxyoctanoyl)-, and 1'-(7-hydroxyoctanoyl)nor nicotine, increased during curing in both varieties. Differences in the trace alkaloid compn. of cured leaf lamina among MC-1, Burley-21 and Basma (Orient) were also studied. In all varieties, N'-formylnor nicotine, nicotyrine, anabasine, and cotinine were present at high concns. relative to the other trace alkaloids. The alkaloids which were present in a relatively high concn. in particular varieties, were N'-formylanabasine in MC-1, N'-hexanoyl-N'-octanoylnor nicotine in Burley-21, and cotinine and N'-formylanatabine in Basma. [on SciFinder(R)]

Matsushita, H., Y. Tsujino, et al. (1979). "New minor alkaloids in flue-cured tobacco leaf (Nicotiana tabacum cv. BY-260-9)." Agric. Biol. Chem. **43**(1): 193-194.

Three minor alkaloids that markedly affect the taste of cigarets when added at ppm levels to tobacco were found in tobacco leaf. N'-carboethoxynor nicotine, N'-butanoylnor nicotine, and N-[4-(dimethylamino)butanoyl]nor nicotine were found at levels of 5, 10, and 1 mg, resp. [on SciFinder(R)]

Mold, J. D., R. E. Means, et al. (1966). "Higher fatty acids of flue-cured tobacco; methyl and cyclohexyl branched acids." Phytochemistry **5**(1): 59-66.

Of the total acids present, 76% are nonpolar satd. or olefinic derivs., with palmitic comprising 20.7, stearic 2.1, oleic 3.5, linoleic 5.8, and linolenic acid 26.2%. Some 4.1% of the acids are homologous monomethyl or cyclohexyl substituted compds. and 5.9% have more complex branched structures. The simple branched acids were further studied by converting them to their Me esters, followed by LiAlH₄ redn. and conversion of the alcs. (via the iodides) to the satd. hydrocarbons. The latter were identified as 2-Me and 3-Me isomers ranging in chain length from C15 to C26 and as 1-cyclohexyl isomers of chain lengths C22-25. [on SciFinder(R)]

Moldoveanu, S. C., C. H. Byrd, et al. (2011). "Analysis of certain nitrogenous compounds in tobacco. Part 1: adenosine, 2,5- and 2,6-deoxy-fructosazines, mannosamine and glucosamine." Beitr. Tabakforsch. Int. **24**(5): 233-242.

Nitrogenous compds. such as amino acids and proteins are frequently analyzed in tobacco since they are considered precursors of toxicants in cigarette smoke. However, much less attention is given to other nitrogenous compds. such as amino sugars and deoxyfructosazines, although their concn. in tobacco can be equal to or even higher than that of most free amino

acids. These nitrogenous compds. may contribute to the formation of toxicants in smoke, or may contribute to the sensory properties of cigarette smoke, reasons for which their anal. is important. This study describes a procedure for the anal. of adenosine, 2,5- and 2,6-deoxyfructosazines (DFs), mannosamine and glucosamine in tobacco. The anal. uses a liq. chromatog.-tandem mass spectrometry (LC/MS/MS) technique. Sample prepn. for anal. consists of the extn. of the tobacco with a soln. of 90% water and 10% methanol, followed by filtration. The sepn. of the analytes was done on a hydrophilic interaction liq. chromatog. HILIC column using an isocratic procedure with a solvent consisting of 78% CH₃CN, 22% H₂O, that also contained 0.1% HCOOH and 0.143 g/L CH₃COONH₄. The measurements were done using electrospray pos. ionization mass spectrometric detection. The anal. procedure was validated and was proven very reliable. A no. of tobaccos were analyzed, including several flue-cured and Burley USA tobaccos, off-shore tobaccos, two Oriental tobaccos, two green tobaccos, as well as tobaccos from com. and Kentucky ref. cigarettes. The ranges for the analytes per g tobacco were found between 0.4 and 20.3 µg/g for adenosine, between 0.0 and 608.5 µg/g for 2,5-DF, between 0.0 and 424.5 µg/g for 2,6-DF, between 12.5 and 415.5 µg/g for mannosamine and between 25.9 and 1885.7 µg/g for glucosamine. The study also indicated that the levels of DFs and that of the amino sugars in tobacco show a very good correlation. This correlation can be explained by the same source of the two classes of compds., namely the reaction of (reducing) sugars and ammonia. [on SciFinder(R)]

Murty, K. S. N., J. C. Tjell, et al. (1986). "Lead and cadmium content of Indian flue-cured tobacco." Plant Soil **95**(2): 281-284.

Studies on levels of Pb and Cd of Indian flue-cured tobacco indicated that the leaf contained very low amts. of these heavy metals as compared to tobacco from other countries. Of the 2 heavy metals, the Pb content of soils was higher than that of Cd; however, in the leaf, the Cd content greater than that of Pb, indicating that soil Cd may be more available to tobacco and hence more readily absorbed by the plant than Pb. [on SciFinder(R)]

Nagai, A., T. Yamamoto, et al. (2012). "Identification of Fructo- and Malto-oligosaccharides in Cured Tobacco Leaves (*Nicotiana tabacum*)." J. Agric. Food Chem. **60**(26): 6606-6612.

Fructooligosaccharides (FOSs) and maltooligosaccharides (MOSs) in cured tobacco leaves (*Nicotiana tabacum*) were detected and quantified using high-performance liq. chromatog. (HPLC) coupled with electrospray ionization (ESI) tandem mass spectrometry (MS/MS). These oligosaccharides were present in several tobacco materials including flue-cured tobacco, sun/air-cured tobacco, and cut filler of com. available tobacco products, but were not detected in air-cured tobacco. The changes in these oligosaccharides during storage were also investigated. The results revealed that MOSs simply decreased under a warm atm. and FOSs increased. In addn., the formation of FOSs in cured tobacco leaves occurred in the presence of sucrose. With regard to FOSs, it has been reported that green tobacco leaves do not contain FOSs such as kestose, nystose, and fructosyl-nystose. The results of a model test suggested that the changes

in FOS amt. were caused by enzymic reactions. [on SciFinder(R)]

Nishikawaji, S., T. Fujimori, et al. (1983). "Sesquiterpenoids from flue-cured tobacco leaves." Phytochemistry **22**(8): 1819-1820.

2,3-Dehydrosolanascone (I) together with the known sesquiterpenoids γ - and δ -cadinene, 2-keto- α -cyperone, solavetivone, solanascone, occidol and rishitin were isolated from flue-cured tobacco leaves. The structure of I was detd. by std. chem. and spectral methods. [on SciFinder(R)]

Noguchi, M., Y. Satoh, et al. (1971). "Storage and aging of leaf tobacco. IX. Changes in the content of amino acid-sugar compounds during aging." Agr. Biol. Chem. **35**(1): 65-70.

The changes in the contents of amino acid-sugar compds. in the flue-cured tobacco leaves were detd. with an automatic amino acid analyzer during 4 years. The contents of amino acid-sugar compds., such as 1-deoxy-1-L-proline-D-fructose, 1-deoxy-1-L-alanine-D-fructose, and asparagine-sugar compd., increased 10-20% during the first 2 years and then declined gradually. [on SciFinder(R)]

Peng, F., L. Sheng, et al. (2004). "Comparison of different extraction methods: steam distillation, simultaneous distillation and extraction and headspace co-distillation, used for the analysis of the volatile components in aged flue-cured tobacco leaves." J Chromatogr A **1040**(1): 1-17.

Steam distillation (SD), simultaneous distillation and extraction (SDE) and headspace co-distillation (HCD) were compared here for their effectiveness in the extraction of volatile compounds from tobacco. The different grades of aged flue-cured tobacco leaves extracted by the three methods respectively were analyzed using GC-MS. Mass spectra or authentic compounds were used to identify around 408 components in various volatile fractions. On the one hand, the qualitative comparison showed that more compounds were detected in HCD extract (391 components) than in SDE extract (377 components), and the approximately quantitative analysis showed that the total amount of volatile components in SDE extract (445.48 microg/g) was much more than that in HCD extract (315.72 microg/g). But on the other hand, HCD was the most efficient for nearly all the highly volatile compounds among the three methods. As to low-volatile compounds such as lactones, long chain aldehydes, ketones, alcohols, and esters, more was detected in SDE extract than in HCD extract. The SD method (322 components, total amount 228.42 microg/g) was the lowest sensitive to all compounds except semi-volatile fatty acids among the three methods.

Prabhu, S. R. and M. K. Chakraborty (1986). "Development of aroma-bearing compounds and their precursors in flue-cured tobacco during curing and post-curing operations." Tob. Res.

12(2): 175-185.

There was a gradual build-up of total free amino acids during curing of flue-cured tobacco. Relative to at-harvest level, certain amino acids decreased while others increased through various stages of curing. Post-curing operations decreased the levels of most of the amino acids. Green leaf contained a relatively low amt. of total carbonyls. Nearly 50% of the carbonyls were generated during the yellowing phase. In subsequent stages a further increase was obsd., max. concn. being in the aged tobacco. Non-volatile carbonyls constituted 75% of the total carbonyls. The pattern of change of the non-volatile carbonyls was similar to total carbonyls. The concn. of volatile carbonyls was max. in aged tobacco. Higher fatty acids showed an irregular pattern of change but C 18:3 acid decreased throughout curing and post-curing stages. The relative changes in total volatile acids indicated a general increase from green leaf to aging except during the drying stage of curing. A gradual build-up of β -methylvaleric and isovaleric acids and a redn. of n-butyric and propionic acids from the middle stage of curing through aging were obsd. [on SciFinder(R)]

Prabhu, S. R., M. K. Chakraborty, et al. (1984). "A note on the generation of carbonyl compounds during curing and aging of flue-cured tobacco." Tob. Res. **10**(2): 202-204.

The total carbonyl content of flue-cured tobacco variety Kanakaprabha, 4th priming leaves, markedly increased during flue curing and aging. A nearly 3-fold increase occurred by the mid-yellowing stage. Individual aldehyde and ketone compds. also increased during curing with the appearance of new compds. at the mid-yellowing stage. [on SciFinder(R)]

Puangnak, W. and P. Chotinuchit (1987). "Changes in chemical constituents during maturation of Thai flue-cured tobacco." Thai J. Agric. Sci. **20**(1): 77-83.

The degree of ripeness of tobacco is important for smoking products. Ripe tobaccos are thought to cure more easily, to respond more readily to aging, and to provide a more flavorful and palatable smoke. Changes in certain chem. constituents of Thai flue-cured tobacco (*Nicotiana tabacum*) were studied during different stages of maturity, including one week on the immature side, 3 days before fully mature, properly mature, and 3 days overmature. Cultural management for the tobacco grown was according to accepted practices at the TTM Expt. Station. These were progressive decline in leaf pigments, total nitrogen, and α -amino nitrogen as maturation progressed. Changes in nicotine during maturation were not significant. Total sugars increased up to the fully mature stage and then decreased at the overmature stage. Percent petroleum ether exts., resins, and waxes decreased in early maturity and increased later on. The ratios total N/nicotine and total sugars/nicotine approached the favorable ratios of 0.6-0.7 and 6-10, resp., as the leaves fully matured. [on SciFinder(R)]

Ramakrishnayya, B. V. and V. Krishnamurthy (1990). "Distribution pattern of potassium in flue-cured tobacco leaf." Indian J. Plant Physiol. **33**(1): 72-75.

Distribution of potassium in leaf according to its position on stalk was studied on flue-cured tobacco. In the normal and healthy plant under sufficient supply of potassium, the K content of leaf decreased from bottom to the top position of the plant while in potassium-deficient plants, a reverse trend was obsd. Severe symptoms of potassium-deficiency were obsd. when K content of leaf fell below 1%. The healthy tobacco plants showed leaf K content above 3%. The K content of leaf in bottom, middle and top position of the plant showed a strong pos. correlation with neutral normal NH₄OAc extractable K content of the surface soil. [on SciFinder(R)]

Rao, B. V. K. and N. C. Gopalachari (1978). "A note on the pattern of distribution of polyphenols and nicotine within the tobacco leaf during curing." Tob. Res. **4**(2): 65-67.

Chlorogenic acid and total flavonoids (as rutin) of flue-cured tobacco increased from the base to the tip of leaves along the margins and from the bottom to the top leaves along the stalk. Chlorogenic acid and, to a lesser extent, flavonoid levels increased in the half-fixing stage of curing, decreased by the end of the fixing stage, then were restored to original levels by the end of curing. Leaf margins contained more nicotine than areas near the midrib, and tips had more nicotine than the base portions. Foliar nicotine levels decreased from the bottom to the top of the stalk. Nicotine levels increased up to the half-yellowing stage of curing, decreased during the half-fixing stage, then increased to the end of curing. [on SciFinder(R)]

Rao, B. V. K., D. G. Kumar, et al. (1978). "Quantitative changes in the polyphenolic constituents of flue-cured tobacco from harvesting to ageing." Tob. Res. **4**(2): 10-12.

Chlorogenic acid and rutin levels of Virginia Gold and Kanakaprabha tobacco were the highest at the end of the leaf-drying stage, and during aging, the levels of both of these constituents decreased. N fertilization did not affect these constituents. Of the 2 varieties, Kanakaprabha contained more chlorogenic acid, and Virginia Gold was richer in rutin. [on SciFinder(R)]

Rashid, A. and M. A. Shah (1975). "Effect of leaf stalk position on chloride content of flue-cured tobacco." Pak. J. Sci. Res. **27**(1-4): 30-31.

Nine varieties of flue-cured tobacco: SC58 McNair-121, Hicks, PD611, NC95, Coker 319, Coker 254, Coker 316 and NC73 were chem. analyzed for the chloride content on leaf stalk position; NC73 contained the lowest chloride. Leaves on the bottom of the plant were the highest and those on the top, the lowest in this respect. [on SciFinder(R)]

Rickert, W. S., P. J. Joza, et al. (2008). "Reductions in the tobacco specific nitrosamine (TSNA) content of tobaccos taken from commercial Canadian cigarettes and corresponding reductions

in TSNA deliveries in mainstream smoke from such cigarettes." Regul Toxicol Pharmacol **51**(3): 306-310.

Tobacco specific nitrosamines (TSNAs) are suspected to cause smoking-related neoplastic diseases. The change from direct-fired to indirect-fired barns (aka kilns) for curing bright (aka Virginia, flue-cured) tobaccos was made to reduce the TSNA concentrations. The effectiveness of such processes in reducing the deliveries of TSNAs to the users of the products should be monitored. However, it is difficult to assess the effects of this reduction on the TSNA levels in mainstream smoke when cigarette blends contain burley tobaccos and other blend components that can increase smoke TSNA concentrations. Canadian cigarettes made prior to and in the few years just after the conversion to indirect-fired curing should not be subject to such interferences. Thus, the TSNA content of tobaccos and mainstream smoke from six brands of Canadian cigarettes produced in 2003, 2004, and 2005 were determined. Reductions in NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone], the most important TSNA in flue-cured tobaccos, levels in the tobacco blends ranged from 60% to 85%. The corresponding reductions in mainstream smoke TSNA levels ranged from 59% to 72% (ISO smoking conditions) and 58-76% (Health Canada Intensive smoking conditions). These results show that other factors (microorganisms, nitrite levels) may be negating the TSNA reductions achieved by indirect-fired curing.

Rohrbach, J. F. (1979). "A survey of some chemical and physical properties of New Zealand flue-cured tobacco." N. Z. J. Agric. Res. **22**(1): 55-59.

Samples of cured leaves from 4 plant positions over 3 yr were analyzed for K, Ca, Mn, Cu, Cl-, reducing sugars, total sugars, total nicotine alkaloids (TNA), and filling value. Results were examd. to det. the effect of soil type, season, and plant position. Comparisons were made with a similar study in Canada, and the correlation between characters detd. Soil type had little effect on the leaf anal., with the exception of Mn which was significantly higher in the Moutere gravels. All characters, except Cl- and Mn, varied significantly with season. Ca, Mg, Cu, Mn, and TNA varied significantly among farms. A comparison with Canadian results showed that New Zealand tobacco was lower in TNA, Mn, and Cl-, higher in Ca, Cu, and about the same in reducing sugars, Mg, K, and filling value. As expected, there was a strong neg. correlation between filling value and reducing sugars, and between reducing sugars and TNA. There was also a definite pos. correlation between filling value and Ca. The advantages of sampling green leaf are discussed. [on SciFinder(R)]

Ryan, W. S., Jr., G. H. Bokelman, et al. (1985). "Influence of genetic and cultural factors on chemical and physical properties of tobacco. II. Cell wall biopolymers." Beitr. Tabakforsch. Int. **13**(2): 88-94.

Five tobacco cultivars, Pennbel 69 (cigar filler), Catterton (Maryland), Coker 319 (bright), Burley 21 (Burley), and Little Sweet Orinoco (sun-cured), were grown and cured under both flue-cured

and dark fire-cured cultural management systems. The cell wall biopolymer compn. of both freeze-dried mature (ripe) leaf and cured tobacco samples was detd. by a std. fractionation procedure. For all 5 tobacco cultivars the levels of most cell wall biopolymers in the freeze-dried mature leaf did not vary significantly as a function of cultural management conditions. However, for Pennbel 69, Catterton and Coker 319 changing from flue-cured to dark fire-cured growing conditions relatively lowered starch contents by 32-74% while increasing the quantities of EtOH solubles and protein. The following general trends were noted for changes in chem. compn. as a function of curing: protein decreased, lignin increased, sol. ash decreased and insol. ash increased. Coker 319 and Little Sweet Orinoco were generally lower in pectin, lignin, and cellulose than the other cultivars regardless of cultural regime. [on SciFinder(R)]

Sakaki, T., K. Fukuhara, et al. (1985). "Studies on tobacco aroma. Part IV. Changes in the composition of headspace volatiles of flue-cured tobacco by aging." Agric. Biol. Chem. **49**(6): 1785-1791.

In order to investigate the headspace volatiles which indicate the aging effect on tobacco, the headspace volatiles of Japanese flue-cured tobacco were collected by active carbon and analyzed by gas chromatog. The data obtained were subjected to principal component anal. (PCA) and stepwise discriminant anal. (SDA). The results of PCA showed that the volatiles indicated the effect of aging on smoking quality. The SDA selected 6 peaks of the volatiles which classified the samples into 4 groups according to the aging period. Thus, the relative proportions of the volatiles, such as furfuryl alc., benzyl alc., and solanone, may be used to judge approx. the aging effects on tobacco. [on SciFinder(R)]

Sakaki, T., K. Niino, et al. (1986). "Studies on tobacco aroma. Part V. Relationship between tobacco headspace volatiles and their smoking quality." Agric. Biol. Chem. **50**(2): 317-323.

To investigate the relationship between tobacco headspace volatiles and their smoking quality, the volatiles of 44 Japanese flue-cured tobacco samples were trapped by active C and analyzed by gas chromatog. (GC). The volatiles related to smoking quality were studied by multiple regression anal. (MRA). Prior to MRA, the information on their GC profiles was condensed into the 6 principal components (PCs) which accounted for 84% of the variance in the 40 GC peaks. The results of MRA using three PCs showed precise predictions for scores of tobacco smoking quality ($r = 0.90$). The samples were roughly sepd. into groups according to their variety and stalk position on the plane for the selected PCs. The influences of the variety and stalk position on the smoking quality were also investigated by stepwise discriminant anal. Those volatiles which are partially governed by variety and stalk position may be used to evaluate the smoking quality of tobacco. [on SciFinder(R)]

Sakaki, T., H. Sakuma, et al. (1984). "Studies on tobacco aroma. Part I. Analysis of the headspace volatiles of tobacco using an ether trap." Agric. Biol. Chem. **48**(11): 2719-2724.

A method for analyzing the headspace volatiles with an ether trap was developed and the headspace volatiles of 4 kinds of tobacco, flue-cured, Burley, domestic (cv Matsukawa), and Turkish, were analyzed. Dried air was passed through cut tobacco and the headspace volatiles swept were absorbed in ice-cooled ether. The ether column of headspace volatiles was analyzed, and 27 components were identified by gas chromatog. (GC) and GC-mass spectra. Of these 27 components, 6 have not previously been reported as components in the tobacco headspace volatiles. The individual headspace volatiles samples of the 4 kinds of tobacco each showed their characteristic GC profiles and odors. The odor and the GC profile of each headspace volatile sample was different from those of the steam distillate of the same tobacco. [on SciFinder(R)]

Schepartz, A. I., J. J. Ellington, et al. (1982). "An analysis of the variation in leaf lipids during the curing of flue-cured tobaccos." Tob. Int. **184**(9): 26-29.

An anal. study was made of the levels of neutral lipids present in 2 flue-cured tobacco varieties, NC 95 and LA 53, during 5 intervals in the curing process: at harvest, half-way through yellowing, end of yellowing, end of leaf drying, and completely cured. Samples were from 4 primings, 3 replications each. Twelve neutral lipid components were assayed on each sample, yielding a total of 1440 pieces of data. In addn., the concn. of Ca was detd. on all samples as a check on changes in the tobacco dry wt. It was concluded that: (1) significant changes occurred in most of the lipids during curing of both varieties (hexane extractables, solanesol, hydrocarbon waxes, and neophytadiene increased; fatty acids decreased); (2) there were some differences between the 2 varieties; and (3) the levels of most of the lipids were strongly dependent upon all 3 factors (variety, priming, and interval in the curing). [on SciFinder(R)]

Schepartz, A. I., R. L. Wilson, et al. (1983). "Variation in the mineral concentration of certain flue-cured tobaccos during curing." Tob. Int. **185**(24): 55-57.

An anal. study was made of the concns. of 13 inorg. elements present in 2 flue-cured tobacco varieties, NC 95 and LA 53, at 5 intervals in the curing process: at harvest, half-way through yellowing, end of yellowing, end of leaf drying, and completely cured. The elements detd. were Ca, P, K, Mg, Mn, Fe, Al, B Cu, Zn, Sr, Ba, and Na. Although these elements would ordinarily be regarded as stable and nonvolatile, variations in concns. were found. Leaves of the first priming appeared to be quite different from those of other primings in the levels of elements found. [on SciFinder(R)]

Schlotzhauer, W. S. (1978). "Liquid chromatographic separation of chlorophyll products in tobacco leaf." Tob. Sci. **22**(41-45): 44-45.

A tobacco leaf fraction contg. chlorophyll degrdn. products was obtained by hexane extn. of flue-cured leaf, and sepn. of the ext. by chromatog. on a silicic acid column. The ext. fraction was further sepd. by high-pressure liq. chromatog. on a Micro-Pak Si-10 column, using a hexane-CHCl₃ phase system. Individual constituents were collected, and identified by their UV spectra. The principal components sepd. by this procedure were chlorophyllin, pheophytin, and carotenoids. [on SciFinder(R)]

Sheen, S. J. and H. R. Burton (1978). "Amino acid composition in soluble tobacco fractions containing brown pigments." J. Agric. Food Chem. **26**(2): 380-385.

Brown pigments in air-cured and flue-cured tobacco and blended cigarettes were isolated into 6 fractions by soly. in acidic and alk. soln. and in EtOH. Amino acid compn. of the pigment fractions analyzed by gas-liq. chromatog. revealed that aspartic and glutamic acids occurred in great quantities, followed by glycine, alanine, valine, and serine in all fractions, irrespective of tobacco type and curing methods. The compn. was comparable between the same fraction of different tobacco types but differed among the fractions. Proline content was extremely high in the nonpptd., EtOH-sol. brown pigment fraction. The major brown pigment in cured tobacco is alkali-sol., acid-precipitable, and EtOH-insol. This pigment consisted of >50% as proteins whose amino acid compn. was similar to that of fraction I protein. Brown pigments of the Kentucky IR1 ref. cigarettes differed from smoke pigments in wt. distribution of pigment fractions and in amino acid compn. The present results suggest that the distribution of proteins and amino acids in brown pigment fractions is not affected by tobacco types but can be modified by cultural practices and curing methods. [on SciFinder(R)]

Siddiqui, I. R., N. Rosa, et al. (1981). "An Amadori compound from tobacco." Carbohydr. Res. **98**(1): 57-63.

The Amadori compd. 1-deoxy-1-[(S)-2-(3-pyridyl)-1-pyrrolidinyl]- β -D-fructopyranose (I) was isolated from flue-cured lamina of Delhi tobacco and its structure established from the results of hydrolysis, GLC-mass spectra of its trimethylsilyl deriv., and ¹³C-NMR spectroscopy. The significance of the compd. in relation to tobacco quality is discussed. [on SciFinder(R)]

Snook, M. E., P. J. Fortson, et al. (1981). "Isolation and identification of phenolic acids from tobacco leaf." Beitr. Tabakforsch. Int. **11**(1): 19-26.

A new chromatog. method utilizing the H bonding properties of Sephadex LH-20 gel in MeOH/CHCl₃, was developed for the isolation of phenolic acids. This method was applied to the characterization of phenolic acids in flue-cured tobacco. Gel chromatog. successfully isolated and concd. the phenolic acids from other acidic substances and allowed identification and quantitation of these compds. by gas chromatog. (GC) and GC-mass spectroscopy, as their

trimethylsilyl derivs. Compds. identified included cis- and trans-p-coumaric acid, cis- and trans-ferulic acid, cis- and trans-coffeic acid, cis- and trans-sinapic acid, o-, m-, and p-hydroxybenzoic acid, o-hydroxyphenylacetic acid, 2,5-, and 3,4-dihydroxybenzoic acid, and 2,3-, 2,5-, and 3,4-dihydroxybenzaldehyde. Caffeic acid was the major compd., probably arising from the degrdn. of the chlorogenic acid. Aliph. acids having ≥ 2 carboxyl groups were also isolated and included the following: malonic, succinic, fumaric, malic, and citric acids. A dihydroxycinnamaldehyde and a dihydroxynaphthoic acid are reported in tobacco leaf for the first time. [on SciFinder(R)]

Sun, J.-g., J.-w. He, et al. (2011). "Comparative Analysis on Chemical Components and Sensory Quality of Aging Flue-Cured Tobacco from Four Main Tobacco Areas of China." Agric. Sci. China **10**(8): 1222-1231.

Complicated changes are happening during the aging process of flue-cured tobacco (FCT) and understanding of the changing components is of great significance in improving the quality, shortening aging time, and enhancing prodn. and economic efficiency in tobacco industry. The aging samples of FCT from four main producing areas of China, i.e., Yunnan, Sichuan, Hubei, and Dongbei, were used to study the changing rule of the components such as alkali, acid, and carbohydrate as well as the aroma and their relationship with sensory quality; and based on the correlation among the components and the sensory quality index, multivariable models were established to predict the aging quality. The sensory quality of FCT increased steadily during the aging time, and at the same time, the acidic components (total org. acids, volatile acids) increased gradually, while the alk. substances (nicotine, volatile alkali), carbohydrate (total sugars, reducing sugar) and pH values showed a downward trend. Correlation anal. found that the sensory quality and pH values were neg. correlated ($P < 0.05$), while the sensory quality with total org. acids and aroma were pos. correlated. The optimal model for predicting the quality of FCT was $y = 56.75 - 0.63x_1 + 50.09x_2 - 13.27x_2^2$, (y: sensory quality; x_1 : pH; x_2 : total org. acids). The av. predicating error was 1.04% with max. predicating error of 2.89% and the accuracy rate of above 97%. [on SciFinder(R)]

Sun, W., Z. Zhou, et al. (2012). "Differentiation of flue-cured tobacco leaves in different positions based on neutral volatiles with principal component analysis (PCA)." Eur. Food Res. Technol. **235**(4): 745-752.

Differentiation of tobacco leaves in different plant stalk positions in China was achieved based on basic chem. compds. and neutral volatiles by principal component anal. (PCA). The continuous flow anal. method was used to det. the quantity of the basic chem. compds. (sugars, total nitrogen and nicotine) of 30 samples. The neutral volatiles of tobaccos were analyzed by GC-MS. It was found that the disparity in basic chem. components was not enough to assess the different quality of tobacco leaves. PCA was used to reveal the differences among these three species based on neutral volatile compds. Tobacco leaves in different positions were differentiated according to the results of PCA. Four principal components were extd. and

altogether accounted for exceeding 80 % of the total variances. The first principal component explained 48.1 % of the total variation and was mainly defined by solanone, megastigmatrienone, 5-methylfurfural, furfuralc., phenylethylalc. and benzylalc. The second principal component explained 18.1 % of the total variation and was mainly defined by β -ionone and farnesylacetone. The plots of sample loadings showed that a good differentiation on the basis of concns. of neutral volatiles was achieved. These results demonstrated that neutral volatiles can be used as the useful variables to characterize the tobacco leaves in different positions. [on SciFinder(R)]

Szedljak, I., K. S. Kohegyi, et al. (2010). "Biochemical studies on curing and fermentation processing period of different tobacco plant (*Nicotiana tabacum* L.) cultivars." Beitr. Tabakforsch. Int. **24**(1): 24-28.

We studied the changes in the activity of the enzymes polyphenol oxidase (PPO) and peroxidase (POX), the concn. of total sol. phenolic compds. and sol. protein content in different tobacco cultivars (Virginia and Burley) during curing. The curing procedure was a special combination of open-air-curing and flue-curing methods and a long fermn. period. In the curing period the changes in data suggested a combination of the biochem. processes and the direct effect of oxygen. A slight increase then a decrease in the concns. of both total sol. phenolic compds. and the sol. protein content were detected. In this period we found no correlation between the concn. of total sol. phenolic compds., the decreasing PPO and the increasing POX activity. In the fermn. period a deactivation of the enzymes (PPO and POX) and a decrease in the concn. of both total sol. phenolic compds. and sol. protein content were found, because the fermn. is not a biochem.-regulated process. Moreover, these results suggest that the end of curing period is the most favorable time for protein isolation from different tobacco cultivars. [on SciFinder(R)]

Tazaki, H., H. Kodama, et al. (1986). "Hydroxysolanascone glucosides from flue-cured tobacco leaves." Agric. Biol. Chem. **50**(9): 2231-2235.

The structures of 13- and 15-hydroxysolanascone- β -glucopyranosides, sesquiterpenoid glycosides isolated from flue-cured tobacco leaves, were deduced from spectral data and by means of chem. conversion. [on SciFinder(R)]

Tazaki, H., H. Kodama, et al. (1989). "Structures of new solanascone glucosides from flue-cured tobacco leaves." Agric. Biol. Chem. **53**(11): 3037-3038.

10 β -Hydroxysolanascone β -glucopyranoside (I) and 9 β -hydroxysolanascone β -glucopyranoside (II) were isolated from flue-cured tobacco and the structures detd. by chem. and phys. properties and enzymic hydrolysis. [on SciFinder(R)]

Tazaki, H., H. Kodama, et al. (1991). "Structure of sesquiterpenoid glucoside from flue-cured tobacco leaves." Agric. Biol. Chem. **55**(7): 1889-1890.

Acetylated sesquiterpenoid glucoside I (R = tetra-O-acetyl- β -D-glucopyranosyl, R1 = Ac) (II) was isolated from flue-cured tobacco leaves. It was characterized by sapon. to the free glucoside I (R = β -D-glucopyranosyl, R1 = H), and further hydrolyzed to the aglycon 1 β -hydroxydebnayol (I; R = R1 = H). [on SciFinder(R)]

Ushasri, V., P. S. N. Murthy, et al. (1986). "Ester content and its relationship with the aroma of tobacco." Tob. Res. **12**(1): 63-67.

Estn. of esters of different tobacco varieties may help in ascertaining the aroma characteristics and the other flavor compds. The ester content of different varieties of flue-cured tobacco ranged from 0.66% (Variety 2063) to 0.77% (HR 62-9). [on SciFinder(R)]

Wahlberg, I., K. Karlsson, et al. (1977). "Tobacco chemistry. Part 38. Effects of flue-curing and aging on the volatile, neutral and acidic constituents of Virginia tobacco." Phytochemistry **16**(8): 1217-1231.

Several volatile compds. are generated and the concns. of many others increase on flue-curing and aging of Virginia tobacco. Oxidative degrdn. of isoprenoids, Maillard reactions, and phenylalanine metab. are the major processes accounting for the formation of the majority of these compds. A no. of alcs., aldehydes and ketones, which are apparently products of fatty acid degrdn., are lost during the postharvest handling. [on SciFinder(R)]

Wahlberg, I., K. Karlsson, et al. (1977). "Tobacco chemistry. Part 39. Effects of flue-curing and aging on the volatile basic constituents of Virginia tobacco." Phytochemistry **16**(8): 1233-1235.

Flue-curing and aging of Virginia tobacco decreased the amts. of nicotine and several of its transformation products. In contrast, the concns. of certain structurally simple pyridines and a few compds., presumably products of sugar-amino acid interactions, were found to increase. Generation of several pyridines and a few other bases during the leaf processing was also obsd. [on SciFinder(R)]

Walker, E. K. and T. T. Lee (1968). "Relation of phenolic constituents to maturity, stalk position, and certain physical characteristics of flue-cured tobacco." Can. J. Plant Sci. **48**(4): 381-391.

Chlorogenic acids, scopoletin, and caffeic acid in green and cured leaves of flue-cured tobaccos harvested from various stalk positions at different stages of maturity were isolated and quant.

detd. Total chlorophyll, which provided the basis of maturity classification, was measured in green samples. Lamina wt., grade index, and visual lightness were detd. in cured samples. Levels of the phenolics were related to chlorophyll, lamina wt., stalk position, grade index, and visual lightness. Chlorogenic acids increased from 33 to 500% during curing; levels in green and cured leaves varied from 0.1 to 1.9% (dry wt.). Chlorogenic acids increased with advance in maturity and elevation in stalk position. Grade index was pos. related to level of chlorogenic acids. Visual lightness was affected adversely by prolongation of the yellowing period, which was assocd. with both low and high levels of chlorogenic acids. Therefore, visual lightness was rather poorly related to level of chlorogenic acids. Cured leaves contained less than 0.05% (dry wt.) caffeic acid; none was detected in uncured leaves. Levels of scopoletin and its glucoside were higher in cured than in green leaves; the amt. of scopoletin was less than 0.03% (dry wt.). Levels of caffeic acid and scopoletin in cured leaves were directly related to time of yellowing which increases with chlorophyll and lamina wt. Levels of scopoletin in green leaves increased with advance in maturity and elevation in stalk position. [on SciFinder(R)]

Wassef, M. K. and J. W. Hendrix (1973). "Preliminary analysis of the lipid content and composition of cured tobacco." Tob. Health Workshop Conf., Proc., 4th: 768-776.

Lipids of cured tobacco samples (burley and flue-cured) were extd. These were fractionated by column chromatog., and further sepd. by 2-dimensional thin-layer chromatog. Phospholipids comprised 7% and 10% of the total lipids extd. from burley tips and lugs, resp. The pattern of the individual lipid components was similar in burley tips and lugs. Phosphatidylinositol appeared to be a major component; phosphatidylserine, phosphatidic acid, mono- and di-galactosyl diglycerides, and a no. (~8) of unknown polar lipids were detected. On the other hand, lipids of flue-cured tobacco (tips and cutters) exhibited a different pattern from those of the cured burley in that considerable amts. of phosphatidylethanolamine, phosphatidic acid, phosphatidylcholine, and more unknown lipids were present. At least 6 of the unknown lipids were glycolipids. It appears that flue-cured tobacco contains twice as much lipid as burley, that flue-cured and burley tobaccos contain some lipid components in common but differ greatly in other components, both qual. and quant., that stalk position has only a minor effect on lipid compn., and that storage time and(or) conditions have a major effect. [on SciFinder(R)]

Wassef, M. K. and J. W. Hendrix (1974). "Lipid content of cured tobacco." Can. J. Bot. **52**(5): 1123-1126.

Samples from cured burley and flue-cured tobacco were examd. for total lipid content and for lipid compn. of fractionated lipid classes. Cured burley tobacco contained 6-8% lipids compared with 14-15% in cured flue-cured tobacco. Neutral lipids constituted 70-80% of burley lipids but only half the flue-cured lipids; the latter had 75% more neutral lipids than the former. Flue-cured tobacco contained considerably more (5-10 times) medium- to high-polarity lipids than burley. Stalk position had only a minor effect on the lipid content of both tobacco types. [on SciFinder(R)]

Wassef, M. K. and J. W. Hendrix (1975). "Glycolipids and phospholipids of cured burley and flue-cured tobacco." J. Agric. Food Chem. **23**(4): 688-690.

Cured flue-cured tobacco contained ~14% lipid, twice the lipid content of cured burley tobacco.

Most of the difference was found to be polar lipids. The major phosphatides of flue-cured tobacco were phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylcholine (PC), and phosphatidylglycerol (PG), with smaller proportions of diphosphatidylglycerol (DPG) and phosphatidylinositol (PI). The major phosphatides of burley tobacco were PI, PE, PA, and PC, with smaller proportions of DPG and PG. The major glycolipids were monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG). The MGDG:DGDG ratio was ~1:2 for burley tobacco and 2:1 for flue-cured tobacco. Unidentified glycolipids constituted a 3rd of the lipid carbohydrate in burley tobacco but were negligible in flue-cured tobacco. Stalk position had little effect on lipid compn. [on SciFinder(R)]

Weeks, W. W., V. A. Sisson, et al. (1992). "Differences in aroma, chemistry, solubilities, and smoking quality of cured flue-cured tobaccos with aglandular and glandular trichomes." J. Agric. Food Chem. **40**(10): 1911-1916.

The chem., aroma, solubilities (hexane and 95% ethanol), and smoking quality of cured tobacco from three typical flue-cured varieties that produce glandular trichomes on the surface of the green leaf and an atypical aglandular breeding line were compared. The glandular tobaccos produced more characteristic tobacco aroma than the aglandular tobacco. More hexane-extractable solute was obtained from the aglandular tobacco than from the glandular flue-cured tobaccos, but more alc.-extractable solute was obtained from the glandular types. A trained smoke panel gave higher scores to the aglandular tobacco than the three glandular varieties. Larger quantities of total volatiles were obtained from the aglandular tobacco. Solanone and oxysolanone identified from the glandular tobacco were absent in the aglandular breeding line. The aglandular tobacco was higher in total alkaloids and lower in reducing sugars than the glandular types. [on SciFinder(R)]

Weston, T. J. (1968). "Biochemical characteristics of tobacco leaves during flue-curing." Phytochemistry **7**(6): 921-930.

During normal flue-curing of ripe tobacco leaves, polyphenol oxidase and peroxidase activities rose rapidly during the first few days of curing, and then decreased sharply so that in the fully cured leaf no polyphenol oxidase activity and very little peroxidase activity remained. The ascorbic acid content decreased steadily during the cure, leading to a transient rise in dehydroascorbic acid, although very little of either remained in the cured leaves. The chlorogenic acid content rose during the 1st 1 or 2 days of curing and later fell slightly. Detached tobacco leaves were supplied with water, with ascorbic acid, or with chlorogenic acid and flue-cured with either a normal or an extended yellowing period. There was very little brown in normal cures, and the color of the leaves was found to be unrelated to either the normal or

addnl. levels of ascorbic acid or chlorogenic acid. With extended yellowing, however, there was a greater production of pigment, which was related to the chlorogenic acid content of the leaves but which was unaffected by ascorbic acid. There was a great excess of enzyme available for the oxidn. of the chlorogenic acid present. Ascorbic acid was probably of little or no significance in preventing oxidn. of chlorogenic acid, but in both normal and extended cures, the ascorbic acid content was related to the luster of the leaves. [on SciFinder(R)]

White, F. H., R. S. Pandeya, et al. (1979). "Correlation studies among and between agronomic, chemical, physical and smoke characteristics in flue-cured tobacco (*Nicotiana tabacum* L.)." Can. J. Plant Sci. **59**(1): 111-120.

Coeffs. of correlation among 23 characteristics were detd. for several flue-cured tobacco (*N. tabacum*) cultivars and advanced generation breeding lines, representing a wide range of variability for each characteristic. The variables were grouped into agronomic, chem., and phys. characteristics of the leaf and smoke properties of cigarettes. Most of the traits studied in these individual groups could be classified into 2 main categories in terms of their relationship to yield, leaf alkaloids, smoke total particulate matter (TPM), and wet tar (WT). Those agronomic characteristics pos. assocd. with yield were neg. correlated with leaf total alkaloids, lamina wt. and smoke TPM, WT and alkaloids on a per cigarette basis. Conversely, grade index and the av. length of the 3 top leaves, which were in neg. assocn. with yield, showed a pos. relationship with leaf total alkaloids and smoke characteristics. Leaf total alkaloids and lamina wt., in pos. assocn. with each other, were pos. correlated with smoke TPM, WT, and alkaloids. However, both of these traits were neg. correlated with yield. [on SciFinder(R)]

Wilkinson, C. A. and W. W. Weeks (1994). "Alkaloid analysis in flue-cured tobacco." Mod. Methods Plant Anal. **15**: 137-151.

A review and discussion, with 42 refs., on the anal. of alkaloids in flue-cured tobacco. Methods of historical or practical significance are highlighted with the most pertinent papers being cited for each method. [on SciFinder(R)]

Williamson, R. E. and G. R. Gwynn (1982). "Variation of polyphenols in flue-cured tobacco cultivars attributed to location, stalk position, and year." Crop Sci. **22**(1): 144-146.

The levels of polyphenols were compared in cured leaves from bottom, middle, and top stalk positions of 13 cultivars grown at 4 locations for 2 yr. There were differences among cultivars for polyphenol level. NC 2326 had the highest level with 4.25% and Speight G-28 had the lowest with 3.58%. Highly significant differences in levels of polyphenols assocd. with stalk position were measured (3.15, 4.11 and 4.42% for the bottom, middle, and top position, resp.). There were significant interactions of locations by the seasons experienced in the 2 yr. Interactions involving cultivars were not significant in most cases, and where they were, it involved higher

order interactions. This was also true for stalk positions. The presence of cultivar differences and the consistency of these differences would encourage breeding efforts but testing over locations and years would be advisable. [on SciFinder(R)]

Xiang, Z., K. Cai, et al. "Simultaneous Extraction and Derivatization of Volatile Organic Acids in Flue-Cured Tobacco with Headspace Solid-Phase Microextraction Followed by GC-MS." Anal. Lett.: Ahead of Print.

Simultaneous extn. and derivatization followed by head-space solid-phase microextrn. (HS-SPME) coupled with gas chromatog./mass spectrometry (GC-MS) was employed to analyze volatile org. acids (VOAs) in flue-cured tobacco samples. Several exptl. parameters e.g., the type of SPME fibers, sampling temp., pre-extn. and derivatization equilibration time, anhyd. sodium sulfate amt. were optimized to improve sampling efficiency. Validation was carried out in terms of limits of detection (LOD), precision, recovery and linearity. The results indicated that the optimized technique was easy, highly efficient and sensitive. Finally, the performance of the proposed method was tested for the extn. and detn. of the VOAs in flue-cured tobacco and the satisfactory results were achieved. [on SciFinder(R)]

Yamamoto, K. and M. Noguchi (1973). "Chemicals studies on ninhydrin-positive compounds in cured tobacco leaves. IV. Isolation of sugar compounds of asparagine, phenylalanine, tryosine, and valine from cured tobacco leaves." Agr. Biol. Chem. **37**(9): 2185-2187.

Four amino sugars were isolated from flue-cured tobacco leaves and their structures were detd. to be: 1-deoxy-1-L-asparagino-D-fructose, 1-deoxy-1-L-valino-D-fructose, 1-deoxy-1-L-phenylalanino-D-fructose, and 1-deoxy-1-L-tyrosino-D-fructose. [on SciFinder(R)]

Zhu, D., J. Han, et al. (2001). "Changes in higher fatty acids and related biochemical characteristics of flue-cured tobacco during aging." Beitr. Tabakforsch. Int. **19**(6): 315-319.

The purpose of this study was to investigate the changes in higher fatty acids and related biochem. characteristics of flue-cured tobacco during the process of aging. The concn. of total lipids in flue-cured tobacco decreased gradually during natural aging, but the concn. of individual principal higher fatty acids in flue-cured tobacco changed much differently during aging. Myristic acid (C14:0) increased constantly during the whole aging process. Stearic acid (C18:0) and oleic acid (C18:1) increased at the initial stages of aging and reached max. contents after one year of aging, but decreased thereafter. Palmitic acid (C16:0), linoleic acid (C18:2), and linolenic acid (C18:3) decreased constantly throughout aging, esp. the linolenic acid content which decreased drastically. Furthermore, the ratio of unsatd. fatty acids (C18:1; C18:2; C18:3) and satd. fatty acids (C14:0; C16:0; C18:0) decreased continuously during aging, from 1.00 at the initial stage to 0.43 at the end of aging, when it seemed to be stabilized. The max. rate of decrease of the ratio was between 12 to 18 mo during aging. The activity of

lipoxygenase (LOX) and peroxidn. value (POV) initially increased and reached their highest levels after 6 mo of aging, but steadily decreased thereafter. The acid value (AV) and malondialdehyde (MDA) content increased during the first 12 mo of aging, then decreased rapidly after 12 and 18 mo, resp. [on SciFinder(R)]

Assessment of tobacco specific nitrosamines in tobacco genotypes. By Guo, Peiguo; Xia, Yanshi; Li, Ronghua; Lu, Yonghua; Qiu, Miaowen; Zhao, Weichai; Yu, Yiwen From Advanced Materials Research (Durnten-Zurich, Switzerland) (2013), 641-642, 871-874, 5 pp.. Language: English, Database: CAPLUS,

The tobacco-specific nitrosamines (TSNAs) are one of the most important groups of carcinogens in tobacco leaf, knowing the difference of TSNAs contents in tobacco genotypes should be beneficial for breeding low TSNAs cultivars which could decrease the harmfulness to human health in tobacco products. For this purpose, 8 genotypes (include burley, sun-cured and flue-cured tobacco) were selected as materials, the TSNAs contents of uncured and cured leaves were assessed with LC-MS/MS. The results showed that TSNAs contents were significantly increased after curing in all genotypes. The mean TSNAs contents of leaves before and after curing in burley were the highest among three types of tobacco and the contents in sun-cured tobacco were the lowest. Coeff. of variation (CV) of TSNAs among genotypes in burley was higher than that in flue-cured and sun-cured tobacco and the CV in sun-cured tobacco was the lowest. The pos. correlation was obsd. in NNN, NAT and total TSNAs contents between uncured and cured leaves in three types of tobacco. Therefore, the TSNAs contents in uncured leaves could be considered as evaluation index to screen tobacco cultivars with the low TSNAs contents.

Differentiation of flue-cured tobacco leaves in different positions based on neutral volatiles with principal component analysis (PCA) By Sun, Weifeng; Zhou, Zhilei; Li, Yue; Xu, Zhiqiang; Xia, Wenshui; Zhong, Fang. From *European Food Research and Technology* (2012), 235(4), 745-752. Language: English, Database: CAPLUS

Differentiation of tobacco leaves in different plant stalk positions in China was achieved based on basic chem. compds. and neutral volatiles by principal component anal. (PCA). The continuous flow anal. method was used to det. the quantity of the basic chem. compds. (sugars, total nitrogen and nicotine) of 30 samples. The neutral volatiles of tobaccos were analyzed by GC-MS. It was found that the disparity in basic chem. components was not enough to assess the different quality of tobacco leaves. PCA was used to reveal the differences among these three species based on neutral volatile compds. Tobacco leaves in different positions were differentiated according to the results of PCA. Four principal components were extd. and altogether accounted for exceeding 80 % of the total variances. The first principal component explained 48.1 % of the total variation and was mainly defined by solanone, megastigmatrienone, 5-methylfurfural, furfuralc., phenylethyl alc. and benzyl alc. The second principal component explained 18.1 % of the total variation and was mainly defined by β -ionone and farnesyl acetone. The plots of sample loadings showed that a good differentiation on the basis of concns. of neutral volatiles was achieved. These results demonstrated that neutral volatiles can be used as the useful variables to characterize the tobacco leaves in different positions.

Flue Cured Tobacco : Toxicology of unburned tobacco

Becker, C. G., D. P. Hajjar, et al. (1985). "Tobacco constituents are mitogenic for arterial smooth-muscle cells." *Am J Pathol* **120**(1): 1-5.

Tobacco glycoprotein (TGP) purified from flue-cured tobacco leaves, tar-derived material (TAR), the water soluble, nondialyzable, delipidized extract of cigarette smoke condensate, rutin-bovine serum albumin conjugates, quercetin, and chlorogenic acid are mitogenic for bovine aortic smooth-muscle cells, but not adventitial fibroblasts. The mitogenicity appears to depend on polyphenol epitopes on carrier molecules. Ellagic acid, another plant polyphenol, inhibited arterial smooth-muscle proliferation. These results suggest that a number of ubiquitous, plant-derived substances may influence smooth-muscle cell proliferation in the arterial wall.

Hoffmann, D., N. H. Harley, et al. (1986). "Carcinogenic agents in snuff." *JNCI, J. Natl. Cancer Inst.* **76**(3): 435-437.

The oral use of snuff has been assocd. with an increased risk for cancer of the oral cavity and pharynx. The 5 most popular US snuff brands were analyzed for alkaloids, volatile and

tobacco-specific N-nitrosamines (TSNA), benzo[a]pyrene [50-32-8], and 210Po. The carcinogenic TSNA in the 5 snuff brands ranged from 9600 to 289,000 ppb. These concns. exceed the nitrosamine concns. of other consumer products by at least 2 orders of magnitude. Po amounted to 0.16-1.22 pCi/g dry snuff. Trace amts. of benzo[a]pyrene (0.1-63 ppb) were indicative of contamination of the tobacco with thermal degrdn. products, probably due to fire curing or flue curing. The findings from this study, the biol. activity of snuff in animal models, and the epidemiol. studies on snuff use and oral cancer strongly suggest the need for redn. of carcinogens and esp. of nitrosamines and 210Po in snuff. [on SciFinder(R)]

Schmeltz, I., S. Abidi, et al. (1977). "Tumorigenic agents in unburned processed tobacco: N-nitrosodiethanolamine and 1,1-dimethylhydrazine." Cancer Lett. (Amsterdam) 2(3): 125-131.

The tumorigenic agents N-nitrosodiethanolamine [1116-54-7], a weak hepatic carcinogen in rats, and MeNNH₂ [57-14-7] were isolated from unburned processed tobacco. N-nitrosodiethanolamine varied in amts. from 0.1 ppb in flue-cured tobacco not treated with the herbicide MH-30 (maleic hydrazide) [123-33-1] to 173 ppb in Burley tobacco to which MH-30 had been applied prior to harvesting. MH-30 is commonly formulated as the diethanolamine salt. Me₂NNH₂ ranged 60-147 ppb, except in the case of Burley tobacco, where none was detected. The source of the nitrosamine in the tobacco appears to be the MH-30. [on SciFinder(R)]

Changes in TSNA contents during tobacco storage and the effect of temperature and nitrate level on TSNA formation

By Shi, Hongzhi; Wang, Ruiyun; Bush, Lowell P.; Zhou, Jun; Yang, Huijuan; Fannin, Neil; Bai, Ruoshi

From Journal of Agricultural and Food Chemistry (2013), 61(47), 11588-11594. Language: English, Database: CAPLUS, DOI:10.1021/jf404813m

Samples of burley, sun-cured, and flue-cured tobacco from the main producing areas of China were examd. for changes in tobacco-specific nitrosamine (TSNA) contents during storage and for the effects of storage temp. and tobacco nitrate level on TSNA formation of cured tobacco. Four TSNA compds. were detd. (N-nitrosonornicotine, N-nitrosoanabasine, N-nitrosoanatabine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone). The TSNA contents in burley and sun-cured tobacco increased substantially during 1 yr under natural storage conditions, with the total TSNA content increasing about 215% in both tobacco types. The fastest increase occurred during the high temp. season. Temp. had significant promoting effects on TSNA formation during storage. Storage temp. as high as 27°C for 12 days was enough to increase TSNA formation, while the most significant effect was seen at temp. >30°C. The increased rate of TSNA accumulation became greater as the temp. increased. The total TSNA content in air-cured burley tobacco after the treatment at 60°C for 24 days was 772% higher vs. the low temp. control. Different types of tobacco showed different results in terms of TSNA formation responses to high temp. The TSNA

formation in flue-cured tobacco did not increase after high-temp. treatment for 36 days, while burley and sun-cured tobacco showed dramatic increases in TSNA content. This difference could be explained by the fact that burley tobacco and sun-cured tobacco usually had >10-times the nitrate content than flue-cured tobacco. As the nitrate levels increased in cured burley tobacco, the TSNA formation during leaf storage at high temp. also increased. Addn. of nitrate to the flue-cured tobacco to the level equiv. to burley tobacco followed by high-temp. treatment increased the TSNA concns. comparably as in burley tobacco. The interaction between high temp. and abundant nitrate content in cured tobacco could be responsible for TSNA formation during storage.

Flue Cured Tobacco : Transfer Studys

Chortyk, O. T., R. F. Severson, et al. (1975). "Chromatographic determination of hydrocarbon waxes in tobacco leaf and smoke." Beitr. Tabakforsch. **8**(4): 204-210.

Paraffins of tobacco leaf were sepd. by column chromatog. on silicic acid. Leaf paraffins were fractionated from other wax constituents by chromatog. in a definite sample to substrate to solvent ratio. This method was used to evaluate the transfer of paraffins and neophytadiene from leaf to smoke in a ref. cigarette. Gas chromatog. sepns. were performed on a high-temp. liq. phase. Gas chromatog. in conjunction with mass spectrometry was used to det. the paraffin compn. of a representative flue-cured tobacco, a ref. cigarette tobacco, and smoke condensate. Thus, paraffins were probably transfered to smoke relatively unchanged, whereas neophytadiene underwent some pyrolytic decompn. [on SciFinder(R)]

Flue Cured Tobacco : Pyrolysis

Baker, R. R. (1975). "Formation of the oxides of carbon by the pyrolysis of tobacco." Beitr. Tabakforsch. **8**(1): 16-27.

Flue-cured Virginia tobacco has been heated in N and N/O mixts. under flow conditions, and the rate of formation of CO and CO₂ has been detd. as a function of temp. heating rate, and proportion of O in the gas. When the tobacco is heated in N at heating rates comparable to those in a smouldering cigarette, 27% of the C content of the tobacco is converted to C oxides. Both C oxides show 2 distinct formation regions: a low-temp. region (about 100°-450°), and a high-temp. region (about 550°-900°). These temp. limits are almost identical to those predicted from studies on the combustion coal of a cigarette burning in air. When tobacco, or the carbonaceous residue remaining after the pyrolysis expts., is heated in N/O mixts., the total amt. of C converted to CO and CO₂ is independent of heating rate, but the relative proportions of the 2 oxides are strongly dependent on heating rate. At the lower heating rate, proportionally less CO, and more CO₂, is produced. Under oxidn. conditions, about 70% of both C oxides formed

in the low-temp. region (100°-450°) are produced by tobacco decompn. reactions, whereas in the high-temp. region about 10-20% of the CO, and 2-9% of the CO₂, are produced by tobacco decompn. [on SciFinder(R)]

Bell, J. H., A. O. Saunders, et al. (1966). "The contribution of tobacco constituents to phenol yield of cigarettes." Tob. Sci. **10**: 138-142.

A study of pyrolysis conditions made possible a selection of exptl. variations which, when tested with flue-cured and burley tobacco, produced a PhOH yield similar to that obtained when these tobaccos are smoked. The results indicate that the carbohydrates of tobacco are important precursors of PhOH in cigarette smoke, but they alone do not account for the total PhOH yield, probably only ~25%. Pyrolysis of tobacco exts. indicates that the more polar components of the leaf contribute greatly to the total PhOH yield. Cigarettes impregnated with glucose-14C were smoked, and the PhOH which was obtained established that the carbohydrate conversion is greater than that estd. from the pyrolysis of the pure compds, being perhaps as high as 41%. [on SciFinder(R)]

Schlotzhauer, W. S. and O. T. Chortyk (1975). "Comparison of pyrolytic products from flue-cured tobacco leaf and a reconstituted tobacco sheet." Beitr. Tabakforsch. **8**(2): 84-88.

Pyrolytic products of com. tobacco sheet, flue-cured tobacco leaf, and stems were compared. The yields of acids, bases, neutrals, and phenolics were detd. The neutrals were further characterized by chromatog. in order to examine pyrolysis fractions corresponding to biol. active cigarette smoke fractions. Individual phenols, carboxylic acids, polynuclear arom. hydrocarbons (PAH), and nicotine were detd. for sheet and leaf pyrolyzate. Analyses of constituents of various pyrolyzate fractions indicated significantly lower amts. of phenols, PAH, and nicotine in sheet and stem pyrolyzates than in leaf pyrolyzates. [on SciFinder(R)]

Schlotzhauer, W. S. and O. T. Chortyk (1981). "Pyrolytic studies on the origin of phenolic compounds in tobacco smoke." Tob. Int. **183**(2): 25-29.

Fractions of flue-cured tobacco were subjected to a pyrolysis method that duplicates cigarette smoke formation to det. the contributions of leaf constituents to the formations of specific phenolic compds. of tobacco smoke. Individual smoke phenolics were formed from specific leaf components or component groups. Cresols and xylenols could be attributed to the thermal breakdown of cellulose [9004-34-6]. Lignin [9005-53-2] was the principle source of guaiacol [90-05-1] and eugenol [97-53-0] in smoke, in addn. to contributing to overall catechol [120-80-9] content. Catechols and hydroquinones were largely derived from chlorogenic acid [327-97-9] and other leaf polyphenols. Pyrolysis of polyphenolic leaf pigments indicated that the use of low polyphenol tobacco should yield a safer smoking product with decreased catechol in the smoke. [on SciFinder(R)]

Schlotzhauer, W. S., E. B. Higman, et al. (1972). "Pyrolysis of tobacco extracts." Chem. Tob. Tob. Smoke, Proc. Symp.: 65-76.

Flue-cured tobacco was extd. sequentially with solvents of increasing polarity, pyrolyzed, then fractions contg. hydrocarbons, phenols, and N-contg. compds. isolated. The leaf lipids were found to contribute preferentially to the levels of aromatic hydrocarbons. Similar preferences for pyrolytic production of volatile phenols was shown by the brown pigments and leaf carbohydrates. Bases arose mainly from pyrolysis of nicotine. A review of the literature on pyrolysis in smoking and health research is included. 36 refs. [on SciFinder(R)]

Schlotzhauer, W. S. and I. Schmeltz (1968). "Pyrogenesis of aromatic hydrocarbons present in cigaret smoke I. Role of the hexane-soluble fraction of tobacco." Beitr. Tabakforsch. **4**(4): 176-181.

Flue-cured tobacco was extd. with hot n-hexane (I) for 16 hrs. Unextd. tobacco and the I ext. were pyrolyzed in quartz tubes, under N, at 860°. The trapped pyrolyzates were washed with 0.5N NaOH and extd. with Et₂O. Ethereal exts. were concd. and their aromatic hydrocarbon content was detd. by gas chromatog. Benzo[a]pyrene (II) was detd. by thin-layer chromatog. and uv spectrography. I-sol. compds. contributed significantly more to aromatic hydrocarbons of the pyrolyzates than would be predicted from the content of the dry leaf. About 67% of the pyrolyzed II may be attributed to the I-sol. components of the leaf. [on SciFinder(R)]

Schlotzhauer, W. S. and I. Schmeltz (1969). "Pyrogenesis of aromatic hydrocarbons present in cigaret smoke. II. Pyrolytic products of some representative constituents of the hexane-soluble fraction of tobacco." Beitr. Tabakforsch. **5**(1): 5-8.

The hexane-sol. fraction of flue-cured tobacco produced on pyrolysis (860°, N₂) a relative high yield of aromatic hydrocarbons, accounting for ~60% of the benzo(a)-pyrene (I) produced on tobacco pyrolysis. The straight-chain satd. compds. n-hexane, stearic acid, and dotriacontane in this fraction produced pyrolyzates distinguished by relatively low yields of total neutrals and the absence of any significant quantities of aromatic products with alkyl side chains. Compds. contg. double bonds such as linolenic acid and its methyl ester, squalene, and phytol which are characterized by methyl groups on the chain produced relatively higher yields of neutral products, including alkyl-substituted aromatic hydrocarbons. Pyrolysis of the plant sterol β -sitosterol produced the greatest amts. of phenanthrene and I. Estn. of I levels in pyrolyzates indicate that phytol is a relatively effective high-temp. precursor of I. The hexane-sol. compds. of flue-cured tobacco produced I levels intermediate between the least effective (stearic acid) and the most effective (β -sitosterol) precursor examd. [on SciFinder(R)]

Schmeltz, I., L. C. Hickey, et al. (1967). "Phenols from pyrolysis of tobacco leaf acids." Tob. Sci. **164**(15): 28-29.

Malic and citric acids (2 of the main acids present in flue-cured tobacco), fumaric acid (also present in tobacco), cis-aconitic acid, Na citrate, and Na lactate were pyrolyzed at 700° in N₂. The phenols formed were analyzed by gas and thin-layer chromatog. All the substances gave rise to phenols, but the yields were lower than those of other phenol-forming material present in tobacco leaf. [on SciFinder(R)]

Scholtzhauer, W. S., R. F. Severson, et al. (1976). "Pyrolytic formation of polynuclear aromatic hydrocarbons from petroleum ether extractable constituents of flue-cured tobacco leaf." J. Agric. Food Chem. **24**(5): 992-997.

A petroleum ether ext. of flue-cured tobacco leaf was sepd. by silicic acid column chromatog. into 8 major fractions which were then pyrolyzed. Polynuclear arom. hydrocarbons (PAH) were isolated from the pyrolyzates by silicic acid column chromatog. and gel filtration. Major components of fractions that produced the highest amts. of PAH were characterized by various techniques, including gas chromatog., column chromatog., uv spectrometry, and mass spectrometry. Fractions contg. sterols, steryl esters, and terpenes, esp. the C₄₅-isoprenoid alc., solanesol, produced the greatest amts. of PAH per gram pyrolyzed. The paraffin fraction and the fraction contg. the most polar constituents produced relatively low levels of PAH. Because of its relatively high concn. in tobacco leaf, solanesol may contribute more than 30% of the total PAH in the pyrolyzate of the total ext. Thus, solanesol may be a major precursor of cigarette smoke PAH. [on SciFinder(R)]

Shigematsu, H., S. Shibata, et al. (1977). "Thermal degradation products of several Amadori compounds." Agric. Biol. Chem. **41**(12): 2377-2385.

Amadori compds., 1-deoxy-1-L-alanino-D-fructose, 1-deoxy-1-L-valino-D-fructose, 1-deoxy-1-(N-γ-aminobutyric acid)-D-fructose, and 1-deoxy-1-L-prolino-D-fructose were pyrolyzed at 200° for 5 min and 1.5 h, resp. Org. solvent ext. of 5 min pyrolyzate and volatile products obtained by 1.5 h pyrolysis were examd. Alkylpyrazines, pyrrolelactones such as 2-(5-hydroxymethyl-2-formylpyrrol-1-yl)propanoic acid lactone and 2-(5-hydroxymethyl-2-formylpyrrol-1-yl)isovaleric acid lactone, pyrrole-2-aldehydes, 2-acetylpyrroles, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 1-formyl- and 1-acetylpyrrolidines, 1-acetyl- and 1-acetonil-2-pyrrolidones, 2-pyrrolidone, 1-acyl substituted 2,3-dihydropyrrolizines, and 5,6,7,8-tetrahydroindolizin-8-one were identified. Isolation and identification of these compds. were made by gas chromatog. and spectroscopic methods. The aromas of degrdn. products were evaluated by organoleptic test and the degrdn. pathway of pyrrole-2-aldehydes was postulated by comparison of the compn. of 5 min pyrolysis products with those of 1.5 h pyrolysis. Most of the compds. identified as the pyrolysis products are identical with the compds. reported

as constituents of flue-cured tobacco leaf and smoke. [on SciFinder(R)]

Torikai, K., K. Torikau, et al. (2005). "Study on tobacco components involved in the pyrolytic generation of selected smoke constituents." Food Chem Toxicol **43**(4): 559-568.

The aim of this study was to investigate the contribution of various tobacco components to the generation of smoke constituents using a tobacco pyrolysis model. We analyzed the amounts of primary tobacco components (sugars, protein, polyphenols, alkaloids, organic acids, inorganics etc.) in flue-cured and burley tobacco leaves. Each of the components was added to the tobacco leaves at the 0.5-fold and 1.0-fold amount naturally present in the leaves. The treated tobacco samples were pyrolyzed at 800 degrees C in a nitrogen atmosphere with an infrared image furnace, and the selected smoke constituents (benzo[a]pyrene, hydrogen cyanide, carbonyl compounds, aromatic amines, volatile organic compounds and phenolics) were quantitatively analyzed by several methods, including high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC-MS). The contribution of each tobacco component to the generation of selected smoke constituents was estimated from a regression line determined by the three yields (no addition, 0.5-fold addition, and 1.0-fold addition). The results of this study can provide useful and comprehensive information on the relationship between tobacco components and selected smoke constituents during pyrolysis.

Cai, J., B. Liu, et al. (2003). "Fast analysis of nicotine related alkaloids in tobacco and cigarette smoke by megabore capillary gas chromatography." J Chromatogr A **1017**(1-2): 187-193.

A novel fast megabore capillary gas chromatographic (MCGC) method for analysis of 7 nicotine related alkaloids in tobacco and cigarette smoke, including nicotine, nornicotine, myosmine, nicotyrine, anabasine, anatabine and 2,3-dipyridyl, was developed. The use of megabore capillary column GC methodology, equipped with flame ionization detector (FID), provided rapid, unambiguous nicotine related alkaloids analysis. One gram flue-cured tobacco (or Cambridge filter pad), 20 ml ether, and 5 ml 10% sodium hydroxide solution, added with n-heptadecane as the internal standard, were placed in a flask, and the flask was capped and placed in an ultrasonic bath for 15 min. A 1 microl volume was analyzed by capillary GC operating in split-injection mode on a mega bore Simplicity-5 column. This simple procedure was compared with the previously reported packed column GC method and the Griffith still-colorimetric method. The application of the method for analysis of various flue-cured tobaccos and cigarette smoke was discussed.

Flue Cured Tobacco : Smoke Chemistry

Bilimoria, M. H. and M. A. Nisbet (1975). "The reducing property of tobacco smoke. 1. Modified flue-cured tobacco and other smoking products." *Beitr. Tabakforsch.* **8**(4): 186-192.

To det. which tobacco constituents give rise to reducing compds. in smoke as well as to find out what factors affected the reducing property of the latter, modifications of flue-cured tobacco by solvent extn. and chem. impregnations were carried out. While no unequivocal evidence was obtained as to the main source of reducing activity, all the evidence suggests that lignin and phenolic compds. derived therefrom were the main contributors to reducing activity. A no. of cigarette parameters, strand width, expansion of tobacco, paper porosity, and burn control additives were studied and had no significant effect, within the limits tested, on the reducing power of the smoke. On the other hand, additives such as glycerol, ethylene glycol, polyethylene glycol, polypropylene glycol, and carbowax gave distinctly lower reducing activities. Examn. of tobacco products showed lower reducing activities in smoke from Cuban, Latakia, and Turkish tobaccos, as well as in smoke from cigarettes made from Indian sun-cured and French dark, fermented tobaccos. The lowest reducing activity was recorded for the highly fermented Perique tobacco. Indian "bidis" had sp. reducing activities similar to those of std. flue-cured cigarettes. Freshly harvested, rapidly dried green tobacco as well as dried beech leaves, gave sp. reducing activities close to those obtained for regular cigarettes. On the other hand, com. available lettuce cigarettes, which undergo a fermentation step in their manuf., gave distinctly lower sp. activity. [on SciFinder(R)]

Boneva, A. (1979). "Chemical ratios linked to the combustibility of cigarettes." Ann. Tab. Sect. 17: 29-35.

Linear correlation coeff. between cigarette combustibility and ratios between characteristic chem. components of the tobacco were high and similar for the 3 tobacco types (sun- and air-dried or flue-cured) studied. An inverse linear relation between the static burning rate (mm/60 s) and cigarette d. was found. The combustibility index $(K_2O + CaO)/(Cl + MgO)_p$ was the most appropriate for grading cigarettes according to their static burning rate. The other 3 indexes established were K_2O/p , CaO/p , and $(K_2O + CaO)/p$. The main combustibility index combines the inorg. elements with both pos. and neg. effects on the combustibility and the d. (p). The correlation coeffs. between this index and combustibility for the 3 tobacco types were 0.84, 0.79, and 0.81, resp. High values of the correlation coeff. were also obsd. for the K_2O/p (0.83, 0.95, and 0.8), CaO/p (0.7, 0.89, and 0.73), and $(K_2O + CaO)/p$ index (0.82, 0.96, and 0.79, resp.). [on SciFinder(R)]

Chortyk, O. T., R. F. Severson, et al. (1975). "Chromatographic determination of hydrocarbon waxes in tobacco leaf and smoke." Beitr. Tabakforsch. 8(4): 204-210.

Paraffins of tobacco leaf were sepd. by column chromatog. on silicic acid. Leaf paraffins were fractionated from other wax constituents by chromatog. in a definite sample to substrate to solvent ratio. This method was used to evaluate the transfer of paraffins and neophytadiene from leaf to smoke in a ref. cigarette. Gas chromatog. sepns. were performed on a high-temp. liq. phase. Gas chromatog. in conjunction with mass spectrometry was used to det. the paraffin compn. of a representative flue-cured tobacco, a ref. cigarette tobacco, and smoke condensate.

Thus, paraffins were probably transferred to smoke relatively unchanged, whereas neophytadiene underwent some pyrolytic decomposition. [on SciFinder(R)]

Ding Yan, S., J. Yan Xizheng, et al. (2006). "Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from U.S. brand and non-U.S. brand cigarettes." *Environ Sci Technol* 40(4): 1133-1138.

Tobacco smoke contains thousands of chemical compounds, including many carcinogenic polycyclic aromatic hydrocarbons (PAHs). To determine the concentration ranges of PAHs in tobacco smoke and to understand what factors alter their levels, we quantitatively measured 14 PAHs in mainstream smoke from a transnational U.S. brand (Marlboro) and from locally popular brand cigarettes from 14 countries. We used standardized machine smoking conditions (35-mL puff volume, 60-s puff interval, and 2-s puff duration), extraction of total particulate matter from the Cambridge filters, and gas chromatography/mass spectrometry detection. Deliveries of total PAHs in mainstream smoke of local brands were statistically significantly higher ($p < 0.01$) than Marlboros in seven countries. In four countries, Marlboro cigarettes had mainstream smoke total PAH levels that were statistically significantly higher ($p < 0.01$) than local brands. In the remaining three countries, the differences in PAH levels were not statistically significant. Under standard machine smoking conditions, PAH levels were negatively correlated with cigarette filter ventilation levels. We found that several local brands containing primarily flue-cured tobacco filler had relatively high mainstream smoke PAH deliveries, in agreement with findings by previous researchers that flue-cured tobacco typically delivers more PAHs than other tobacco types. We also observed that PAHs were inversely correlated with total carcinogenic tobacco-specific nitrosamines and nitrate content, but these correlations were not statistically significant at the 95% confidence interval. The findings suggest that tobacco blend and nitrate levels may influence PAH deliveries, but other factors may confound this relation.

Fenner, M. L. and J. Braven (1968). "The mechanism of carcinogenesis by tobacco smoke. Further experimental evidence and a prediction from the thiol-defence hypothesis." *Brit. J. Cancer* 22(3): 474-479.

Smoke from cigarettes made from flue- or air-cured tobacco reacted with cysteine (I) to yield varying amounts of I and 2-methyl-L-thiazolidine-4-carboxylic acid plus 4 unknown materials. The MeCHO concn. in smoke from flue-cured tobacco was higher than in that from air-cured tobacco. The higher concn. of plant sugars in flue-cured tobacco may lead to greater amounts of MeCHO in the smoke, thereby inactivating I and losing its protective effect. [on SciFinder(R)]

Griest, W. H. and M. R. Guerin (1977). Influence of tobacco type on smoke composition, Oak Ridge Natl. Lab., Oak Ridge, TN, USA.: 25 pp.

Cigarette smoke quantity and composition were affected by both the physical properties and chemical

nature of the tobacco. Flue-cured tobacco exhibited a greater d. than did Burley, which resulted in a larger no. of std. puffs per cigarette for the former and, thus, increased per cigarette deliveries of most smoke constituents. The greater carbohydrate and polyphenolic content of flue-cured tobaccos contributed to an increased yield of acidic constituents in the smoke. The increased nitrogenous component of Burley tobacco led to a more alk. smoke and one enriched in oxides of nitrogen. A quant. elucidation of the relation between tobacco type and smoke compn. from literature results is complicated by the large no. of variables influencing smoke compn. which are generally unspecified in the reports. Smokes from cigarettes contg. straight Burley and straight Bright tobaccos were compared based on anal. results from this lab. With few exceptions, smoke compn. was often influenced more by processing variables and agronomic practices than by general tobacco "type". [on SciFinder(R)]

Guerin, M. R. and G. Olerich (1976). "Direct gas chromatographic determination of catechol in cigarette smoke." *Tob. Sci.* 20: 19, 20-11.

Catechol (I) [120-80-9] was detd. in the pyridine ext. of the total particulate matter (TPM) collected by CM113A Cambridge filters on a Philip Morris smoking machine as its trimethylsilyl diether by gas chromatog. The anal. had a precision of 2-4% relative std. deviation. A Kentucky Ref. Cigarette delivered 0.19 mg I/cigarette (0.44 wt.% of TPM). Flue-cured tobacco delivered much more I than burley tobacco. Nicotine [54-11-5] and glycerol [56-81-5] can be detd. in TPM simultaneously with I. [on SciFinder(R)]

Hammond, D. and R. J. O'Connor (2008). "Constituents in tobacco and smoke emissions from Canadian cigarettes." *Tob Control* 17 Suppl 1: i24-31.

BACKGROUND: There is relatively little information available about the chemical constituents of tobacco and individual toxic emissions from cigarettes and other tobacco products. OBJECTIVE: To characterise 21 constituents in whole tobacco and 41 constituents in the smoke emissions of Canadian cigarettes, as well as to compare differences between domestic and imported brands. METHODS: All data were released as part of Canada's Tobacco Reporting Regulations. Data are reported for 247 brands tested in 2004. RESULTS: The results indicate significant differences in the constituent levels of domestic and imported cigarette tobacco. Levels of ammonia compounds were significantly higher in imported "US blended" tobacco compared to domestically manufactured brands. Toxic emissions for tobacco-specific nitrosamines were significantly higher for imported cigarettes under both the ISO and Canadian Intense testing methods; however domestic cigarettes had higher levels of other toxic constituents, including benzo[a]pyrene. The findings also highlight the extent to which nicotine, heavy metals and tobacco-specific nitrosamines are "transferred" from the whole tobacco to the smoke. CONCLUSIONS: The findings illustrate important differences between domestically manufactured Virginia flue-cured cigarettes and imported US blended cigarettes. Although the findings suggest that domestic cigarettes had lower levels of constituents such as ammonia, which are associated with increased "additives", Canadian cigarettes were by no means

"additive-free." Overall, these findings provide important benchmarks for making historical and international comparisons across brands on key constituents.

Ishiguro, S. and S. Sugawara (1977). "Studies on compositions of smoke components of lamina and midrib cigarettes. Part III. Comparisons of volatile N-containing compounds in the smoke of lamina and midrib of flue-cured tobacco leaves." *Agric. Biol. Chem.* 41(2): 377-382.

Smoke condensates of lamina and midrib cigarettes of flue-cured tobacco leaves were subjected to glass capillary column gas chromatog. without fractionation. A flame thermionic detector, which is specific for N-contg. org. compds., was used. **By this method, pyridines, pyrroles, and lactams were detd. simultaneously.** Nicotine was a major component, in both smokes. Degrn. products of nicotine predominated in lamina smoke, whereas pyrazines and oxygenated N-compds. predominated in midrib smoke. In this study, several N-contg. compds. were found for the first time in tobacco smoke. [on SciFinder(R)]

Ishiguro, S. and S. Sugawara (1978). "Studies on compositions of smoke components of lamina and midrib cigarettes. Part V. Comparisons of smoke components in the semivolatile phase from lamina and midrib cigarettes of flue-cured tobacco leaves." *Agric. Biol. Chem.* 42(8): 1527-1531.

Dichloromethane exts. of lamina and midrib cigarette smoke condensates were subjected to glass capillary column gas chromatog. Most peaks in the gas chromatogram were identified and the major components were quant. detd. The compositional differences between the 2 types of smoke were then compared. In midrib cigarette smoke the major components appear to be derived from cell wall substances by pyrolysis while lamina cigarette smoke consist of many sugar degn. products and essential oil components which would be directly transferred from tobacco. [on SciFinder(R)]

Ishiguro, S. and S. Sugawara (1978). "Studies on the compositions of smoke components of lamina and midrib cigarettes. Part IV. Comparisons of smoke components from lamina and midrib cigarettes of flue-cured tobacco leaves by the trimethylsilylation method." *Agric. Biol. Chem.* 42(2): 407-410.

Direct gas chromatog. detn. of tobacco smoke was developed. **Tobacco smoke condensate was collected on a glass fiber filter and the components were converted into their trimethylsilyl derivs. and then subjected to glass capillary column gas chromatog. By this method, all tobacco smoke components, including unstable phenolic substances and water-sol. polyhydroxy compds., were simultaneously detd.** Compositional differences between lamina and midrib smoke of flue-cured tobacco leaves were also clarified by the method. The results indicate that there are quant. differences in nicotine, phenols, levoglucosan, quinic acid γ -lactone, and the other major components between lamina and midrib smoke. [on SciFinder(R)]

Neurath, G. B. and M. Duenger (1975). "Measurement of nitrogen oxides in tobacco smoke by means of the chemiluminescence method." IARC Sci. Publ. 9(N-Nitroso Compd. Environ., Proc. Work. Conf., 1973): 177-179.

The contents of nitrogen oxides in tobacco smoke were detd. by the chemiluminescence method. A total of 37 different tobacco products were tested. Normal German blended cigarettes had NO [10102-43-9] contents of 540 ng/ml smoke. The max. value was found in a Spanish filter cigarette with 1520 ng NO/ml in the smoke, and the min. value in a flue cured tobacco filter cigarette with 180 ng NO/ml smoke. [on SciFinder(R)]

Saint-Jalm, Y. and P. Moree-Testa (1980). "Study of nitrogen-containing compounds in cigaret smoke by gas chromatography-mass spectrometry." J. Chromatogr. 198(2): 188-192.

The N-contg. compds. from the smoke tar fractions of nonfilter, flue cured Virginia tobacco and air cured black tobacco cigarettes were isolated and identified by gas-chromatog.-mass spectrometry. In addn. to 44 previously identified compds., 10 compds. not previously reported in tobacco were found. These were 4-ethylpyridine, N,N-dimethylacetamide, N-ethylacetamide, 1,2-dimethylimidazole, 2-methylimidazole, 2-ethylimidazole, 1-methylisoquinoline, 2-aminopyridine, 3-aminopyridine, and 4,4'-bipyridyl. [on SciFinder(R)]

Schmeltz, I., R. L. Stedman, et al. (1964). "Composition studies on tobacco. XX. Bases of cigarette smoke." J. Sci. Food Agric. 15(11): 774-781. cf. CA 61, 12340h.

The bases of cigaret smoke from tobaccos of different types and of different organoleptic properties were investigated. The volatile bases found were pyridine, α -picoline, 2,6-lutidine, β - and (or) γ -picoline, 2,4-and (or) 2,5-lutidine, 2,3-lutidine and (or) 2,4,6-collidine, 3-ethylpyridine, and 3-vinylpyridine. Alkaloids found were nicotine, nornicotine, myosmine, anabasine, and 2,3'-bipyridyl. Flue-cured, Burley, Turkish, and Maryland tobaccos were examd., as well as blends. The presence of Turkish tobaccos in a cigarette blend appeared to enhance the evolution of some of the more volatile bases compared to the individual tobaccos. Nicotine was the major component of the alkaloidal fraction. There appeared to be some relation between smoke flavor and the amts. of volatile bases and nicotine. However, in the case of the volatile bases, the differences among samples were relatively small. The values were compared with those for cigar smoke, and both cigarette and cigar smokes were found to be similar on the basis of/g. tobacco smoked. [on SciFinder(R)]

Sloan, C. H. and G. P. Morie (1974). "Determination of nitrogen oxides in cigarette smoke with a nitrate ion electrode." Tob. Sci. 18: 101-102.

A rapid and accurate means of detg. NO and NO₂ (as NO) in cigarette smoke is described. The gas phase of the smoke (the part of the smoke passing the Cambridge filter) is collected in a partially evacuated gas bottle contg. 1% H₂O₂ (pH 3.5). The bottle is shaken to quant. oxidize the N-oxides to N₂O₅, which hydrolyzes to HNO₃. Nitrate was detd. with an Orlon nitrate ion electrode, the concn. being read directly in ppm on an Orion Model 407 selective-ion meter. Smoke values found were 537 for Burley, 465 for dark tobacco, 274 for nonfilter 85-mm cigarettes, 279 for 85-mm filter cigarettes, and 104µg/cigarette for flue-cured tobacco. [on SciFinder(R)]

Spincer, D. and B. C. Chard (1971). "Determination of formaldehyde in cigarette smoke." Beitr. Tabakforsch. 6(2): 74-78.

A sensitive method for the detn. of formaldehyde in cigarette smoke based on the spectrophotometric detection of the product formed by the reaction of formaldehyde with p-rosaniline and SO₂ is described. The procedure is suited for the anal. of smoke from cigarettes made of flue-cured, burley, and fermented tobacco. Smoke from cigarettes made of flue-cured tobacco contained more formaldehyde than smoke from cigarettes made of burley or fermented tobacco. The formaldehyde retention by conventional paper and cellulose acetate filters was approx. 80% of the nominal total particulate matter retention of these filters, and the presence of C in the filter increased formaldehyde retention. The recovery of formaldehyde was markedly affected by smoking parameters and the use of Cambridge filters. [on SciFinder(R)]

Spincer, D. and D. T. Westcott (1976). "Formation of nitrosodimethylamine in smoke from cigarets manufactured from different tobacco types." IARC Sci. Publ. 14(Environ. N-Nitroso Compd. Anal. Form., Proc. Work. Conf., 1975): 133-139.

Smoke from United Kingdon flue-cured blend, flue-cured blend + 5% KNO₃, air-cured and cigar tobaccos contained 53, 262, 280 and 173 µg N oxides/cigarette, resp.; 15, 15, 25 and 15 µg dimethylamine [124-40-3]/cigarette; 106, 90, 32 and 34 µg HCO₂H [50-00-0]/cigarette; and 4, 30, 62, and 43 ng nitrosodimethylamine [62-75-9]/cigarette, resp., with pH 4.9, 4.0, 5.8 and 6.1, resp. The levels of nitrosodimethylamine in the smokes could not be predicted by detg. the compn. and levels of precursors in the tobaccos or smokes. The data do not support hypotheses that smoke from cigarettes of United Kingdom flue-cured tobaccos should contain more nitrosamine than that of other tobaccos because of the relatively low pH of these tobaccos, and that HCO₂H catalyzes nitrosodimethylamine formation from dimethylamine and nitrite at pH 6-7. [on SciFinder(R)]

Stabbert, R., K.-H. Schafer, et al. (2003). "Analysis of aromatic amines in cigarette smoke." Rapid Commun Mass Spectrom 17(18): 2125-2132.

A method for the analysis of o-toluidine, o-anisidine, 2-naphthylamine, and 4-aminobiphenyl in

cigarette mainstream smoke has been developed, which combines the sensitivity of their pentafluoropropionyl (PFP) derivatives in negative ion chemical ionization (NICI) mode with the selectivity of the gas chromatography/tandem mass spectrometry (GC/MS/MS) technique. The use of four deuterated analogues as internal standards along with the application of the standard addition method results in accurate and precise results; the interday precision for the aromatic amines was 3-10% and the accuracy ranged from 97-100%. This method was applied to two American-blend University of Kentucky reference cigarettes, eight American-blend market cigarettes, a bright (flue-cured) tobacco cigarette, and an electrically heated cigarette smoking system (EHCSS). For the American-blend cigarettes there was a linear correlation between aromatic amine yields and mainstream smoke 'tar' ('tar' = total particulate matter - (nicotine + water)), whereas the bright tobacco cigarette and the EHCSS demonstrated significantly lower aromatic amine yields on an equal 'tar' basis. The results support the hypothesis that the nitrogen content of the tobacco, and above all the cigarette combustion temperature, are determining factors for the yields of aromatic amines in smoke.

Sugawara, S., U. Kobashi, et al. (1972). "The chemical evaluation of tobacco quality. III. Evaluation of tobacco quality from gas chromatographic analysis of the particulate phase of cigarette smoke." *Tob. Sci.* 16: 95-97.

Sixty kinds of flue-cured tobacco were used for the manuf. of unblended cigarets which were smoked both by a panel and a smoking machine. The particulate phase was analyzed by gas chromatog. Several pairs of peaks of the chromatograms were selected and the logs. of the ratios of the areas of individual pairs were correlated to the resp. organoleptic evaluations. The correlation coeffs. obtained were generally higher than those obtained from a comparison of the ratios of the peak areas themselves. Using the log. values, linear equations for the evaluation of the individual organoleptic characteristics were computed; and the results were applied to 20 kinds of flue-cured tobacco, including tip, leaf, and cutter grades for each. The calcd. values were compared with the resp. organoleptic results; and the correlation was significant. [on SciFinder(R)]

Tso, T. C. and G. B. Gori (1972). "Effect of tobacco characteristics on cigarette smoke composition." *Chem. Tob. Tob. Smoke, Proc. Symp.*: 51-63.

Two groups of exptl. cigarets were used to examine the effects of leaf tobacco characteristics on cigaret smoke compn. One group consisted of 32 straight flue-cured cigarets, the other of 23 blends involving many variables. Correlations among leaf and smoke variables and multiple regression of total tar, total particulate matter, and smoke nicotine with leaf variables were measured. The data suggest that total N, K, and cellulose content in flue-cured cigarets are major factors affecting total particulate matter delivery. Waxes, polyphenols, and free histidine are the major factors affecting the total particulate matter delivery in the blend cigarets. [on SciFinder(R)]

Yoshida, D. (1978). "Effect of cultural and curing practices of tobacco on the contents of tar in the smoke." Tob. Res. 4(2): 1-6.

Smoke of cigarettes made from flue-cured tobacco cultivars Bright Yellow and Hicks contained more tar than that from the air-cured Japanese cultivars Matsukawa and Daruma and from Burley 21. The tar contents of smoke from cigarettes made with air-cured Bright Yellow, Hicks and Shiroensyu tobacco leaves, however, did not differ from those of flue-cured leaves of the resp. cultivars. Tar content of smoke increased with advancing leaf maturity. Smoke of cigarettes made from untopped plants contained less tar than that from topped plants. The tar content of smoke of cigarettes made from middle leaves of the plant decreased with increasing rate of N fertilization, but variable results were obtained with the upper leaves of the plant. Tar content of cigarette smoke was significantly pos. correlated with cigarette wt. and the nicotine content of the tobacco as well as with the content of alc.- benzene-, hexane-, and Me₂CO-extractable components in the leaves; tar content was neg. correlated with hot water-extractable residues in the leaves. [on SciFinder(R)]

The influence of tobacco blend composition on carbon monoxide formation in mainstream cigarette smoke. By Djulancic, Nermina; Radojicic, Vesna; Srbinovska, Marija From Arhiv za Higijenu Rada i Toksikologiju (2013), 64(1), 107-113. Language: English, Database: CAPLUS,

The aim of this study was to examine the impact of three main tobacco types (flue-cured FC, air-cured AC and sun-cured SC) and two tobacco-based materials (reconstituted tobacco - recon RT and expanded stem) on the formation of carbon monoxide (CO) in the gas phase of mainstream cigarette smoke. The results showed that the type of tobacco examd. had a significant impact on the amt. of carbon monoxide prodn. in the gas phase of cigarette smoke. AC and SC tobaccos had the most evident impact. The amt. of tobacco in mixts. M₁, M₂ and M₃ as well as the addn. of expanded stems had an impact on the amt. of CO formed in the cigarette smoke. There is weak correlation between CO content in the smoke and the chem. compn. of the tobacco. Draw resistance had an impact on CO prodn. The research results are of great importance, since tobacco selection is the first step in the prodn. of cigarettes with reduced emission of harmful elements contained in the smoke.

Flue Cured Tobacco : In vitro studys

Bilimoria, M. H., M. J. Johnson, et al. (1973). "Inhibition of radical-initiated polymerization of vinyl acetate by tobacco smoke and some polycyclic hydrocarbons." Beitr. Tabakforsch. 7(3): 158-164.

Smoke condensates from different tobacco types can be differentiated by their relative efficiencies in retarding the polymerization of vinyl acetate. Flue-cured tobacco smoke had the highest activity; smoke from reconstituted tobacco had the lowest; air-cured, stem and perique tobacco had intermediate values. Of the polycyclic compds., carcinogenic substances generally showed higher activities than noncarcinogens. Some weak carcinogens such as anthracene were, however, unexpectedly active inhibitors of the polymerization. A polyarom.-hydrocarbon-enriched fraction of smoke was not any more active than a crude condensate. The mechanisms by which tobacco smoke inhibits the polymerization is discussed. [on SciFinder(R)]

Bombick, D. W., K. Putnam, et al. (1998). "Comparative cytotoxicity studies of smoke condensates from different types of cigarettes and tobaccos." Toxicol In Vitro 12(3): 241-249.

The neutral red assay, a rapid and accurate method for estimating the cytotoxicity of chemicals, has been used to assess the cytotoxicity of cigarette smoke condensate (CSC), a complex chemical mixture containing over 3000 identified compounds. The first objective was to optimize the neutral red assay for evaluation of CSCs. This study also assessed and compared the cytotoxicity of smoke condensates from three reference cigarettes which differ in 'tar' content; cigarettes of different tobacco type composition; an ultra-low tar cigarette (R1); and an RJR test cigarette which heats but does not burn tobacco. Finally, this study investigated the cytotoxicity of a specific CSC component, nicotine, and its metabolite, cotinine. Exposure times of 24 hours or longer using CHO cells provided optimal conditions for evaluation of CSC cytotoxicity. The

cytotoxicity of CSCs from reference cigarettes was similar. CSC from cigarettes comprised of flue-cured tobacco exhibited greater cytotoxicity than CSC from cigarettes comprised of burley tobacco. CSC from the R1 cigarette exhibited similar cytotoxicity compared with 1R4F CSC. The CSC from a cigarette that heated but did not burn tobacco (RJR test cigarette) demonstrated no cytotoxicity in CHO cells. Finally, nicotine and cotinine were not cytotoxic to CHO cells. The neutral red assay has been proved useful for quantifying differences in cytotoxicity of smoke condensates from cigarettes which vary in 'tar' yield and for assessing specific smoke constituents.

Bridges, R. B. and L. Hsieh (1986). "Effects of cigarette smoke fractions on the chemotaxis of polymorphonuclear leukocytes." J Leukoc Biol **40**(1): 73-85.

The effects of cigarette smoking fractions on polymorphonuclear leukocyte (PMN) chemotaxis were determined using the ⁵¹Cr-assay. Water-soluble fractions (WSF) of cigarette smoke produced from several tobacco types differed in inhibitory potencies (i.e., flue cured greater than or equal to Maryland greater than or equal to blended greater than Burley greater than or equal to Turkish) corresponding to the respective unsaturated aldehyde content of the smoke from these tobaccos. Fractionation of cigarette smoke condensate (CSC) demonstrated that the more polar fractions were potent inhibitors of chemotaxis whereas those containing nicotine and the polycyclic hydrocarbons were weak inhibitors of chemotaxis. Unlike the inhibitory effects of WSFs, CSC fractions did not inhibit random migration and their inhibition of chemotaxis could not be completely prevented by reduced glutathione. These data suggest that while the alpha, beta-unsaturated aldehydes present in the vapor phase of smoke are among the most potent inhibitors of in vitro PMN chemotaxis, other polar, nonvolatile constituents of cigarette smoke also inhibit chemotaxis and by a mechanism which differs from that of the unsaturated aldehydes.

Clapp, W. L., B. S. Fagg, et al. (1999). "Reduction in Ames Salmonella mutagenicity of mainstream cigarette smoke condensate by tobacco protein removal." Mutat Res **446**(2): 167-174.

The mutagenic activity of cigarette smoke condensates (CSC) made from tobacco before and after removal of protein was assessed by the Ames Salmonella assay in bacterial strains TA98 and TA100. Removal of protein and peptides from flue-cured tobacco via water extraction followed by protease digestion reduced the mutagenicity of the resultant CSC by 80% in the TA98 strain and 50% in the TA100 strain. Similarly, reductions of 81% in TA98 and 54% in TA100 were seen following water extraction and protease digestion of burley tobacco. The significant reductions in Ames mutagenicity following protein removal suggest that protein pyrolysis products are a principal contributor to the genotoxicity of CSC as measured in this assay.

Comber, R. and P. Grasso (1973). "Effects of chemical irritants and tobacco smoke condensate on the chorioallantoic membrane of the fertile hen's egg." Chem.-Biol. Interactions **6**(1): 25-34.

The effects of various materials on the chorioallantoic membrane of the 10-day incubated fertile hen's egg were studied. The materials produced thickening of the membrane in the following decreasing order of potency: croton oil, formaldehyde [50-00-0], flue-cured tobacco smoke condensate, acetic acid [64-19-7], cigar tobacco smoke condensate, phenol [108-95-2], Na chloride [7647-14-5], carrageenan, colloidal silica, and glycerol [56-81-5]. Histol. study indicated that croton oil and tobacco smoke condensate induced local hemorrhage and necrosis, followed by hyperplasia. A test procedure for comparing the irritant activity of tobacco smoke condensates was developed. The procedure involved measuring the max. thickness of the membranes at 72 hr after application of the condensates. [on SciFinder(R)]

Enzell, C. R., E. Bergstedt, et al. (1971). "Tobacco chemistry. 4. Chemical and ciliotoxic studies of smoke from freeze-dried tobacco." Beitr. Tabakforsch. **6**(1): 41-50.

Freeze-dried tobacco had a higher filling capacity than flue-cured tobacco but there was little difference in taste or amts. of particulate matter, dry condensate, and nicotine in smoke derived from equal wts. of the tobaccos. The "phenol" content of the smoke was lower for freeze-dried cigarettes. The ciliotoxic effect of the smoke on rabbit trachea in vitro was similar with both tobaccos. A gas chromatog.-mass spectrometric study showed no significant differences between the gas phases of the smoke derived from each tobacco. [on SciFinder(R)]

Fukano, Y., M. Oishi, et al. (2006). "Analysis of the expression of heme oxygenase-1 gene in human alveolar epithelial cells exposed to cigarette smoke condensate." J. Toxicol. Sci. **31**(2): 99-109.

Airway epithelium is exposed to inhaled exogenous sources. Injury of the alveolar epithelium by cigarette smoking is presumed to be an important process in the pathogenesis of smoking-related pulmonary diseases. Current mechanistic assays that measure the toxicity of cigarette smoke focus on carcinogenesis. However, there is a need to design assays relevant to other disease processes. Oxidative stress is implicated in the pathogenesis of many respiratory diseases including chronic obstructive pulmonary disease. Therefore, we evaluated whether in vitro studies of cigarette smoking are appropriate to examine HO-1 mRNA expression. The human lung epithelial cell line A549 was exposed to the particulate fraction of cigarette smoke (Cigarette Smoke Condensate; CSC) and examd. for the induction of HO-1 mRNA. HO-1 gene expression by CSC is increased dose-dependently. In comparison of the induction of HO-1 mRNA by CSC prepd. from flue-cured or Burley tobacco, CSC from flue-cured tobacco seems to tend to induce an mRNA of HO-1 higher than CSC from Burley tobacco. The adaptation of HO-1 mRNA expression assay as a biol. relevant indicator of cigarette smoke-induced stress may be exemplified in this study whereby CSC derived from cigarette smoke pos. correlated with an increase in HO-1 expression and the difference of the type of tobacco can be detected.

[on SciFinder(R)]

Gairola, C. (1982). "Genetic effects of fresh cigarette smoke in *Saccharomyces cerevisiae*." Mutat Res **102**(2): 123-136.

Ability of fresh cigarette smoke from University of Kentucky reference cigarette 2R1 to induce gene conversion, reverse mutation and mitotic crossing-over in strain D7 of *Saccharomyces cerevisiae* was examined. A closed cell suspension-recycle system using 2 peristaltic pumps interconnected to a single-port reverse-phase smoking machine was developed to provide complete exposure of cells to smoke within 0.2--10 sec of its generation. The exposed cells showed a dose-dependent increase in the frequency of all the 3 genetic endpoints examined. Cell age was an important factor with younger cells being more sensitive than older. Filtration studies showed that the gas phase possessed as much as 25% of the total whole-smoke activity. Activated charcoal reduced the activity of smoke in direct proportion to its amount in the filter. Acetate filter did not appreciably alter the activity. A comparison of whole smoke from various cigarettes showed that: (1) the nicotine content of a cigarette does not affect the genetic activity of smoke; (2) burley and flue-cured tobaccos have differential activity in gene conversion and reverse mutation systems; and (3) the genetic effects of whole smoke are not peculiar to tobacco pyrolysis because similar effects are produced by smokes from lettuce and other non-tobacco cigarettes. It is concluded that the yeast D7 system can be used effectively for the quantitative evaluation of genetic effects of smoke from different cigarettes, and both whole cigarette smoke and its gas phase possess mutagenic as well as recombinogenic activity that can be modified by the use of filters.

Hayes, J. R., D. R. Meckley, et al. (2007). "Effect of a flue-curing process that reduces tobacco specific nitrosamines on the tumor promotion in SENCAR mice by cigarette smoke condensate." Food Chem Toxicol **45**(3): 419-430.

A 30-week dermal tumor promotion study was conducted to evaluate the dermal tumor-promoting potential of cigarette smoke condensate (CSC) collected from cigarettes containing flue-cured tobacco cured by a heat-exchange process (HE) relative to that of cigarettes containing flue-cured tobacco cured by the traditional direct-fire process (DF). Heat-exchange process cured tobacco contains significantly lower concentrations of tobacco specific nitrosamines (TSNAs) compared to traditional direct-fire cured tobacco. Mainstream CSCs were collected by cold trap from smoke generators using the Federal Trade Commission puffing regimen. Groups of 40 female SENCAR mice were initiated by a single application of 75 micro g 7,12-dimethylbenz[a]anthracene (DMBA) to the shaved dorsal skin. CSCs were then applied to the skin three times/week for 29 weeks at 9, 18, or 36mg tar/application. End-points included body weights, clinical observations, organ weights, dermal tumor development and histopathology. The numbers of dermal tumors and the numbers of tumor-bearing mice for each CSC were statistically different from the DMBA/acetone control group and increased with increasing dose. When corresponding doses of each CSC were compared, only the

DMBA/mid-dose HE CSC group was statistically significantly different (lower) from the corresponding DMBA/mid-dose DF CSC group. In this assay, the dermal tumor-promotion potential of CSC from heat-exchange flue-cured tobacco did not differ from that of traditional direct-fire flue-cured tobacco CSC.

Morin, A., N. Poirier, et al. (2010). "Effects of flue-curing on cigarette smoke condensates mutagenicity." Beitr. Tabakforsch. Int. **24**(2): 72-77.

Flue-curing is a post harvest conditioning process which strongly affects the tobacco leaf chem., and consequently the chem. properties of tobacco smoke. Several studies identified the major changes in tobacco chem. occurring during flue-curing. It is not known how flue-curing contributes to changes in bioactivity of cigarette smoke condensate (CSC). In this study, tobacco leaves collected throughout the twelve days of flue-curing were used to prep. cigarettes that were smoked to generate CSC samples. The assessment of mutagenicity was performed using the Bacterial Reverse Mutation/Ames test with *Salmonella typhimurium* TA98 in the presence of S9 metabolic activation. CSC from cured leaves were significantly more mutagenic than CSC from uncured leaves. The no. of revertants was pos. influenced by the duration of the curing. The effect of the duration of curing on the no. of revertants was more pronounced with increasing CSC concn. [on SciFinder(R)]

Richter Patricia, A., P. Li Albert, et al. (2010). "Cytotoxicity of eight cigarette smoke condensates in three test systems: comparisons between assays and condensates." Regul Toxicol Pharmacol **58**(3): 428-436.

Cytotoxic properties of tobacco smoke are associated with chronic tobacco-related diseases. The cytotoxicity of tobacco smoke can be tested with short-term predictive assays. In this study, we compare eight mainstream cigarette smoke condensates (CSCs) from commercial and experimental cigarettes in three different cytotoxicity assays with unique and overlapping endpoints. The CSCs demonstrated cytotoxicity in all assays. In the multiple cytotoxicity endpoint (MCE) assay with TK-6 cells, the cigarette varieties that had the highest EC50s for reduced cell growth also showed a positive dose-response relationship for necrotic cells. In the IdMOC multiple cell-type co-culture (MCTCC) system, all CSCs reduced the viability of the cells. Low concentrations of some CSCs had a stimulatory effect in lung microvascular endothelial cells and small airway epithelial cells. In the neutral dye assay (NDA), except for a 100% flue-cured tobacco CSC, there was little consistency between CSCs producing morphological evidence of moderate or greater toxicity and the CSCs with the lowest EC50s in the MCE or MCTCC assays. Overall, cigarettes made with flue-cured tobacco were the most cytotoxic across the assays. When results were expressed on a per-mg of nicotine basis, lower tar cigarettes were the most cytotoxic in primary human respiratory cells.

Yoshida, D. and T. Matsumoto (1980). "Amino-alpha-carbolines as mutagenic agents in

cigarette smoke condensate." Cancer Lett **10**(2): 141-149.

Two mutagenic agents, 2-amino-9H-pyrido[2,3-b]indole and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (amino- α -carbolines) have been isolated from cigarette smoke condensate for this study. The former agent varied in amounts from a low of 25 ng/cigarette in the smoke of flue-cured tobacco, to a high of 258 ng/cigarette in a cigarette of Japanese domestic variety. The latter ranged in amounts from 9 to 37 ng/cigarette. The contents of these mutagens in the smoke condensate were positively related to an increase in mutagenic activity of *Salmonella typhimurium* TA 98.

Flue Cured Tobacco : Dermal or Inhalation Carcinogenesis

Bartsch, H., N. Caporaso, et al. (1990). "Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers." *J Natl Cancer Inst* **82**(23): 1826-1831.

In 100 healthy volunteers, we have studied the relationship between the type (air- or flue-cured) and number of cigarettes smoked and different biomarkers relevant to the risk of bladder cancer, including the levels of 4-aminobiphenyl (ABP) hemoglobin adduct (a marker of internal dose), urinary mutagenicity in *Salmonella typhimurium* TA98, and the N-acetylation phenotype (a marker of susceptibility). ABP is a potent bladder carcinogen that is N-acetylated as an overall detoxification step. Levels of the ABP hemoglobin adduct were higher in smokers of black tobacco (air-cured) than in smokers of blond tobacco (flue-cured), confirming our earlier study. In addition, "slow" acetylators had higher levels of the ABP hemoglobin adduct for the same type and quantity of cigarettes smoked. Urinary mutagenicity was also associated with quantity of cigarettes but not with the acetylation phenotype. Convex dose-response relationships were found between the amount smoked and ABP hemoglobin adduct levels or urinary mutagenicity. In 15 nonsmokers who reported exposure to environmental tobacco smoke, ABP hemoglobin adduct levels, unlike urinary mutagenicity, were found to be an aspecific exposure indicator.

Bartsch, H., C. Malaveille, et al. (1993). "Black (air-cured) and blond (flue-cured) tobacco cancer risk. IV: Molecular dosimetry studies implicate aromatic amines as bladder carcinogens." *Eur J Cancer* **29A**(8): 1199-1207.

Tobacco smoking causes a major fraction of male urinary bladder cancers and the relative risk of bladder cancer is reported to be two to three times higher for smoking of black (air-cured) than for smoking of blond (flue-cured) tobacco. In molecular dosimetry studies to examine the hypothesis that aromatic amines in tobacco smoke are primarily responsible for bladder cancer, the higher bladder cancer risk in smokers of black tobacco was correlated with two to five times higher exposure to carcinogenic aromatic amines present in black tobacco smoke, notably 4-aminobiphenyl (ABP). For the same amount of smoking, black tobacco smokers had levels of ABP-haemoglobin (Hb) adducts 1.5 times higher and excreted a 1.8-fold higher level of urinary

mutagens. These mutagens were characterised as aromatic amines, and included the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a known mutagen and multiorgan/species carcinogen. In smoking volunteers, the ABP-Hb adduct level depended significantly on the acetylator and P-450IA2 phenotypes, being 1.3- to 1.5-fold lower in fast acetylators, slow/intermediate P-450IA2 individuals. The N-(deoxyguanosine-8-yl)-ABP adduct was a major smoking-related DNA adduct in bladder biopsies from surgical patients. It was also tentatively identified in exfoliated urothelial cells of smoking volunteers, who showed a significant and linear correlation between adduct levels of ABP with Hb and with deoxyguanosine in urothelial DNA; both were related to number of cigarettes smoked per day. Levels of several smoking-related DNA adducts in urothelial cells were 2-20 times elevated in smokers. Similar convex dose-response relationships have been found between the number of cigarettes smoked and the relative risk for bladder cancer and between the levels of ABP-Hb adducts and markers of recent smoking. A possible explanation is that fast and slow acetylators have different susceptibility to aromatic amine carcinogens. Case-control studies have consistently revealed an excess of variable magnitude of slow acetylators in subgroups exposed occupationally to carcinogenic aromatic amines. Altogether, results from these studies reinforce the association between cigarette smoking, carcinogen-DNA adducts in urothelial cells, and implicate primary aromatic and possibly heterocyclic amines as bladder carcinogens.

Benhamou, E. and S. Benhamou (1993). "Black (air-cured) and blond (flue-cured) tobacco and cancer risk. VI: Lung cancer." *Eur J Cancer* 29A(12): 1778-1780.

Boffetta, P. (1993). "Black (air-cured) and blond (flue-cured) tobacco and cancer risk. V: Oral cavity cancer." *Eur J Cancer* 29A(9): 1331-1335.

Boffetta, P. (2008). "Tobacco smoking and risk of bladder cancer." *Scand J Urol Nephrol Suppl*(218): 45-54.

Tobacco smoking is the main known cause of urinary bladder cancer in humans. In most populations, over half of cases in men and a sizeable proportion in women are attributable to this habit. Epidemiological studies conducted in different populations have shown a linear relationship between intensity and duration of smoking and risk. Quitting smoking reduces the risk of bladder cancer. Smoking black (air-cured) cigarettes results in a higher risk than smoking blond (flue-cured) tobacco cigarettes; results on inhalation patterns and use of filter are not consistent. Cigar and pipe smoking also increases the risk of bladder cancer; data on other tobacco products are limited. The evidence for non-transitional bladder carcinoma is limited, but consistent with an increased risk. The available evidence does not point towards a different carcinogenic effect of tobacco smoking in men and women or in whites and blacks. Data on involuntary smoke and use of smokeless tobacco products are limited, but do not suggest an increased risk of bladder cancer.

Davies, R. F. and J. K. Whitehead (1970). "A study of the effects of altering the tar-nicotine ratio in experimental tobacco carcinogenesis." *Br J Cancer* 24(1): 191-194.

There was no statistically significant difference in specific mouse skin carcinogenicity between smoke condensate from plain, flue-cured tobacco cigarettes with a normal tar to nicotine ratio and condensate from filter-tip cigarettes made from selected flue-cured tobaccos with a reduced tar to nicotine ratio.

De Stefani, E., E. Barrios, et al. (1993). "Black (air-cured) and blond (flue-cured) tobacco and cancer risk. III: Oesophageal cancer." *Eur J Cancer* 29A(5): 763-766.

Relative risks of oesophageal cancer for smoking were higher in communities smoking mainly black tobacco, when compared with results from populations comprising mainly users of blond tobacco. Also, hand-rolled cigarette smoking, which could be considered as a proxy indicator of black tobacco smoking, was also associated with higher risk of oesophageal cancer, in comparison with the use of commercial (manufactured) cigarettes. Finally, the use of pipes and cigars showed odds ratios of higher magnitude than those associated with cigarettes. This indirect evidence of a higher risk of oesophageal cancer due to the use of black products was confirmed in three recent hospital-based case-control studies. These investigations were able to compare the effect of both types of tobacco; relative risks for black tobacco were two to three times higher than risks associated with blond tobacco smoking, after controlling for major potential confounders. Laboratory evidence suggests that swallowing tobacco condensates could be a major risk factor for oesophageal cancer. Also, the higher content of tobacco-specific N-nitroso compounds in black tobacco, including organospecific substances, could explain its higher carcinogenic effect on the oesophageal mucosa.

Duuren, B. L. V., A. Sivak, et al. (1966). "The tumor-promoting agents of tobacco leaf and tobacco smoke condensate." *J. Natl. Cancer Inst. (1940-1978)* 37(4): 519-526.

Exts. from flue-cured cigaret tobacco leaf were taken successively with Et₂O, CHCl₃, and MeOH. These exts., a reconstituted whole ext., and known tobacco phenols were tested for tumor-promoting activity on mouse skin after an initiating treatment with 7,12-dimethylbenz[*a*]anthracene. The Et₂O ext., the MeOH ext., and the reconstituted whole ext. of unburned tobacco leaf showed notable tumor-promoting activity. In parallel expts., whole cigaret smoke condensate showed promoting activity but no tumorigenicity without initiation; eugenol, a known tobacco leaf phenol, showed weak promoting activity. Several tobacco tar aromatic hydrocarbons were tested for initiating activity with croton resin used as the promoter. Benzo[*a*]pyrene, benzo[*b*]fluoranthene, and chrysene were potent initiators, whereas benzo[*m,n,o*]fluoranthene and 1-methylpyrene were inactive. [on SciFinder(R)]

Elson, L. A., T. E. Betts, et al. (1972). "Sugar content and the pH of the smoke of cigarette, cigar, and pipe tobaccos in relation to lung cancer." *Int. J. Cancer* 9(3): 666-675.

Flue-cured tobacco used in cigarets had a high sugar content, resulting in an acid smoke which became increasingly acidic during the course of smoking; air-cured tobacco used in cigars had a low sugar content, producing a smoke which became more alk. during smoking. The use of either type of tobacco in a pipe gave smoke which became progressively more alk. The relatively lower incidence of lung cancer in cigar and pipe smokers may be related to the fact that nicotine is more readily absorbed as the free base (alk. pH) than as the salt (acid pH). Therefore, to obtain the same degree of "nicotine satisfaction" the smoker of cigarets would smoke more and experience greater exposure to the carcinogenic effects of tobacco smoke than would be the case with cigar or pipe smokers. [on SciFinder(R)]

Lee, P. N., B. A. Forey, et al. (2009). "Does use of flue-cured rather than blended cigarettes affect international variation in mortality from lung cancer and COPD?" *Inhalation Toxicol.* 21(5): 404-430.

A review. We compared risk of lung cancer and chronic obstructive pulmonary disease (COPD) assocd. with flue-cured and blended cigarettes. Mortality and smoking data were collected for 1971-2000 by sex, age, and period for three countries with a mainly flue-cured market and four with a blended market. Epidemiol. relative risk ests. for current and ex smoking were summarized. Smoking statistics and mortality were compared between flue-cured cigarette and blended cigarette countries. Unadjusted mortality rates were generally lower in blended cigarette countries early on, with the difference diminishing or reversing by the 1990s. Differences by cigarette type were rarely significant, due to variations, particularly for COPD, between countries within cigarette type. Current smoking prevalence was generally lower in blended cigarette countries in 1971-1975, with the difference reducing over time. Differences by type were never significant, with blended cigarette countries varying markedly. Ex-smoking increased over time and was lower for blended cigarette countries, generally not significantly. Consumption per smoker was somewhat lower for blended cigarette countries. Relative risk ests. for smoking, derived mainly from U.S. and UK studies, varied little by cigarette type. Conclusions based on estd. smoking-related excess mortality were similar to those based on unadjusted mortality rates. There was little indication of any difference between flue-cured and blended cigarettes on risk of lung cancer or COPD. Our approach could have detected differences of about 40% for male lung cancer, or twofold differences for females or for COPD, had they existed. Between-country differences in rates of two major diseases predominantly caused by smoking cannot materially be explained by whether the countries use flue-cured or blended cigarettes. [on SciFinder(R)]

Roe, F. J. C., J. C. Clark, et al. (1970). "Comparative carcinogenicity for mouse-skin of smoke condensates prepared from cigarettes made from the same tobacco cured by two processes." *Brit. J. Cancer* 24(1): 107-121.

Applications of either flue-cured and redried (FC) or air-cured and bulk fermented (AC) tobacco condensates (60 mg 3 times a week until death) to the clipped dorsal skin of mice proved to be carcinogenic as compared to controls. Chem. anal. of the 2 tobaccos and 2 condensates revealed only small differences in compns.; it was noteworthy that the concn. of reducing sugars was almost as high in the AC tobacco as in the FC tobacco. The risk of development of skin tumors, particularly malignant skin tumors, was higher in FC-treated mice than in AC-treated mice, but the difference may have been due to the use of equal wts. of condensates rather than the use of exts. from equal nos. of cigarettes, since the AC cigarette produced more condensate. The rates of detection of pulmonary tumors also varied between groups but, this does not necessarily imply that the incidence rate of pulmonary tumors varied. There was no evidence that the detection or incidence rates of any other neoplasm, including malignant lymphoma, were affected by treatment with either of the condensates. [on SciFinder(R)]

Sancho-Garnier, H. and S. Theobald (1993). "Black (air-cured) and blond (flue-cured) tobacco and cancer risk II: Pharynx and larynx cancer." *Eur J Cancer* 29A(2): 273-276.

Two case-control studies have examined the relationship between black or blond tobacco smoking and the occurrence of pharynx or larynx cancer. The first study was carried out in several European countries. Tobacco smoking was found to be associated with higher risks for supraglottic and epilarynx cancer localisations than for pharynx, glottic and subglottic localisation. In all localisations, risk was twice as high again in users of black tobacco after adjusting for alcohol and for lifetime average daily dose of tobacco. The other study was carried out in Uruguay. After taking into account age, age at start of smoking, duration of smoking, years since stopping smoking and filter use, risks were found to be higher in black tobacco smokers than in blond tobacco smokers. All known studies which have been performed in countries where blond tobacco is generally smoked showed lower risks even when adjusted for alcohol. Use of black tobacco appears to be associated with higher risks of cancer of the pharynx and larynx than blond tobacco use.

Vineis, P. (1991). "Black (air-cured) and blond (flue-cured) tobacco and cancer risk. I: Bladder cancer." *Eur J Cancer* 27(11): 1491-1493.

Four case-control studies in different Latin countries have reported risks of bladder cancer 2-3 times higher for smokers of black (air-cured) than for smokers of blond (flue-cured) tobacco. This observation is interesting in the light of a higher concentration of arylamines in black tobacco. The relative risk dropped very rapidly after discontinuation of smoking, and there was also an effect of age at start, with higher risks associated with earlier onset of the habit. Overall, black tobacco seems to act both on early and late stages of bladder carcinogenesis.

Vineis, P., N. Caporaso, et al. (1990). "Acetylation phenotype, carcinogen-hemoglobin adducts,

and cigarette smoking." *Cancer Res* 50(10): 3002-3004.

Levels of 4-aminobiphenyl-hemoglobin adducts in smokers of blonde (flue-cured) and black (air-cured) tobacco have been found to be proportional to bladder cancer risk. In addition, risk of bladder cancer due to exposure to occupational carcinogens is elevated in genetically determined slow acetylators. In this study of normal male volunteers, 4-aminobiphenyl-hemoglobin adducts were found to be related to both the quantity and the type of tobacco smoked, as well as to the acetylator phenotype (independently of smoking habits). The demonstration that both the genetically determined slow acetylator phenotype and tobacco smoking are independently associated with levels of the carcinogen 4-aminobiphenyl in adducted hemoglobin suggests a single mechanism to explain the contribution of genetic susceptibility and environmental exposure in bladder carcinogenesis.

Wynder, E. L. and D. Hoffmann (1965). "Reduction of tumorigenicity of cigaret smoke. An experimental approach." *JAMA, J. Am. Med. Assoc.* 192(2): 88-94.

Methods for the redn. of tumorigenicity of cigarette smoke condensates were presented. The tar yield can effectively be lowered by using more finely cut tobacco, particularly stems and reconstituted tobacco. Smoke condensates from cigarettes made with flue- or sun-cured tobaccos contain more benzopyrene and phenols and are more highly tumorigenic than cigarettes of air-cured tobaccos. The incorporation of $\text{Cu}(\text{NO}_3)_2$ and $\text{Ni}(\text{AcO})_2$ caused modified combustion and reduced benzopyrene and phenol yields. The more coarsely tobacco was cut the greater was its toxicity. Ciliotoxic muscle-affecting components present in the particulate and gaseous phases were phenols, formic and acetic acids, and aldehydes, particularly acrolein. Cellulose acetate filters removed up to 90% of the phenols and some acidic components. Compressed charcoal filters decreased aldehydes. A moist cellulose acetate filter was more effective than a dry one. A combination of filters resulted in less toxic smoke. [on SciFinder(R)]

Gaworski *et al.*, (1999). Toxicologic evaluation of flavour ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. *Toxicology* 139:1-17

Four comparative two-stage SENCAR mouse skin painting bioassays were conducted with cigarette smoke condensate (CSC) preparations to evaluate the effect of common American cigarette flavoring ingredients on tumor promotion. Each independent study employed a unique flavoring combination applied to tobacco at exaggerated levels, and in total resulted in an evaluation of 150 ingredients. Groups of 30-50 female SENCAR mice each were initiated topically with 50 microg of 7,12-dimethylbenz(a)anthracene (DMBA), and promoted thrice weekly for 26 weeks with either 10 or 20 mg of CSC from test cigarettes containing ingredient mixtures. For comparison, separate groups of mice received concurrent treatment with CSC from reference cigarettes prepared without added ingredients. Negative and positive controls

were treated with acetone or 12-0-tetradecanoyl-phorbol-13-acetate (TPA) as a promoter, respectively. CSC-only groups served as promotion controls. Tumors developed in > 80% of the TPA-treated mice by study week 11, with a < 3% background tumor formation in the acetone treated controls at termination. Tumor incidence in CSC-only promotion control groups was < 20%, with no apparent difference between reference and test CSC groups. Approximately 70% of the DMBA-initiated mice promoted with 20 mg CSC developed tumors. Tumors first appeared around week 9, with about five tumors/tumor bearing animal. Tumor incidence, latency and multiplicity were CSC dose related, with a lower tumor incidence (approximately 50%), longer latency (12 weeks), and reduced tumor burden (four tumors/tumor bearing animal) at the 10 mg CSC dose level. While tumor incidence, latency and multiplicity data occasionally differed between test and comparative reference CSC groups, all effects appeared to be within normal variation for the model system. Furthermore, none of the changes appeared to be substantial enough to conclude that the tumor promotion capacity of CSC obtained from cigarettes containing tobacco with ingredients was discernibly different from the CSC obtained from reference cigarettes containing tobacco processed without ingredients.

Flue Cured Tobacco : Addictive properties

Sanders, E., R. Weitkunat, et al. (2012). "Does the use of ingredients added to tobacco increase cigarette addictiveness?: a detailed analysis." *Inhal Toxicol* 24(4): 227-245.

The possibility that ingredients added to tobacco contribute to the addictiveness of cigarette smoking was evaluated by comparing cessation rates of smokers of traditional blended cigarettes to those of smokers of flue-cured cigarettes. Such a comparison is a valid means of assessing cigarette ingredients as traditional blended cigarettes contain ingredients (>20), whereas flue-cured cigarettes contain no or very few ingredients. Separate analysis of 108 treatment groups and 108 control groups from randomized clinical trials of nicotine replacement therapy (NRT) were performed by multiple logistic regressions. The results of these analyses demonstrated slightly higher quit rates for smokers of blended cigarettes (OR = 1.90, 95% CI 1.70-2.13 and OR = 1.32, 95% CI 1.14-1.53 for treatment and control groups, respectively). The control groups were also investigated using classification tree analysis from which no difference in quit rates were observed for smokers of either type of cigarette. Further analyses showed that studies that utilized a high level of psychological support in conjunction with NRT produced at least a two-fold increase in quit rates compared to studies that utilized a low level of psychological support. It was also demonstrated that there is a large difference when results were reported by sustained abstinence compared to point prevalence. Additional meta-analyses found the pooled OR for NRT treatment to be in exact agreement with a recent review that assessed the effectiveness of NRT. Overall these results strongly suggest that ingredients used in the manufacture of traditional blended cigarettes do not increase the inherent addictiveness