

## Air cured Tobacco

### Air cured Tobacco – Chemistry & Toxicity of Unburnt Materials

Golia, E. E.; Dimirkou, A.; Mitsios, I. K. (2009) "Heavy-metal concentration in tobacco leaves in relation to their available soil fractions" Communications in Soil Science and Plant Analysis, 40(1-6), 106-120

Tobacco (*Nicotiana tabacum*) is one of the most important agricultural products in Greece. The accumulation of heavy metals in tobacco leaves in accordance with a possible risk of transferring them to people by smoke is well known. To monitoring heavy-metal contents in soils, as well as in tobacco leaves, a 6-yr survey was conducted in the Thessaly region (central Greece). The concn. of zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), iron (Fe), and manganese (Mn) was measured both in soils and in air-cured (Burley), flue-cured (Virginia), and sun-cured (Oriental-filling) tobacco leaves in lower and upper leaves (primings). Regression anal. was conducted between soil and tobacco heavy-metal content to examine the possibility of predicting tobacco heavy-metal levels based on the results of soil anal. DTPA-extractable levels. The results showed a high correlation between Oriental tobacco heavy-metal content and DTPA-extd. heavy-metal level in soils. Although regressions based on the pooled data from the three tobacco types adequately predicted tobacco heavy-metal levels, better fits were obtained when regression models were used for Oriental tobacco sep. Statistically significant neg. correlations were recorded between heavy-metal concns. in Oriental tobacco leaves and soil pH, in all primings. Therefore, low soil pH value leads to an increased availability of heavy metals in tobacco leaves.

Staaf, M.; Back, S.; Wiernik, A.; Wahlberg, I.; Long, R. C.; Young, J. H. (2005) "Formation of tobacco-specific nitrosamines (TSNA) during air-curing: conditions and control". Beitraege zur Tabakforschung International, 21(6), 321-330.

The present review deals with studies performed during several consecutive years on the effect of air-curing on tobacco-specific nitrosamine (TSNA) formation and quality of tobacco. Temp., relative humidity, water content and water activity data were collected during curing of dark tobacco in traditional air-curing barns and bulk-curing barns of different sizes, and chem. anal. of the cured tobacco were performed. The results from the screening study of the traditional curing conditions support the idea that a high continuous relative humidity during the entire curing period results in high TSNA levels in the tobacco, while a drier climate produces tobacco with lower amt. of TSNA and nitrite. The study also demonstrates that a high continuous relative humidity at the beginning of the yellowing phase followed by a pronounced decline of relative humidity at the crit. period for TSNA formation, i.e., when the plant cell membrane breaks down due to moisture loss and the cell contents become available to nitrite-forming microorganisms, results in tobacco with relatively low TSNA levels. Control of air flow is important during the curing process and a uniform air flow will increase the rate and amt. of moisture loss from the tobacco, and also reduce possible gas-phase reactions between alkaloids and gaseous nitric oxides, derived from microbially generated nitrite, during air-curing of dark tobacco. Curing expts., in minibarns and bulk barns with controlled conditions, aiming to mimic a redn. in humidity at the end of yellowing confirm that a decline in humidity at the crit. period reduces the accumulation of TSNA in the cured tobacco, while still producing a satisfactory tobacco quality in a shorter time than traditional air-curing.

Wisniowska-Kielian, Barbara; Kielian, Artur (2003) "Comparison of trace element contents in leaves of different tobacco types". Chemia i Inzynieria Ekologiczna, 10(9), 1031-1038.

Microelement and heavy metal contents were compared in leaves of Polish flue-cured

tobacco (Virginia and Burley cvs.) and air-cured tobacco (Mocny Skroniowski and Kentucky cvs.). These two types of tobacco differ in their soil requirements and nutritional needs. Both types require intensive phosphorus and potassium treatment, so far applied as single component fertilizers, but recently recommended as multi-component ones. These fertilizers bring to the soil as ballast easily available forms of metallic trace elements, which may be absorbed in excessive quantity and accumulate in tobacco leaves. A considerable diversification in tobacco contents of trace elements (Zn, Cu, Cd, Pb, Ni and Cr) was obsd. depending on the usable type, cultivars variety, place of cultivation, date of harvest and quality of tobacco raw material.

Cui, Mingwu; Burton, Harold R.; Bush, Lowell P.; Sutton, Tommy G.; Crafts-Brandner, Steve J. (1994) "Effect of Maleic Hydrazide Application on Accumulation of Tobacco-Specific Nitrosamines in Air-cured Burley Tobacco". Journal of Agricultural and Food Chemistry, 42(12), 2912-16

A two year study was initiated to det. the effect of maleic hydrazide application rates (0.5x, 1.0x, 1.5x, and 2.0x the recommended rate) and methods (single, split, and reduced vol. application) on accumulation of tobacco-specific nitrosamines (TSNA) and the correlation between TSNA and their precursors in burley tobacco. With increased MH application rates, alkaloids and nitrate levels decreased, whereas nitrite level was not affected. TSNA were significantly lower (30-50%) in the lamina from the top stalk position than the hand-suckered control. N'-Nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT) were lowered by 15-51%, 36-65%, 38-61%, and 30-58%, resp., at the top stalk position. TSNA content was significantly correlated with the content of alkaloid in the lamina. These results suggest that MH application may alter precursors-TSNA relationship which results in decreases of TSNA in air-cured burley tobacco

Burton, Harold R.; Dye, Naewanna K.; Bush, Lowell P. (1994) "Relationship between Tobacco-Specific Nitrosamines and Nitrite from Different Air-cured Tobacco Varieties" Journal of Agricultural and Food Chemistry, 42(9), 2007-11

Three tobacco varieties of differing agronomic characteristics were grown and air-cured during the 1988-1990 growing seasons. The leaves were sepd. into lamina and midvein from three stalk positions. The replicated samples were analyzed for individual alkaloids, nitrate, nitrite, N'-nitrosonornicotine, N'-nitrosoanatabine, N'-nitrosoanabasine, and 4-[(methylnitroso)amino]-1-(3'-pyridyl)-1-butanone. The air-cured flue-type tobacco contained consistently lower quantities of the individual tobacco-specific nitrosamines (TSNA). There were no statistical differences in TSNA and stalk position or TSNA and curing season. Correlation coeffs. were detd. between nitrite and individual TSNA. Their coeffs. showed there was a pos. relationship between nitrite levels and individual TSNA concns. in the tobacco tissue. From this 3-yr study there was no relationship between alkaloids or nitrate and individual TSNA. Thus, accumulation of TSNA in tobacco is limited by the amt. of nitrite accumulated during air-curing.

Chamberlain, W. J.; Chortyk, O. T. (1992) "Effects of curing and fertilization on nitrosamine formation in Bright and Burley tobacco". Beitraege zur Tabakforschung International, 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'-nitrosonornicotine (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.

Bell, Paul F.; Mulchi, C. L.; Chaney, R. L. (1992) " Microelement concentrations in Maryland air-cured tobacco" Communications in Soil Science and Plant Analysis, 23(13-14), 1617-28.

Samples of cured tobacco collected on farms over the period 1980 to 1983 were analyzed for their Zn, Mn, Pb, Ni, Cd and Cu contents. Mean concns. for individual metals in mg/kg were: Zn (43.6), Cu(7.6), Mn(192), Pb(1.92), Ni(1.20), and Cd(2.26). Approx. 5% of the samples contained Zn, Cu, Ni, Pb, or Cd concns. higher than expected for these elements in Maryland tobacco. Some 25% of the samples contained Mn levels within the range 332-2400 mg/kg which were likely caused by low soil pH conditions. A total of 5% of the cured samples were borderline deficient in Zn ( $\leq 18$  mg/kg), and 27% were considered deficient in Cu ( $\leq 5.2$  mg/kg). Problems assocd. with inadequate liming programs and the use of croplands for municipal waste disposal were discussed.

Andersen, Roger A.; Fleming, Pierce D.; Burton, Harold R.; Hamilton-Kemp, Thomas R.; Sutton, Tommy G. (1989). " N'-Acyl and N'-nitroso pyridine alkaloids in alkaloid lines of burley tobacco during growth and air-curing". Journal of Agricultural and Food Chemistry, 37(1), 44-50

N'-Acyl and N'-nitroso pyridine alkaloids were quantified by gas chromatog. (GC) in burley tobacco genotypes varying in accumulation of pyridine alkaloids. Leaves were sampled during field growth and air-curing. N'-substituted alkaloid identities were confirmed by GC and GC-mass spectroscopy; N'-acetylanatabine was newly identified. Alkaloid derivs. in lamina were in the following order of decreasing content averaged over the sampling dates: acylated compds. formylhornicotine, n-octanoylnornicotine, n-hexanoylnornicotine, formylanatabine, acetylnornicotine, n-butanoylnornicotine, and acetylanatabine; nitrosamines nitrosoanatabine, nitrosonornicotine, and 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone. Generally higher levels of N'-substituted pyridine alkaloids were found in lamina of the KY 78379 line (high nornicotine and total alkaloids) and B21 cultivar (high nicotine and total alkaloids) compared to LAB21 (low total alkaloids). N'-Acyl alkaloids occurred in green as well as cured leaves; N'-nitroso alkaloids were mainly present during curing. Thus, acylated alkaloids, unlike nitroso alkaloids, are apparent products of plant metab. during late growth stages.

By Rao, B. V. Kameswara; Murthy, P. S. N.; Chakraborty, M. K. (1986) " Chemical studies on Lanka tobacco and its smoke: 2. Alkaloids". Tobacco Research, 12(2), 196-201

Lanka tobacco, an indigenous air-cured type of tobacco, was thoroughly examd. for its alkaloid compn. The primary alkaloids identified by GC-MS are nicotine, nornicotine, anabasine, and anatabine, the remaining 8 being other pyridine derivs. Fermn. of Lanka tobacco resulted in a loss of 36.9% in total alkaloids.

Andersen, Roger A.; Kemp, Thomas R. (1985) " Accumulation of 4-(N-methyl-n-nitrosamino)-1-(3-pyridyl)-1-butanone in alkaloid genotypes of burley tobacco during postharvest processing: comparisons with N'-nitrosonornicotine and probable nitrosamine precursors" Cancer Research, 45(11, Pt. 1), 5287-93

The concn. relationships of 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)(I)

[64091-91-4], N'-nitrosonornicotine (NNN) [16543-55-8], and their probable precursors, i.e., nitrite, nitrate, and alkaloids, were detd.: (a) after the growth of Ky 14 burley tobacco under different shade conditions followed by air curing; and (b) during prepn. of air-cured and homogenized-leaf-cured (HLC) burley tobaccos from conventionally grown tobaccos of different alkaloid genotypes. NNK contents ranged from 0.2 to 0.5 µg/g in air-cured Ky 14 tobacco lamina from leaves grown under 0-65% shade (100, 65, and 35% of natural daylight). The highest NNK concns. were from 45% shade-grown lamina from lower leaf positions on stalks. Concns. of NNK did not correlate significantly with those of either nitrate or total alkaloids calcd. over all shade treatments and stalk positions. During HLC tobacco processing, the following significant correlations of NNK with precursor content changes were found for each of 4 burley alkaloid genotypes calcd. over the 4 successive stages of processing: NNN ( $r = -0.9$ ). NNK also correlated neg. with nicotine [54-11-5] concn. changes ( $r = -0.9$ ) in the low-alkaloid and high-alkaloid isolate [30000-36-3] during processing. After a 20-h incubation period under aerobic conditions followed by a 1-h standing period without aeration, substantial increases of NNK were obsd. in each burley line. The increased NNK contents ranged from 9-fold for the low-alkaloid isolate to 99-fold for the nornicotine [494-97-3]-converter line. Increases in NNK content (27-69%) also occurred during the air drying stage; further increases occurred during a 15-mo storage period at ambient conditions. After the HLC process and prolonged storage, maximal NNK contents were obsd. in each tobacco genotype in the following order of increasing NNK content: Ky 14 cultivar, 79 µg/g; low-alkaloid line, 80 µg/g; nornicotine converter line, 102 µg/g; and high-alkaloid line, 177 µg/g. At the beginning of a controlled environmental storage period used for high-alkaloid and low-alkaloid isolate air-cured and HLC tobaccos, NNK contents correlated with nitrite ( $r = 1.0$ ) and nitrate ( $r = -0.9$ ) calcd. over the 2 curing regimens. Increased NNK contents were obsd. in HLC high-alkaloid line tobacco but not in the air-cured high-alkaloid line after 52 wk of storage at 20° and 12% moisture. NNK accumulation varied among tobacco alkaloid genotypes and occurred mainly during the following HLC processing step: standing period following incubation; forced-air drying and prolonged storage (aging) for HLC tobaccos. It seems probable that low levels of NNK and NNN in burley tobacco could be achieved by growth of a low-alkaloid genotype and use of postharvest treatment procedures that minimize nitrite formation and nitrate and alkaloid conversion (disappearance).

Matsushima, Sanji; Ohsumi, Tomoko; Sugawara, Shiro (1983) "Composition of trace alkaloids in tobacco leaf lamina" Agricultural and Biological Chemistry, 47(3), 507-10

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 nornicotine derivs., i.e. N'-hexanoyl-, N'-octanoyl-, 1'-(6-hydroxyoctanoyl)-, and 1'-(7-hydroxyoctanoyl)nornicotine, 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'-formylnornicotine, 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'-octanoylnornicotine in Burley-21, and cotinine and N'-formylanatabine in Basma.

Hamilton, J. L.; Bush, Lowell; Lowe, R. H. (1982) "Nitrate concentration changes during senescence and air curing of burley tobacco" Tobacco International, 184(24), 44-8.

Seasonal effects during senescence, sequential changes during air curing on the stalk, and methods of curing of burley tobacco on leaf nitrate concn. were investigated. Nitrate concn. decreased during the 3 wk period between topping and harvesting. The amt. of decrease was greater when soil moisture was adequate compared to when soil moisture was deficient. Nitrate concn. in the leaves decreased during the 1st and 2nd days of curing. After the 2nd

day nitrate level increased until the leaf started to brown and then decreased to near the original level. It is postulated that when browning of the leaves starts, cells lose their integrity and nitrate reducing organisms enter the cell and reduce the nitrate. Nitrate concn. was lower in stalk-cut conventionally air-cured burley tobacco than in primed leaf with air-cured or accelerated-cured with heat. Primed air-cured leaves showed an increase in nitrates until the leaves started to brown, but the decrease during the latter stage of curing was not as large, probably because the leaves dried faster. Nitrate loss in leaves processed by the homogenized leaf curing (HLC) method was related to incubation temp. Nitrate concn. decreased from 44 to 64% depending on stalk position when incubated at 31°. Increasing the temp. to 46° resulted in little change in nitrate concn.

Matsuzaki, Toshiaki; Koiwai, Akira; Kawashima, Nobumaro; Matsuyama, Susumu (1981) "Changes in fatty acids and glycerolipids of tobacco leaves during air-curing". Agricultural and Biological Chemistry, 45(10), 2317-21

Changes in the lipid components of tobacco leaves during air-curing were investigated. In the air-curing system the contents of total fatty acids and linolenic acid decreased markedly. Digalactosyldiglyceride, monogalactosyldiglyceride, sulfoquinovosyldiglyceride, and phosphatidylglycerol, which are components of chloroplast membranes, were degraded almost completely during the process. Phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine (PC), and phosphatidylinositol (PI) were relatively resistant to the degradn. process during the yellowing stage, but they also decreased markedly thereafter. PC and PI remained until the final drying stage, although their contents were low. In most polar lipids a considerable decrease was obsd. in the ratio of linolenic acid. Free fatty acid content increased gradually.

Davis, D. L.; Atkinson, W. O.; Smiley, J. (1974) "Maleic hydrazide residue from air-cured burley tobacco". Crop Science, 14(1), 109-12.

Residue levels of maleic hydrazide (I) [123-33-1] on air-cured tobacco were decreased by allowing longer periods of time between application and harvest, lower application rates, and the use of single rather than split applications. Residue levels were higher from the upper portions of the plant and from the laminae than from midvein tissue. Levels were 21-152.3 µg l/g dry wt. Treatment of burley tobacco with sucker retardants significantly increased yields over nontreated hand-suckered plots, esp. at late harvest dates.

Sheen, S. J. (1973) "Determine the effect of genetical background and nitrogen nutrition on the polyphenols and carbohydrates composition of tobacco plants" Tob. Health Workshop Conf., Proc., 4th (1973), 706-16.

The major polyphenols, brown pigments, lignin, sol. reducing sugars, starch, and cellulose were detd. in 81 air-cured leaf samples of 3 Burley 21 tobacco genotypes. Lignin content was unaffected by rate of N fertilization. The phenolic constituents were increased at the lowest N treatment (112.3 kg/ha) tested. The quantities of all polyphenols increased with ascending order of leaf stalk position. No starch was detected in any of the 3 genotypes, irresp. of fertilizer rate and leaf stalk position. The amts. of reducing sugars (fructose, glucose, and sucrose) varied from 2.73 to 3.68 mg/g of dry wt. Significant variety X stalk position interactions were obsd. in the levels of chlorogenic acid, total polyphenols, lignin, total sol. sugars, and cellulose.

Chakraborty, M. K. (1968) "Compositions of the volatile oils obtained from different varieties of tobacco". Indian Journal of Technology, 6(9), 281-2.

The volatile oil contents of some important varieties of tobacco used in cigarette manuf. were



compared. With respect to oil content, air-cured tobaccos are richer than sun-cured tobaccos and the latter are superior to flue-cured tobaccos. Carbonyls are particularly high in air-cured tobaccos. On the basis of volatile oil compn., Natu tobacco, an indigenously grown air-cured type, is particularly suitable for prepg. cigarette tobacco blends.

Andersen R A; Burton H R; Fleming P D; Hamilton-Kemp T R; Gay S L (1987) " Effects of air-curing environment on alkaloid-derived nitrosamines in burley tobacco " IARC scientific publications, (84), 451-5

Levels of nitrite and pyridine alkaloid-derived total tobacco-specific nitrosamines (TSNA) were significantly higher in tobacco leaf (normal or late harvest) air-cured at 32 degrees C/83% relative humidity (RH) than in more moderate environments, i.e., 15 degrees C/50% RH and 24 degrees C/70% RH. These constituents increased appreciably from day 10 to day 21 of the cure. The near-concurrent appearances of maximal total contents of TSNA [sum of N'-nitrosonornicotine (NNN), N'-nitrosoanatabine (NAT), N'-nitrosoanabasine (NAB) and 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)] and nitrite supports the concept that nitrite is a limiting and proximal precursor of total TSNA during the curing of tobacco. During a long curing period (50 days) at 32 degrees C/83% RH, nicotine and anatabine contents decreased, but TSNA contents increased in leaves of all harvest dates and stalk positions. These results support the view that nicotine and anatabine are precursors of TSNA. Measurement of nitrite and individual alkaloids during post-harvest processing of tobacco leaf may provide an index of the potential accumulation of alkaloid-derived nitrosamines.

Variation dynamics of total nitrogen and nicotine of burley tobacco at various growing stages under different nitrogen nutrition levels. By Chai, Jiarong. From Agricultural Science & Technology (2012), 13(11), 2327-2331. Language: English, Database: CAPLUS

[Objective] This study aimed to investigate the variation dynamics of total nitrogen and nicotine of burley tobacco at various growing stages under different nitrogen nutrition levels to provide ref. for optimizing fertilization technologies for burley tobacco. [Method] Fresh burley tobacco leaves were collected at various growing stages and dried to measure the content of total nitrogen, protein and nicotine by using INTEGRAL automated chem. analyzer and analyze the correlation with nitrogen application level. [Result] Regardless of the nitrogen application level, the content of total nitrogen and protein showed a downward trend since root extending stage and reached the min. at mature stage; the content of nicotine showed an upward trend since early vigorous growing stage and increased to the max. at mature stage; the content of total nitrogen, protein and nicotine all increased after air curing; the content of total nitrogen, protein and nicotine showed pos. correlation with nitrogen application level, while total nitrogen/nicotine was neg. correlated. The results indicate that nitrogen level is closely related to the total nitrogen and nicotine of burley tobacco at growing period and post-air curing, rational application of nitrogen is an important measure to regulate the content of total nitrogen and nicotine of tobacco. [Conclusion] This study provides scientific basis for rational fertilization of burley tobacco.

### **Air Cured Tobacco : Pyrolysis**

Bell, J. H., A. O. Saunders, et al. (1966). "The contribution of tobacco constituents to phenol yield of cigarets." 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)]

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 N<sub>2</sub>. 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)]

### Air Cured Tobacco : Smoke Chemistry

Rao, B. V. Kameswara; Murthy, P. S. N.; Chakraborty, M. K. (1986) " Chemical studies on Lanka tobacco and its smoke: 2. Alkaloids" Tobacco Research, 12(2), 196-201

Lanka tobacco, an indigenous air-cured type of tobacco, was thoroughly examd. for its alkaloid compn. The primary alkaloids identified by GC-MS are nicotine, nornicotine, anabasine, and anatabine, the remaining 8 being other pyridine derivs. Fermn. of Lanka tobacco resulted in a loss of 36.9% in total alkaloids.

Hoffmann, D.; Woziwodzki, H. (1968) " Chemical studies on tobacco smoke. IV. Quantitative determination of free nonvolatile fatty acids in tobacco and tobacco smoke". Beitraege zur Tabakforschung, 4(4), 167-75.

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. C<sub>6</sub>H<sub>6</sub>, washing with C<sub>6</sub>H<sub>6</sub>, and evapg. in vacuo. The residue was dissolved in Et<sub>2</sub>O, shaken with 2N KOH, followed by H<sub>2</sub>O. The combined aq. layers were acidified with 5N HCl and the free acids were extd. with Et<sub>2</sub>O. 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-C<sub>6</sub>H<sub>6</sub> (1:1) followed by C<sub>6</sub>H<sub>6</sub>. 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 C<sub>12</sub>, C<sub>14</sub>, C<sub>15</sub>, and C<sub>20-26</sub> 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.

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. M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> 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.

Effect of sugar content on acetaldehyde yield in cigarette smoke. By Cahours, Xavier; Verron, Thomas; Purkis, Steve. From Beitrage zur Tabakforschung International (2012), 25(2), 381-395. Language: English, Database: CAPLUS



The relationship between cigarette blend sugar and acetaldehyde formed in its smoke is a matter of current regulatory interest. This paper provides a re-anal. of data from 83 European com. cigarettes studied in the 1970s and more modern data on sugar levels and acetaldehyde yields from a series of 97 European com. cigarettes contg. both inherent sugar and in other cases inherent and added sugar. It also provides data from 65 exptl. cigarette products made from single curing grades of tobacco, having a wide range of inherent sugar levels but no added sugar. This study has shown that there is no relationship between acetaldehyde yields and blend sugar content even if a multivariate anal. is carried out taking into account Nicotine Free Dry Particulate Matter (NFDPM) as a co-factor. Such analyses should take into consideration each of the known contributory factors in order to avoid misleading conclusions. No distinction was found between the mainstream acetaldehyde yields from dark air-cured, flue-cured or US blended style cigarettes irrespectively of their sugar content after taking account of differences in NFDPM yields. Similarly, no distinction was found between mainstream acetaldehyde yields of cigarettes made from single grades of either flue-cured, sun-cured or air-cured tobaccos with no sugar added. This work supports the conclusion that structural material in the tobacco plant is the main source of acetaldehyde in mainstream smoke after combustion during cigarette smoking.

#### **The influence of tobacco blend composition on carbon monoxide formation in mainstream cigarette smoke**

By Djulancic, Nermina; Radojicic, Vesna; Srbinovska, Marija

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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. M<sub>1</sub>, M<sub>2</sub> and M<sub>3</sub> 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.

#### **Air Cured Tobacco : In vitro studies**

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-alpha-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.

## Air 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.

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.

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.