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Chapter 8
Leaf Chemistry

8A Basic Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types

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INTRODUCTION

In 1960, a little over 200 chemical constituents had been identified in tobacco leaf of all types and less than 450 had been reported in smoke. Today, approximately 3000 have been identified and characterized in tobacco leaf and some 4000 in smoke. Estimates are that the total number of chemical constituents in leaf exceeds 4000 and there are over 6000 in tobacco smoke. It is not the purpose of this section to comprehensively review all of the known constituents, but rather to provide an insight into the known composition and chemistry of tobacco types that impact tobacco quality and differentiate tobacco types. Emphasis will be placed on the major tobaccos utilized commercially: Virginia (flue-cured), air-cured (burley and cigar) and Oriental.

The physical and chemical properties of leaf tobacco are influenced by genetics, agricultural practices, soil type and nutrients, weather conditions, plant disease, stalk position, harvesting and curing procedures. A change in any of these factors can markedly alter the chemical composition of leaf and thus affect smoking quality (Tso, 1972; Tso, 1990).

It is now generally accepted that the metabolic carbon–nitrogen balance in living plants is due to continuing transformations based on the Krebs tricarboxylic acid cycle. In the Krebs cycle, carbon dioxide from air is assimilated through photosynthesis in the tobacco leaf while inorganic nitrogen (nitrate and/or ammonia) is assimilated through the roots from the soil. Soil nitrate is converted to ammonia which is utilized in the Krebs cycle to form amino acids which serve as a nitrogen pool for the formation and transformation of a multitude of nitrogenous chemicals important in the development of aroma and flavor quality. Dawson (1952) has suggested a concept based on the Krebs cycle to account for inherited and culturally induced variations in gross tobacco composition. Using this concept, he rationally suggested that for tobaccos where the nitrogen supply is abundant, such as in cigar and burley tobacco production, there should be an abundant formation of protein, amino acids and nicotine. For Oriental tobacco, where growth is maintained with limited supplies of nitrogen nutrients and water, there is an accumulation of acetate in the Krebs cycle resulting in the biosynthesis of terpenoids via mevalonic acid as well as a higher production of carbohydrates, 'aromatic' acids and resins at the expense of nitrogen constituents. Flue-cured tobacco is intermediate in that the phytochemistry during the plant's life cycle is balanced by a moderate supply of nitrogen which is depleted as the plant reaches maturity.

Examination of representative analyses of the major cigarette tobacco types as presented by Harlan and Moseley (1955) (Table 8.1) provides an overview of the major differences in aged flue-cured, burley, Maryland and Oriental tobaccos.

Although average reducing sugar content in flue-cured and Oriental cigarette tobaccos today rarely runs as high as reported in Table 8.1, the basic analytical trends still remain valid. Thus, in air-cured burley, Maryland and cigar tobaccos the carbohydrates have been virtually depleted via metabolism of the living cells, whereas the protein and α-amino nitrogen are obviously higher than in flue-cured or Oriental tobaccos. Conversely, the flue-cured and Oriental

Table 8.1 Composition of cigarette tobaccos: representative analyses of cigarette tobaccos (leaf web after aging, moisture-free basis).

Component (%)[1]	Flue-cured, type 13	Burley, type 31	Maryland, type 32	Oriental[2]
Total volatile bases as ammonia	0.282	0.621	0.366	0.289
Nicotine	1.93	2.91	1.27	1.05
Ammonia	0.019	0.159	0.130	0.105
Glutamine as ammonia	0.033	0.035	0.041	0.020
Asparagine as ammonia	0.025	0.111	0.016	0.058
α-Amino nitrogen as ammonia	0.065	0.203	0.075	0.118
Protein nitrogen as ammonia	0.91	1.77	1.61	1.19
Nitrate nitrogen as NO_3	trace	1.70	0.087	trace
Total nitrogen as ammonia	1.97	3.96	2.80	2.65
pH	5.45	5.80	6.60	4.90
Total volatile acids as acetic acid	0.153	0.103	0.090	0.194
Formic acid	0.059	0.027	0.022	0.079
Malic acid	2.83	6.75	2.43	3.87
Citric acid	0.78	8.22	2.98	1.03
Oxalic acid	0.81	3.04	2.79	3.16
Volatile oils	0.148	0.141	0.140	0.248
Alcohol-soluble resins	9.08	9.27	8.94	11.28
Reducing sugars as dextrose	22.09	0.21	0.21	12.39
Pectin as calcium pectate	6.91	9.91	12.41	6.77
Crude fiber	7.88	9.29	21.79	6.63
Ash	10.81	24.53	21.98	14.78
Calcium as CaO	2.22	8.01	4.79	4.22
Potassium as K_2O	2.47	5.22	4.40	2.33
Magnesium as MgO	0.36	1.29	1.03	0.69
Chlorine as Cl	0.84	0.71	0.26	0.69
Phosphorus as P_2O_5	0.51	0.57	0.53	0.47
Sulfur as SO_4	1.23	1.98	3.34	1.40
Alkalinity of water-soluble ash[3]	15.9	36.2	36.9	22.5

[1] In % except for pH and alkalinity.
[2] Blend of Macedonia, Smyrna, and Samsun types.
[3] Milliliters of 1 N acid per 100 g tobacco.
Source: Harlan and Moseley (1955).

tobaccos possess significant amounts of reducing sugars (which are virtually absent in the air-cured tobaccos) and lesser amounts of protein and α-amino nitrogen. Substantial changes in the chemical composition of tobacco leaf occur following harvest and during subsequent processes.

CARBOHYDRATES: STARCH, SUGARS, SUGAR ESTERS, CELLULOSE, AND PECTIN

(a) Starch and sugars

In flue-cured tobacco, respiration in the primed leaf is arrested by the controlled desiccative dehydration during flue-curing which causes enzyme inactivation. Nevertheless, considerable change has occurred (Table 8.2) in the leaf (during the period after priming and early stages of curing) as starch loss occurs via enzymatic hydrolysis with a concomitant increase in reducing sugar (Bacon, *et al.*, 1952). This is illustrated for the starch depletion during cure of Virginia tobacco (Fig. 8.1) and the concurrent generation of reducing sugars (Long & Weybrew, 1981) (Fig. 8.2).

In burley tobacco the starch accumulation during growth is only about 25% the amount in Virginia tobacco (Weybrew & Hamann, 1977) and this is nearly depleted completely during the catabolic respiration of the plant while air-curing leaving negligible sucrose, and reducing sugars in the cured leaf.

Table 8.2 Changes in composition of Virginia tobacco during the flue-curing process (% of dry weight).

Constituents	Green	Yellowed	Cured
Starch	29.30	12.40	5.52
Free reducing sugars	6.68	15.92	16.47
Levulose	2.87	7.06	7.06
Sucrose	1.73	5.22	7.30
Crude fiber	7.28	7.16	7.24
Total nitrogen	1.08	1.04	1.05
Protein nitrogen	0.65	0.56	0.51
Nicotine	1.10	1.02	0.97
Ash	9.23	9.24	9.25
Calcium	1.37	1.37	1.37
Oxalic acid	0.96	0.92	0.85
Citric acid	0.40	0.37	0.38
Malic acid	8.62	9.85	8.73
Resins	7.05	6.53	6.61
Pectinic acid	10.99	10.22	8.48
pH of tobacco	5.55	5.64	5.55

Source: Bacon, *et al.* (1952).

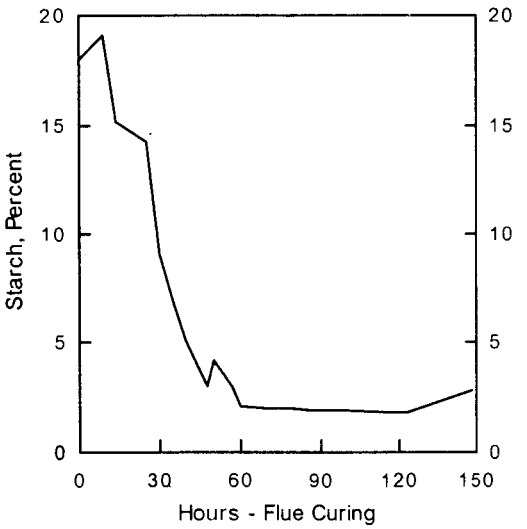


Fig. 8.1 Change in lamina starch during flue-curing of Virginia tobacco (adapted from Long & Weybrew, 1981).

Starches are generally polymers of two polysaccharides: amylose and amylopectin. Corn starch has approximately 27% amylose and 73% amylopectin, whereas tobacco has been found to have approximately 23% amylose and 77% amylopectin (Johnstone & Plimmer, 1959). The amylose portion is estimated to have a chain length of 40 to 47 anhydro-

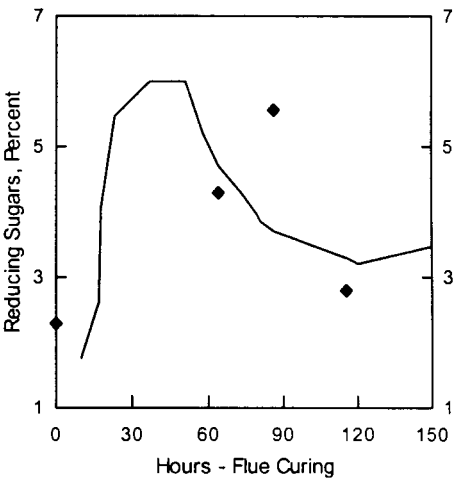


Fig. 8.2 Change in lamina reducing sugars during flue-curing of Virginia tobacco (adapted from Long & Weybrew, 1981).

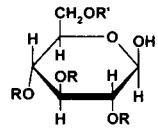
glucose units, while the amylopectin has about 26 glucose units.

(b) Sugar esters

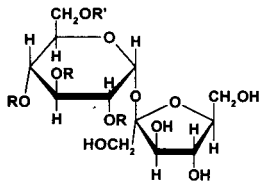
The first report of sugar esters in Oriental tobacco came in 1970 with the isolation, structure elucidation and synthesis of 6-0-acetyl-2,3,4-tri-0-[(+)-3-methylvaleryl]-beta-D-glucopyranose (a glucose tetraester) by Schumacher (1970). This and the more predominant sucrose tetraesters (STE) of lower carboxylic acids (Fig. 8.3) are now considered to be some of the most important aroma precursors responsible for Oriental flavor (Leffingwell & Leffingwell, 1988).

In 1981, Severson, *et. al.*, found that the cuticular waxes of a tobacco budworm resistant tobacco contained a series of STE which are the probable precursors of 6-0-acetyltriacylglucopyranosides (glucose tetraesters (GTE)) isolated from Oriental tobacco (Severson, *et al.*, 1981; Severson, *et al.*, 1985a; Severson, *et al.*, 1985b).

Einolf and Chan have quantified the accumulation of STE for Coker 319 (Virginia), Kentucky 14 (burley) and Smyrna (Oriental) tobaccos utilizing flue-curing, air-curing and sun-curing respectively to mimic the field-curing practices for each tobacco type (Einolf & Chan, 1984). The graphic representation (Leffingwell



GLUCOSE TETRAESTERS



SUCROSE TETRAESTERS

R = C_3 -C_8 Carboxylate

R' = Acetate

Fig. 8.3 Sugar esters present in Oriental tobacco.

& Leffingwell, 1988) (Fig. 8.4) shows that accumulation of STE for Oriental and burley is greater than for Virginia tobacco, with the Oriental and burley maxima occurring at full maturity.

It has been demonstrated that the tetraesters of Oriental STE tended to have higher amounts of the more aromatic C_5 and C_6 carboxylic acids (Severson, *et al.*, 1985b).

From a smoke aroma and flavor standpoint, it is interesting that GTE and STE readily release free carboxylic acids on thermolysis while totally esterified sucrose and glucose esters (such as glucose pentaisovalerate and the sucrose octaesters) do not easily release their acid moieties on thermolysis.

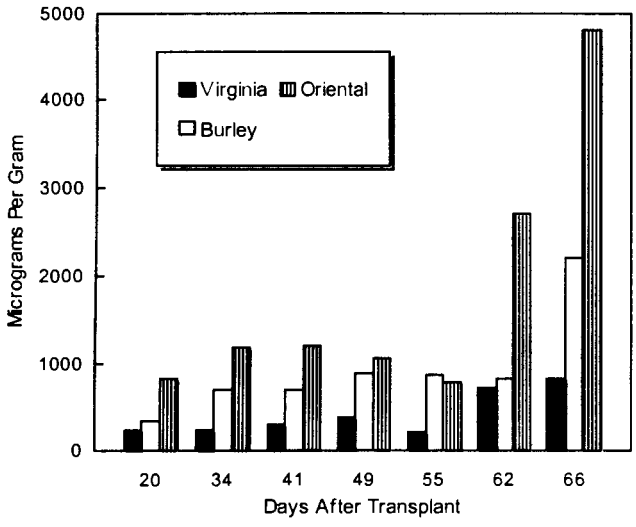


Fig. 8.4 Sucrose tetraesters in tobacco types (adapted from Einolf & Chan, 1984).

(c) Glucosides

The suggestion that nonvolatile aroma precursors of tobacco existed in the form of glucosides was made by the observation that materials such as 3-hydroxydamascone and 3-keto-ionol were released from tobacco by hydrolysis (Green, *et al.*, 1980). Very little information on the hydrolytic release of aroma compounds from fruits and other plant materials by either the acid or enzymatic hydrolysis of glycosidically bound terpenoid and norterpenoid materials was known prior to the mid-1970s. Today, it is clear that many plants, including tobacco, which have sugars (mono- and disaccharides) generate nonvolatile materials with an inter-sugar link to aroma materials arising from the oxidative and metabolic transformation of carotenoid pigments (Williams, 1993). Many tobacco identical aroma compounds have now been found in fruits, wines and other products, primarily bound as glucosides. Heckman, *et al.* (1981) demonstrated that a series of tobacco aglucone isolates are significantly increased by enzymatic hydrolysis of glucosides from Virginia tobacco (Table 8.3).

The commercial enzymatic hydrolysis of tobacco scrap and dust has been used to generate tobacco aroma materials from the glucosidically bound precursors in tobacco by one of the major flavor companies. The use of glucosides for flavoring tobacco has been patented (Anderson, *et al.*, 1976), and commercial use of the glucoside of ethyl vanillin (Herron, 1987) (an artificial flavor about three times more powerful than vanillin) for improving sidestream aroma was employed at one time in the US in cigarettes.

(d) Cellulose

It has been estimated (Green, 1977) that cigarette blends contain approximately 10% of cellulose and hemicellulose, which corresponds closely to the values obtained for a series of flue-cured varieties (Collins & Legg, 1977). However, it must be noted that the tobacco stalk, stem and mid-rib portion of the leaf are much higher in cellulose than the lamina portion of the leaf.

Table 8.3 Aglucone increase from enzymatic hydrolysis of glucosides in Virginia tobacco (mg/100 g).

Aglucone	Virginia control	Enzyme-treated	Percent increase
3-Methylbutanol	0	104	N/A
Benzyl alcohol	1815	3167	74
2-Phenylethanol	887	1744	97
2-Methoxy-4-vinylphenol	498	792	59
4-Vinylphenol	1023	1255	23
3-Hydroxyldamascone	2469	2697	9
4-Vinylsyringol	1615	2850	77
3-Keto-α-ionol	7257	10993	52
4-(4-Hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butyn-2-ol	1783	1805	1
3-Hydroxy-5,6-epoxy-β-ionol	248	698	181
4-(3-Hydroxybutenylidene)-3,5,5-trimethylcyclohex-2-en-1-one	426	1146	169
3-(2-Hydroxyethyl)-phenol	155	1207	679
6-Hydroxy-3-keto-α-ionone	67	109	63
Vomifoliol	416	701	69

Source: Heckman, *et al.* (1981).

Cellulose is a polysaccharide of the general formula $(C_6H_{10}O_5)_n$ which usually consists mainly of glucose units. Acid hydrolysis of cellulose fractions from Japanese Bright (Virginia) tobacco leaf yielded only glucose from one fraction and mainly galactose from another, while the stem cellulose yielded mainly arabinose and glucose (Johnstone & Plimmer, 1959).

Normally, high cellulose content in a tobacco blend is a negative to smoking quality in that it tends to impart a sharp stinging harshness and a 'burnt paper' odor to the smoke. The cellulose content of stems plays an important part in the manufacture of reconstituted tobacco because the fiber provides structural strength. The degree of polymerization (DP) of cellulose from leaf lamina varies considerably from that of mid-rib and stems (DP of 1100–1650 for lamina versus 1600–1800 for leaf mid-ribs). These values are considerably lower than that found for wood fiber (DP of about 3000). In addition, the DP for Virginia tobacco tends to be lower than for air-cured tobaccos (Stedman, 1968). Pyrolysis studies on the generation of formic acid from tobaccos also suggest that Virginia cellulose may have a shorter mean chain length than burley (Fenner, 1988).

As tobacco stems play an important role in the modern economics of tobacco manufacture, being employed as both cut rolled expanded stems and as a cellulose source in reconstituted tobacco, it is noted that Virginia or flue-cured stems are preferred to air-cured stems for their smoking quality. This may be due to the difference in cellulose polymerization, as well as other materials present (such as higher nitrate (and combustibility)) in air-cured stems.

(e) Hemicellulose

Hemicelluloses are a group of polysaccharides found in the cell walls of all plants in association with lignin. Whether lignin and hemicellulose are bonded or the hemicellulose is mechanically entrapped by lignin is unclear (Anon, 1989). The hemicellulose of Japanese Bright (Virginia) tobacco leaf was separated into two fractions which on hydrolysis gave mainly arabinose, galactose and glucose, along with lesser amounts of xylose (Johnstone & Plimmer, 1959). Hemicellulose is subject to extreme variations as its composition can change with growth and maturation and is influenced by cultural and environmental factors.

(f) Pectin

The tobacco pectins are the 'glue' that holds tobacco leaf together. Pectin comprises 6 to 12% of tobacco weight and as such contributes importantly both to the structural stability of leaf and to pyrolysis products that contribute to the smoke chemistry.

The pectin molecule is basically a polymeric carbohydrate comprised of 1,4-linked α-D-galactouronic acids with various degrees of methylation of the carboxylic acid groups. Dispersed within the polygalacturonic acid backbone are neutral sugar moieties such as galactose, rhamnose and arabinose.

Bokelman and coworkers (Sun, *et al.*, 1987) found that the main pectic polysaccharide fraction from tobacco has a backbone of 1,4-linked α-D-

galactopranosyluronic acid interspersed with 2-linked L-rhamnopyranosyl residues. Approximately 22% of the galacturonic acid was methylated. The main pectic chain was determined to be branched at carbon-4 of rhamnose with the neutral side chains containing terminal and 4-linked β-D-galactopyranosyl and α-L-arabinofuranosyl residues.

Lauterbach and Moldoveanu (1991) determined the degree of both methylation and amidation by pyrolysis gas chromatography-mass spectrometry (GC-MS) for the soluble pectic fractions of commercial grades of cured Virginia, burley and Oriental tobaccos with the following results:

Tobacco	Methoxylation (%)	Amidation (%)
Virginia	20	24
Burley	4	34
Oriental	12	16

It has long been known that the pectin fractions of tobacco are modified chemically by curing procedures (especially air-curing and cigar fermentation where ammonia can interact with the pectic substances present).

From a commercial aspect, pectins play an important role in the manufacture of reconstituted tobacco. One of the two types of major commercial reconstituted tobacco processes involves the treatment of a tobacco slurry with diammonium phosphate to solubilize the tobacco pectin. When the tobacco slurry is then cast onto a hot metal belt, a portion of the ammonia is volatilized, 'setting' the tobacco pectin to 'glue' the reconstituted sheet together. Because the ammonia generated plays an important role in natural tobacco flavor formation, this reconstituted process not only provides a means of extending tobacco utilization, it also provides a superior reconstituted tobacco from a smoking quality standpoint by increasing the flavorful pyrazines. The role of ammonia in tobacco will be discussed below.

CARBOHYDRATES AND SMOKE CHEMISTRY

Carbohydrates in the forms previously described can account for 40 to 50% of tobacco weight and contribute significantly to smoking quality. As mentioned, the sugar esters are a major contributor to the Oriental tobacco flavor, in that they thermally release the potent lower fatty acids characteristic of Turkish smoke. In addition, it has been noted that of the 147 pyrolysis products reported from cellulose, starch, mono- and di-saccharides and pectic substances that 121 have been found in tobacco smoke (Heckman, *et al.*, 1981).

SENSORY ROLE OF CARBOHYDRATES, AMMONIA AND NICOTINE – PH OF TOBACCO SMOKE

Pyrolysis of carbohydrates in tobacco smoke has long been known empirically to change the sensory impression of certain types of tobaccos. Even before cigarettes became popular, sugars were added to air-cured tobaccos used for pipe smoking to mitigate the harshness of nicotine in the smoke. And, today, as 50 years ago, tobaccos are purchased and blended based on certain chemical criteria (sugar and nicotine content) as well as the more subjective criteria of appearance and smoke flavor. In addition, it is a common practice to add sugars (usually invert sugar (Glucose + fructose) or partially inverted sugar which still contains some sucrose) in American blend cigarettes. Sugars are known to 'balance' the smoke flavor, primarily by modifying the sensory impact of nicotine and other tobacco alkaloids (Leffingwell, 1976).

In the section on nitrogenous constituents of tobacco, the interaction of amino acids and ammonia in the development of important compounds in both Virginia and air-cured tobaccos (including amino-sugar compounds) will be discussed.

However, at this point a brief discussion on the effect of the major acid forming constituents (carbohydrates) of tobacco smoke and the interaction with the major basic substances of tobacco and smoke (nicotine and ammonia) is in order.

Fenner (1988) showed the striking differences in the generation of both formic acid (Fig. 8.5) and ammonia (Fig. 8.6) from Virginia and burley tobaccos on pyrolysis over the temperature range encountered in a cigarette.

In Fig. 8.5, we see the generation of formic acid in Virginia tobacco to be far greater than for burley with the large transition at 190°C being due to simple sugars, while the transition at 250°C is due to pectin plus hemicellulose and the transition at 310°C. is due to cellulose.

Conversely, Fig. 8.6 demonstrates that burley tobacco generates significantly more ammonia than Virginia. The ammonia transition at 190°C. may simplistically be considered as a labile or exchangeable

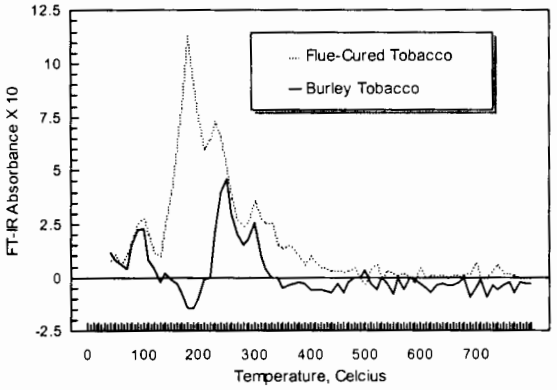


Fig. 8.5 Thermal generation of formic acid (adapted from Fenner, 1988).

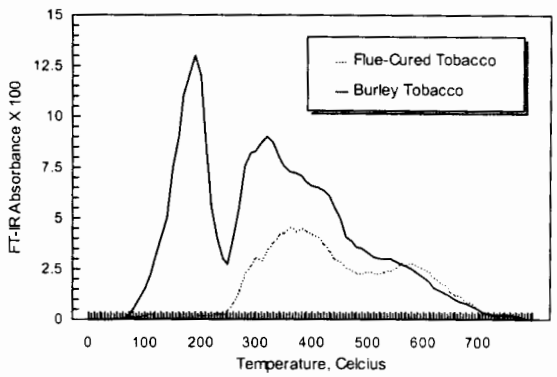


Fig. 8.6 Thermal generation of ammonia (adapted from Fenner, 1988).

ammonia source (e.g. ammonium salts), while the 350°C. transition is amine derived and the 550°C transition is amide-derived ammonia.

Acid forming constituents (carbohydrates) and basic constituents (ammonia) can have a significant effect on smoke pH. Figure 8.7 shows the cumulative effect of successive puffs on the pH of water through which smoke has been passed. In a study of over 150 brands of cigarettes, Elson, *et al.* (1972), concluded that brands with low sugar content generated more alkaline smoke than those with high sugar content (which become progressively more acidic).

As nicotine is a major volatile base present in

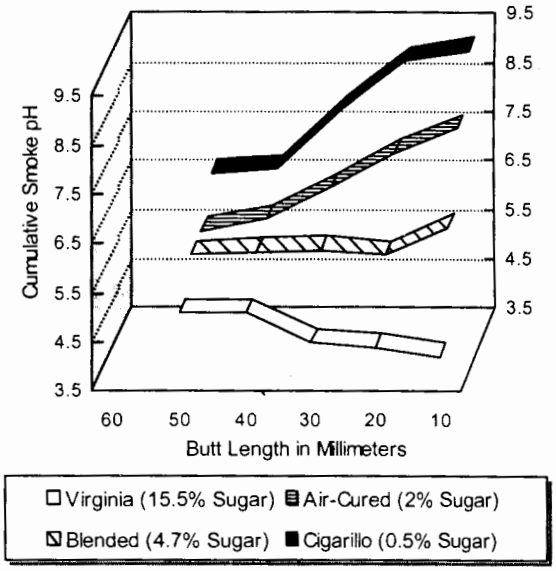


Fig. 8.7 Cigarette smoke pH (cumulative puffs) (adapted from Elson, *et al.*, 1972).

cigarette smoke, the pH of smoke plays an exceedingly important role on the sensory impression imparted.

The higher ammonia level of air-cured tobacco containing low or negligible sugar content would presumably have two major effects. It has an effect on the ratio of nicotine/nicotine salts delivered on smoking relative to high sugar and/or more acidic tobacco types wherein the nicotine and other alkaloids are complexed (more) as salts in the smoke.

$$\text{Acids} \rightarrow$$

$$\text{Nicotine Alkaloid} \longleftrightarrow \text{Nicotine Alkaloid salt}$$

$$\text{(Free base)} \quad \leftarrow \text{Ammonia} \quad \text{(Salt complex)}$$

Since the pH of smoke in air-cured tobacco is considerably more alkaline than flue-cured or Oriental, the ratio of nicotine base to nicotine salts increases. This causes the sensory and physiological perception of increased nicotine strength (and harshness). It should also be noted that the pH of air-cured tobacco smoke increases with succeeding puffs from such cigarettes. Accordingly, the increased alkalinity of straight air-cured cigarettes renders them virtually unacceptable to nearly all smokers as the high smoke pH imparts an alkaloid harshness with a flavor distortion which can be unpleasant. Conversely, many American blend cigarette smokers find the 'acidic' smoke of straight Virginia cigarettes to be

unbalanced. 'Nicotine alone does not determine smoking flavor' (Leffingwell, 1976).

NITROGENOUS CONSTITUENTS

The data in Table 8.4 for air-cured cigar tobacco show the gross chemical changes in nitrogen compounds during air-curing (Frankenburg, 1946; Frankenburg, 1950).

Table 8.4 Changes of nitrogenous compounds in air-cured cigar tobacco (percent of harvested dry weight).

	Primed leaf		Stalk cured	
Type of nitrogen	Before curing	After curing	Before curing	After curing
Total	5.61	5.34	4.70	3.80
Protein (insoluble)	3.69	1.65	3.80	1.85
Soluble	1.92	3.69	0.90	1.95
Amino	0.23	0.80	0.15	0.15
Ammonia plus amide	0.15	1.07	0.05	0.80
Alkaloid	0.35	0.32	0.40	0.40
Nitrate	0.63	0.77	0.20	0.25
Remainder	0.56	0.73	0.10	0.35

Source: Frankenburg (1946).

In general, there occurs some loss in total nitrogen, a reduction in protein via hydrolysis to amino acids, and formation of amino acid amides (as reflected by an increase in soluble nitrogen). Changes occurring in burley tobacco curing (Hamilton, 1974; Hamilton & Lowe, 1978) are similar to those in stalk-cured cigar tobacco.

Flavor quality for both flue-cured and burley tobaccos is dependent on a variety of complex interacting factors related to genetics, agricultural practices, soil types, nutrients, weather conditions, plant disease, stalk position as well as harvesting and curing practices. In general, elevated levels of proteins and amino acids appear to contribute negatively to smoke flavor. This is particularly true of burley tobaccos grown in cool moist climates with insufficient sunshine which are over-fertilized, harvested and air-cured prematurely. In such cases the normal catabolic changes during senescence and curing which result in the enzymatic hydrolysis of fraction 1 protein to amino acids (which then can undergo further catabolic changes) are at least partially arrested. Such immature tobaccos possess a proteinaceous aroma on smoking which is unpleasant. Even secondary fermentation of such poor tobaccos often cannot provide sufficient improvement to make them usable. By comparison to normal burley, in which approximately 50% of total protein undergoes hydrolysis to amino acids, such poor tobaccos may exhibit less than 15% protein hydrolysis during air-curing (Heinzer, 1986). Normal air-curing of fully mature burley shows a rapid drop in protein (Fig. 8.8) (Hamilton & Lowe, 1978; Long & Weybrew, 1981) with a concomitant initial increase in free amino acids for the first few days in cure (Fig. 8.9) (Burton, *et al.*, 1983). Then, there is a decrease and finally a leveling off in total free amino acid concentration. The decrease in free amino acid content after the fifth day of air-curing is due to both metabolic deamination and decarboxylation as well as nitrogen translocation. Even though a slight decrease from a maximum in total free amino acids occurs during the cure, some individual amino acids show a net decrease while others have a net increase (Burton, *et al.*, 1983). It should be noted that the ammonia level of air-cured tobacco rapidly increases during this period (Fig. 8.10) (Burton, *et al.*, 1983).

The role of ammonia on smoke pH and nicotine perception has been discussed above, but ammonia also plays an important role in the formation of aroma and taste compounds.

It is important to remember that, before curing, air-

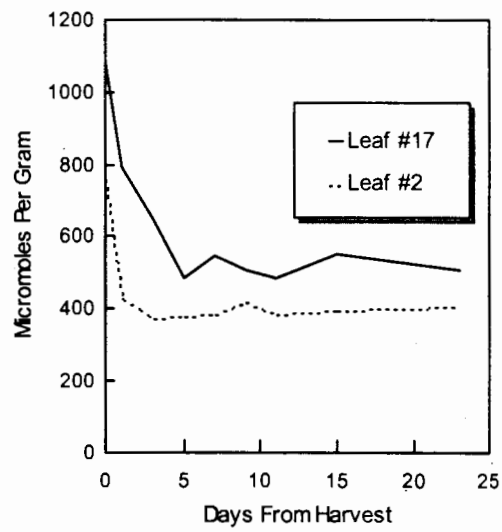


Fig. 8.8 Protein decreases during burley air-curing (adapted from Long & Weybrew, 1981).

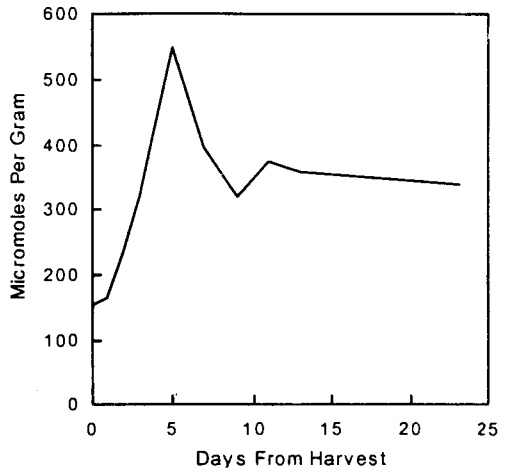


Fig. 8.9 Total free amino acid changes during burley air-curing (adapted from Burton, *et al.*, 1983).

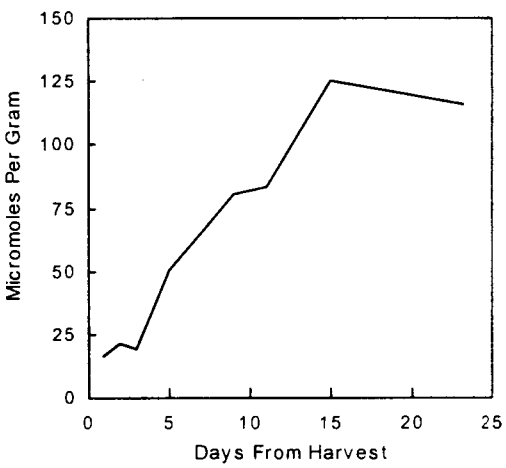


Fig. 8.10 Ammonia increases during burley air-curing (adapted from Burton, *et al.*, 1983).

cured burley or cigar tobaccos may contain 3 to 4% protein nitrogen, while flue-cured tobacco contains only 15 to 20% of that amount. In addition, about 50% of the protein in burley may undergo hydrolysis during air-curing while only about 20% of the protein is hydrolyzed in curing flue-cured tobaccos. Thus, burley tobacco possesses a relative abundance of free amino acids compared to flue-cured tobacco. Representative free amino acid analysis (Table 8.5) of high

Table 8.5 Free amino acids in high quality smoking grade blends (mg/g).

Amino acid	Flue-cured	Burley
Aspartic acid	0.13	7.84
Threonine	0.04	0.43
Serine	0.06	0.17
Asparagine	1.12	10.30
Glutamic acid	0.10	1.78
Glutamine	0.82	0.38
Proline	4.11	0.45
Glycine	0.02	0.14
Alanine	0.32	0.35
Valine	0.06	Trace
Isoleucine	—	0.06
Leucine	Trace	0.10
Tyrosine	0.68	0.84
Phenylalanine	0.24	0.50
Lysine	0.03	0.33
Histidine	0.11	0.45
Arginine	—	0.26
Tryptophan	—	0.50

Source: Leffingwell (1976).

smoking quality blends of flue-cured and burley tobaccos with similar nicotine content illustrates this dramatically (Leffingwell, 1976). In burley, we find the main free amino acids present to be aspartic acid, asparagine and glutamic acid, while for flue-cured, proline, asparagine and glutamine are dominant. In total, 43 amino acids have been found in tobacco leaf.

Cigarette tobaccos are almost never utilized in consumer products unless they have undergone a period of aging ranging from 12 to 60 months. Of course, inventory control is an important factor requiring maintenance of adequate stocks to allow periodic blend changes to occur; but, freshly cured and redried leaf is not used primarily because the smoke changes dramatically from a raw, somewhat irritating and disagreeable taste to a smoother, more rounded flavor during the aging process. Aging is ordinarily carried out at ambient temperatures and about 12% moisture. In areas with relatively long-term high ambient temperatures the desired aging effects are produced in shorter periods than in colder climates (Bates, *et al.*, 1974), and it is common for companies located in cold climate regions to store and age their tobaccos in southern locales. The role that nitrogen compounds play in the improvement of tobacco flavor quality with aging is important and the reasons for this will become apparent.

The role of certain amino acids in flue-cured tobacco

will be briefly discussed. Protein hydrolysis with formation of free amino acids occurs also at the yellowing and early stages of drying during flue-curing, although flue-cured tobacco contains only about 20 to 25% of the total amount of protein/amino acids as burley tobacco. The major amino acid in flue-cured tobacco, proline, seems to be an anomaly in that as much as a 25-fold increase has been observed during the curing schedule for both flue-cured and air-cured (Maryland) tobaccos (Weybrew, *et al.*, 1966). This appears to be greater than can be accounted for by proteolysis of fraction 1 protein – a hypothesis (Hamilton & Lowe, 1978) being that proline is partially formed by a metabolic conversion of the pyrrolic portion of chlorophyll (which is rapidly decreasing during this stage).

In flue-cured tobaccos, proline reacts with the reducing sugar, glucose, to form l-deoxy-l-(L-prolino)-D-fructose (Noguchi, *et al.*, 1971), an Amadori compound, which along with other amino-sugars comprises as much as 1.5 to 2.0% of the dry aged tobacco weight. This compound is significant because it has been shown to undergo low temperature degradation primarily to the probable precursor (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)) of the important volatile flavorant, maltol, found in Virginia tobacco (Leffingwell & Leffingwell, 1988). It has been shown that the smoking properties of DDMP are identical to that of maltol (Leffingwell, 1976).

Figures 8.11 and 8.12 show the change in two of the major Amadori compounds in Virginia tobacco grown in Japan over a 4-year aging period (Noguchi, *et al.*, 1971). The major Amadori compounds present tend to show a gradual increase peaking at about 2 years and then a decrease. During the same period, the amino acid content of the tobaccos shows decreasing values each year. Of course, it is also known that the flavor of Virginia tobacco improves with aging, it rarely being used for cigarettes prior to 12 to 14 months aging, and preferably 18 to 24 months, which interestingly corresponds quite well to these graphs. Amadori compounds are also known to generate a number of flavorful pyrazines and pyrroles on pyrolysis.

Amino acids as well as ammonia interactions with sugars or carbonyl compounds can form a variety of chemical entities. For example, a number of groups have reported the isolation of fructosazines and deoxyfructosazines from Virginia tobacco (Shigematsu & Kitami, 1978; Heckman, *et al.*, 1981) and these are known to be formed by the interaction of ammonia with glucose and fructose under weakly acidic conditions. On pyrolysis, these polyhydroxypyrazines generate products such as water, acetic acid, acetol, furans,

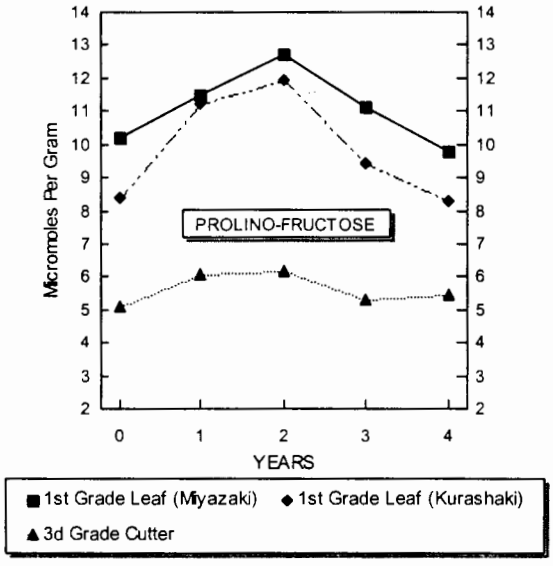


Fig. 8.11 Changes in Amadori compounds (prolino-fructose) during aging of flue-cured tobacco (adapted from Noguchi, *et al.*, 1971).

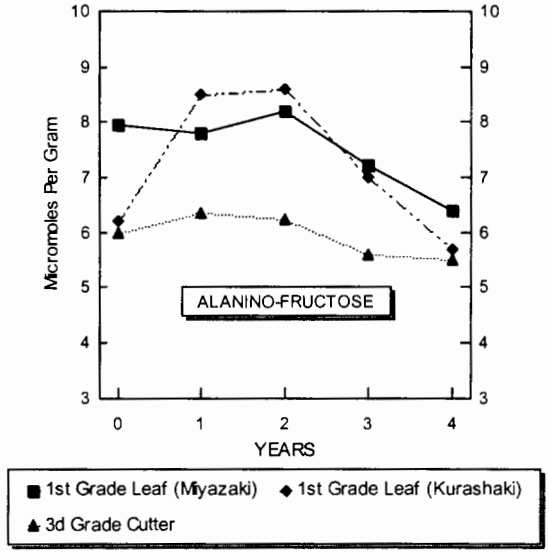


Fig. 8.12 Changes in Amadori compounds (alanino-fructose) during aging of flue-cured tobacco (adapted from Noguchi, *et al.*, 1971).

pyrroles and a number of simple pyrazines (including the major tobacco leaf pyrazines, 2,5- and 2,6-dimethylpyrazine, trimethylpyrazine and tetramethylpyrazine) (Heckman, *et al.*, 1981). Although pyrazines as a class represent a very small percentage of tobacco weight, they are known to be very important to the flavor and aroma of roasted foodstuffs and tobacco.

Bright and co-workers demonstrated that the weight percentages of five amino acids were significantly reduced by heat treatment, while the roasting of tobacco caused dramatic increases in pyrazine levels (especially those high in ammonia or ammonia precursors) under conditions where percent of nicotine remained constant (Tables 8.6 and 8.7) (Bright, *et al.*, 1975).

Table 8.6 Change in amino acid levels of tobacco after roasting (complete cigarette blend).

Amino acid	Before (%)	After (%)	Δ%
Aspartic acid	1.39	0.82	–41
Proline	0.65	0.31	–52
Lysine	0.32	0.10	–69
Histidine	0.24	0.16	–33
Arginine	0.16	0.06	–62

Source: Bright, *et al.* (1975).

Table 8.7 Effects of roasting on dimethylpyrazine concentration.

	Concn before roasting (ppb)	Concn after roasting (ppb)
Virginia	0.1	0.3
Turkish	0.1	0.8
Burley	0.1	460
Reconstituted leaf	0.1	200

Tobacco samples were shredded, without additives and roasted for 4 hours at 120° ± 3°C.
Source: Bright, *et al.* (1975).

Wahlberg, *et al.* (1977) have examined the chemical changes occurring during the flue-curing and aging of Virginia tobacco. One of the observations was that a number of pyrazines, pyrroles and pyridines increased during the flue-curing and aging process (Enzell, *et al.*, 1977). As pyrroles, pyridines and pyrazines can arise in tobaccos by a number of chemical pathways ranging from the heating or aging of proteins and amino acids to the interaction of amino acids and/or ammonia with sugars or carbonyl constituents, suffice it to say, no single pathway is involved and that this complex transformation is dependent on a variety of factors and chemical mechanisms (Leffingwell & Leffingwell, 1988). The generation of certain pyridines (e.g. methyl nicotinate, 3-cyanopyridine and 3-acetylnicotine) can be derived from degradation of nicotine.

TOBACCO ALKALOIDS

The major tobacco alkaloids are nicotine, cotinine, nornicotine, myosmine, nicotyrine, anabasine and anatabine. This subject is covered in greater detail elsewhere in this book (Chapter 8B) and will not be discussed here except to note that nicotine can range in concentration from 0.5 to 8% in the major cultivated tobacco species, *N. tabacum* and *N. rustica*. The interaction of smoke pH and the sensory perception of nicotine is discussed above.

PLASTID PIGMENTS

The major pigments of tobacco are the chlorophylls and carotenoid pigments, plastid pigments found in a wide variety of plants. The chlorophyll and carotenoid contents and changes during the latter stages of growing (and the curing processes) have been studied in significant detail for both burley and Virginia tobaccos.

(a) Chlorophylls

As shown in Fig 8.13 for burley tobacco, the major green pigments are chlorophyll-A and chlorophyll-B, both of which decrease as the tobacco reaches maturity, proceeds into senescence and continues to decrease during barn curing (Burton & Kasperbauer, 1985). Studies on Virginia tobacco show similar trends as the tobacco reaches maturity (Court & Hendel, 1984).

Leaf color in the field as well as for cured leaf is considered to be important in judging the quality of leaf and this has been extensively studied (Tso, 1972; Tso, 1990).

(b) Carotenoid pigments

The major carotenoid pigments in tobacco are lutein, β-carotene, neoxanthin and violaxanthin. In addition to being major color pigments (red-orange to yellow), the

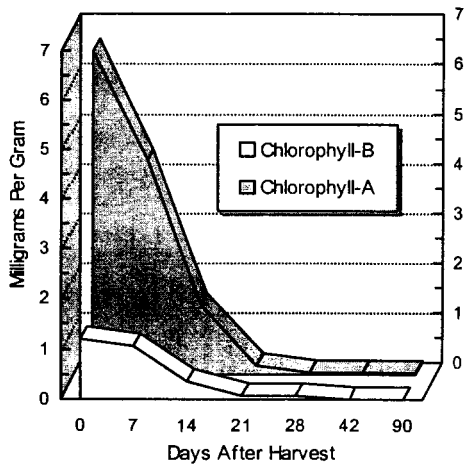


Fig. 8.13 Changes in chlorophyll content of burley tobacco during air-curing (adapted from Burton & Kasperbauer, 1985).

carotenoids are the precursors to many of the volatile aroma components of tobacco.

Figure 8.14 shows the decrease in carotenoids during maturation, senescence and curing for burley tobacco (Burton & Kasperbauer, 1985). Unlike the chloroplastid pigments, the carotenoids do not undergo as extensive degradation (metabolism). Lutein and carotene undergo about 65% degradation in burley.

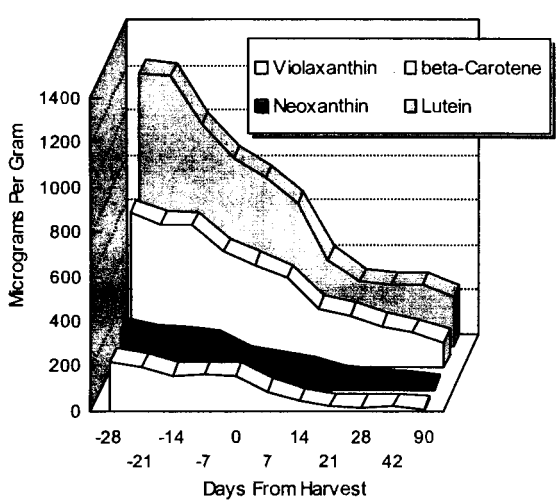


Fig. 8.14 Changes in carotenoid pigments of burley tobacco during air-curing (adapted from Burton & Kasperbauer, 1985).

More extensive degradation of lutein has been reported for Virginia tobacco (Court & Hendel, 1984; Court, *et al.*, 1993).

TOBACCO ISOPRENOIDS

Wahlberg and Enzell (1987) have comprehensively reviewed the subject of tobacco isoprenoid constituents, therefore only comments on some of the more important materials will be included in this section.

(a) Degraded carotenoids

A large number of carotenoid metabolites are encountered in tobacco. The degradation process of the carotenoid pigments in tobacco has been hypothesized to be probably both enzymatic and autooxidative in nature. As indicated in Fig. 8.15 for carotene, the carotenoid chain may be cleaved in a number of locations resulting in a variety of C_9 to C_{13} chemicals, many of which possess important aroma properties. Table 8.8 (Wilson, *et al.*, 1982; Leffingwell & Leffingwell, 1988) illustrates the quantitative amounts found for a number of important nor-carotenoids for various tobacco types.

The degradation of both lutein and β-carotene by both photo-oxidation and high pressure oxidation (at elevated temperature) has been shown to generate

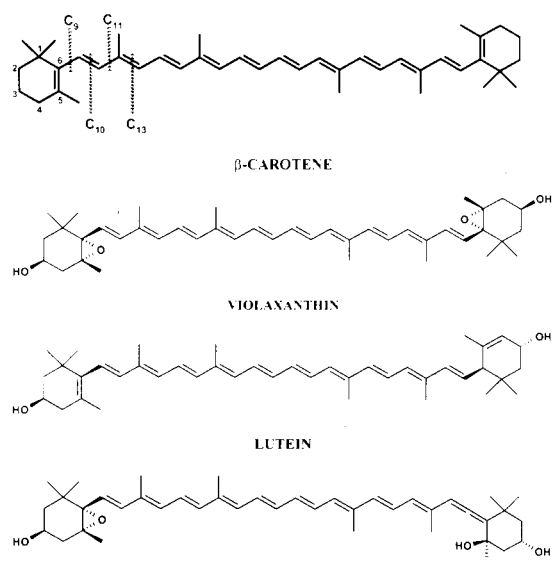
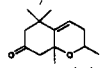
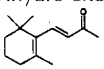
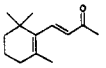
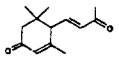
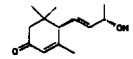
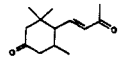
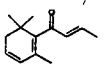
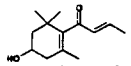
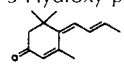
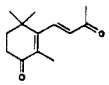


Fig. 8.15 Carotenoid consituents of burley tobacco.

Table 8.8 Major tobacco carotenoid degradation products.

Compound	Virginia (ppb)	Burley (ppb)	Oriental (ppb)
 Isophorone	112	29	33
 4-Ketoisophorone	47	17	42
 Safranal	32	4	43
 Dihydractinodiolide	150	26	73
 Oxoeudalane	139	25	34
 Dihydro-oxoeudalane	47	27	73
 α-Ionone	+	+	+
 β-Ionone	+	+	+
 3-Keto-α-ionone	+	NR	NR
 3-Keto-α-ionol	+	4	+
 3-Ketodihydroionone	51	31	23
 Damascenone	168	42	39
 3-Hydroxy-β-damascone	24	11	+
 (5 Isomers)	1272 Megastigmatrienones	248	415
 4-Keto-β-ionone	NR	+	NR

NR = not reported; + = present, but quantitation not available. Table adapted from Wilson, *et al.* (1982) and Leffingwell & Leffingwell (1988).

many of the same nor-carotenoid components found in tobacco (Heij, *et al.*, 1992).

(b) Acyclic isoprenoids and nor-derivatives

Solanesol (a C_{45} polyprenol) is a major component of tobaccos, generally ranging in quantity from 0.4 to 4%. While this polyprenol was first isolated from tobacco, it is now considered to be a ubiquitous leaf component in the plant kingdom. The progression of solanesol change during growth and air curing for middle stalk burley tobacco is shown in Fig. 8.16 (Long & Weybrew, 1981).

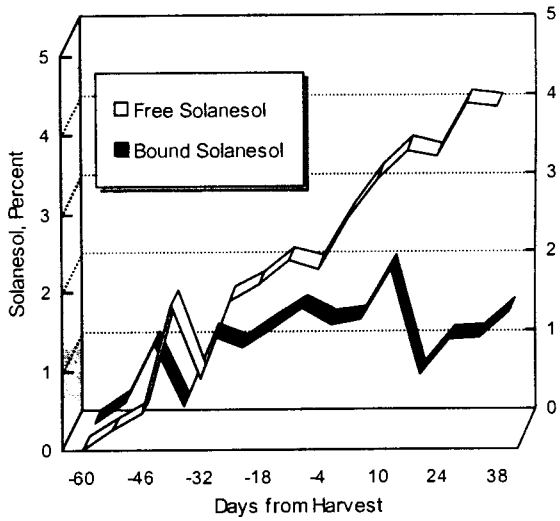


Fig. 8.16 Changes in solanesol levels of burley tobacco during air-curing (adapted from Long & Weybrew, 1981).

It should be noted that solanesol is present as both the free alcohol and in bound forms primarily as acetate, palmitate and linolate esters. Solanesol normally ranges from 1 to 2% in Virginia tobacco and from 1% to as much as 4% in burley tobacco.

The concentration of solanesol in burley tobacco has been shown to be effected by genetic lines and, more importantly, by production practices. Water stress, through decreased soil moisture, leads to an increase in solanesol content as does the length of senescence from time of topping (Burton, *et al.*, 1989).

Neophytadiene (a C_{20} isoprenoid polyene) is a tobacco diterpene whose concentration increases significantly upon curing and aging. Generally, low levels are present in green tobacco leaf, but neophytadiene

increases during the yellowing and curing phases. Fig. 8.17 shows the neophytadiene change in curing burley tobacco (Burton & Kasperbauer, 1985) and Fig. 8.18 shows the change in flue-curing Virginia tobacco (Long & Weybrew, 1981).

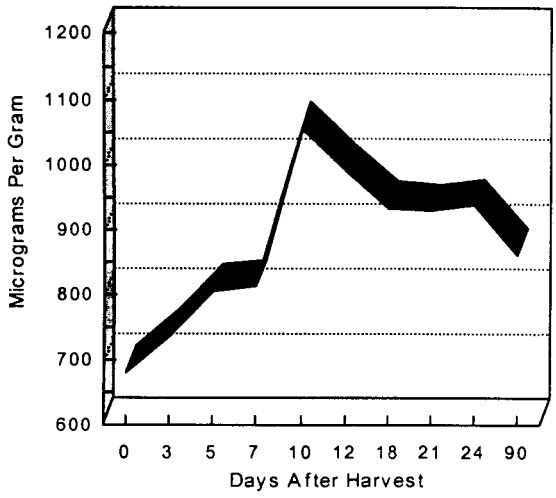


Fig. 8.17 Changes in neophytadiene content of burley tobacco during air-curing (adapted from Burton & Kasperbauer, 1985).

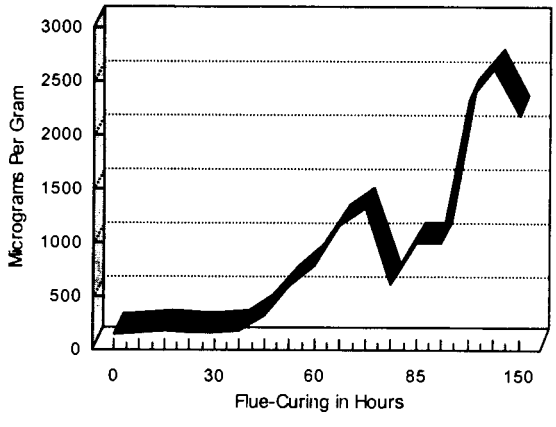


Fig. 8.18 Changes in neophytadiene content during curing of the flue-cured tobacco.

It has been assumed that neophytadiene is produced by dehydration of phytol. Phytol is a metabolite from chlorophyll hydrolysis, but it has been concluded that only 15 to 30% of neophytadiene generated during flue-curing could be accounted for from this source in the case of flue-cured tobacco (Amin, 1979). Neophytadiene has been suggested to be a tobacco flavor enhancer in that it may act as a flavor carrier by entrapping volatiles in the tobacco smoke aerosol (Leffingwell & Leffingwell, 1988).

Among other major acyclic isoprenoids are linalool, linalool oxide, geranylacetone and farnesylacetone. Table 8.9 provides comparative data on some acyclic isoprenoids, nor-derivatives and neophytadiene obtained by the steam distillation of various tobacco types (Wilson, *et al.*, 1982).

Table 8.9 Some major acyclic isoprenoids and nor-isoprenoid products isolated from the steam distillation of tobacco types.

Compound	Virginia (ppb)	Burley (ppb)	Oriental (ppb)
Linalool	281	160	—
Linalool oxide	118	—	—
Geranyl acetone	181	84	130
Farnesylacetone	16	22	trace
6-Methylhepta-3,5-dien-2-one	40	17	—
6-Methylhept-5-en-2-one	78	17	78

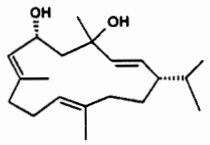
(c) Carbocyclic diterpenoids and their degradation products

Two major classes of diterpenoids are found in tobacco, the monocyclic cembranoids and the bicyclic labdanoids (Wahlberg & Enzell, 1987). These diterpenoids are produced in the glandular heads of the trichomes on the leaf surface as well as in tobacco flowers and are part of the surface cuticular waxes where they occur along with the sucrose tetraesters, wax hydrocarbons, esters and alcohols.

Virginia and burley tobaccos contain only the cembranoids, while Oriental and cigar tobaccos contain both labdanoids and cembranoids. The variety specific occurrence is due to the fact that *Nicotiana tabacum* L. is a hybrid of *N. tomentosiformis (which produces labdanoids) and N. sylvestris* (which produces cembranoids). It has been shown that the biosynthesis of the cembranoids is controlled by two dominant genes, whereas labdanoid synthesis is controlled by a single dominant gene (Wahlberg & Enzell, 1987).

(d) Cembranoids and their degradation products

More than 50 cembranoids have been isolated from tobacco. The cembra-2,7,11-triene-4,6-diols are the major cembranoids of tobaccos (Fig. 8.19) which, for example, have been found in Virginia to be present at 0.23% by weight (Court, *et al.*, 1993).



CEMBRA-2,7,11-TRIENE-4,6-DIOLS (2 ISOMERS)

Fig. 8.19 Cembranoids in tobacco.

Perhaps more important to the quality of tobacco flavor are the cembranoid metabolite products produced by oxidative processes and retro-aldol reactions. More than 60 degradation products of cembranoids have been isolated from tobacco. Of these degradation products, some of the major metabolites are shown in Table 8.10.

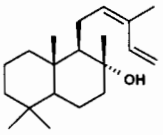
Table 8.10 Some major cembranoid degradation products isolated from the steam distillation of tobacco types.

Compound	Virginia (ppb)	Burley (ppb)	Oriental (ppb)
Solanone	1725	493	1497
Solanol	545	84	177
Norsolanadione	156	26	137
Solanascone	24	33	trace
Solavetivone	18	15	trace
6-Methylhepta-2,5-dione	—	—	101

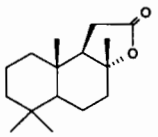
Solanone, a major cembranoid metabolite in tobacco, is considered to be an important tobacco flavor constituent.

(e) Labdanoids and their degradation products

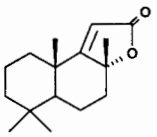
(Z)-Abienol is a major labdanoid in the green leaf of Oriental tobacco and has been shown to be a plant growth regulator. During air- and sun-curing, the concentration of abienol decreases significantly while numerous other labdanoids and labdanoid degradation products are formed. In cured leaf about 20 labdanoids and an additional 25 or more degradation products are found. (Z)-Abienol has been shown to undergo various oxidative processes to many of these products (Wahlberg & Enzell, 1987). Figure 8.20 shows some important labdanoid derived products in Oriental tobacco.



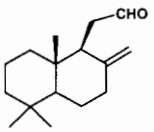
(Z)-ABIENOL



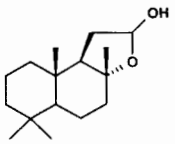
NORAMBREINOLIDE



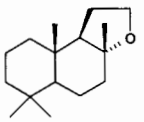
DEHYDRO-NORAMBREINOLIDE



γ-BICYCLOHOMOFARESAL



SCLARAL



AMBROX

Fig. 8.20 Some major labdanoid-derived constituents in Oriental tobacco.

In 1959, norambreinolide (sclareolide), an important constituent of Oriental tobacco and cigar tobacco, was patented (Schumacher, 1959) as possessing a cedar character on smoking, although isolation from tobacco (cigar leaf) was not reported until the early 1970s (Kaneko, 1971). It is now known that norambreinolide and related compounds, such as sclaral, are responsible for the characteristic cedar-amber notes of Havana leaf and Oriental tobacco. The important fragrance compound Ambrox® is found to a much lesser extent in

Oriental tobacco, but this material is a key component in ambergris derived from whales; the exotic aroma and fixative value of ambergris is largely due to this material.

The combination of labdanoid-derived flavorants and sucrose tetraesters present in Oriental tobacco comprises the major flavor-forming components of this variety (Leffingwell & Leffingwell, 1988). Within the Oriental types, the Greek and Macedonian types tend to be higher in labdanoid-derived compounds, while the Turkish Smyrna (Izmir) types are higher in sucrose tetraesters containing the 3-methylvaleric acid component.

CARBOXYLIC ACIDS

The major carboxylic acids in tobacco are citric, malic, oxalic and malonic which in total can comprise 14 to 18% in burley and cigar tobacco, 7 to 10% in Maryland, 6 to 8% in Oriental and 5 to 10% in Virginia leaf, after curing. A substantial portion of such acids are complexed as salts with nicotine, ammonia and inorganic anions of calcium, potassium and sodium. The bar diagram (Fig. 8.21) adapted from Kalianos (1976) graphically shows the differing acidity profiles by tobacco type.

Considerable effort has been expended trying to relate such acids to tobacco quality. Several studies indicate that there is an inverse relationship to the smoking quality of Virginia tobacco and the quantity of citric and oxalic acids (Kalianos, 1976), although this is probably just an indicator, and is not due to the absolute amounts of these acids present in leaf.

The volatile C_2–C_8 acids are known to be important aroma compounds in many fruits, foodstuffs and tobacco. Table 8.11 shows the relative amounts of the major volatile organic acids for various tobacco types (Kalianos, 1976).

Table 8.11 Volatile organic acids in tobacco.

Acid	Virginia (μg/g)	Burley (μg/g)	Oriental (μg/g)	Threshold (ppb)
Formic acid	597	288	587	45 000
Acetic acid	877	372	688	22 000
Propionic acid	13	12	24	20 000
Isobutyric acid	32	29	72	8 100
Butyric acid	2	0	0	240
2-Methylbutyric acid	247	26	313	1 600
Isovaleric acid	116	20	202	120
2-Butenoic acid	12	1	3	NA
Valeric acid	1	0	4	3 000
3-Methylvaleric acid	4	1	1372	300*
Hexanoic	5	3	5	3 000
Heptanoic acid	5	5	6	3 000
Octanoic acid	5	12	5	3 000
Nonanoic acid	16	21	24	3 000
2-Furoic acid	32	36	125	NA
Benzoic acid	22	14	25	85 000
Phenylacetic acid	36	0	65	10 000

* Author's estimate.

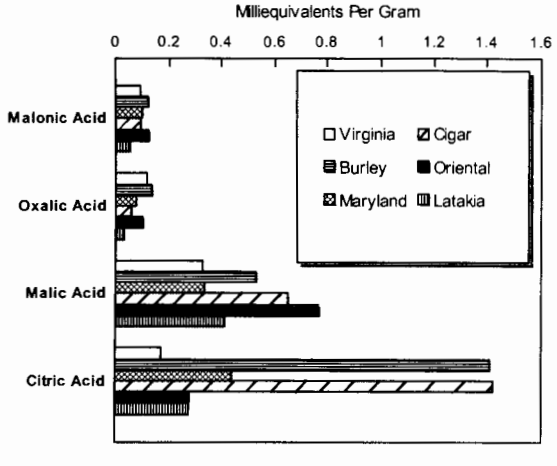


Fig. 8.21 Major organic acids in different tobacco types (adapted from Kalianos, 1976).

It should be noted that the Oriental sample above is an Izmir type which should have a high 3-methylvaleric acid content and that the reported values must include the bound acids released by hydrolysis of sucrose tetraesters.

One of the ways to assess a compound's odor or flavor potency is to evaluate its minimum detectable threshold level. In the table above are included the detection thresholds (Leffingwell & Leffingwell, 1991) for many of the tobacco acids. If one divides the relative concentration of a material by the threshold one can approximate the relative odor potency. For example, for acetic acid, 688/2200 = 0.03, while for 3-methylvaleric acid, 1372/300 = 4.57, indicating the aroma contribution of the latter would be about 152 × that of the acetic acid.

Table 8.12 shows the concentrations of free acids in an Oriental tobacco blend and that of the correspond-

Table 8.12 Increase in aromatic acids in Oriental tobacco smoke due to thermolysis of sucrose tetraesters.

Acid	Tobacco (μg/g)	Smoke (μg/g)	Change (%)
Propionic acid	17.0	66.8	+293
Butyric acid	2.5	9.7	+288
Valeric acid	4.3	3.6	−16
2-Methylbutyric acid	2.1	12.4	+490
Isovaleric acid	5.1	19.7	+286
3-Methylvaleric acid	12.0	62.0	+417
Hexanoic acid	7.5	3.9	−48
Octanoic acid	1.7	6.2	+265
Phenylacetic acid	48.9	19.9	−59

Source: Heckman, *et al.* (1981).

ing cigarette smoke (Heckman, *et al.*, 1981). This provides an indication of the increase in free acids due to thermolysis of the sucrose tetraesters (Leffingwell & Leffingwell, 1988).

Without question, the volatile tobacco acids are some of the most important contributors to smoke quality and the powerful contribution from Oriental means that additions to cigarette blends at even low percentages can have a profound effect on product acceptability.

The higher fatty acids (myristic, palmitic, stearic, oleic, linoleic and linolenic) comprise about 0.75 to 1.1% in Virginia tobacco and about 0.5% in burley, with palmitic being about 25% of these total acids. Table 8.13 lists the average amounts of fatty acids found in a 2-year study on Virginia tobacco grown at Delphi, Ontario, Canada (Court, *et al.*, 1993).

Table 8.13 Fatty acids in Virginia tobacco.

Acid	%
Myristic acid	0.02
Palmitic acid	0.28
Stearic acid	0.05
Oleic acid	0.11
Linoleic acid	0.18
Linolenic acid	0.35

TOBACCO PHENOLICS

(a) Polyphenols

The amount of total phenols varies considerably by tobacco type suggesting considerable genetic and cultural variability. In a 2-year study the average polyphenol amounts found were as listed in Table 8.14 (Smeeton, 1987).

Table 8.14 Polyphenols by tobacco type.

Tobacco	1980 (%)	1981 (%)
Virginia	3.13	3.75
Burley	1.78	2.05
Cigar wrapper	2.13	2.65
Cigar filler	2.03	3.40
Cigar binder	2.27	3.54
Maryland	2.09	3.25
Dark fire-cured	2.78	3.64
Oriental	1.83	2.09

Source: Smeeton (1987).

Major polyphenolics found in tobacco include chlorogenic acid, rutin, scopoletin and scopolen, along with materials such as quercetin and kaempferol. The biogenesis of plant phenolics with relationship to the pathways relevant to the major groups in tobacco for compounds formed via shikamic acid has been reviewed by Enzell & Wahlberg (1980). The amounts of polyphenols for Virginia NC 95 and Burley 21 have been reported (Table 8.15) (Sheen, *et al.*, 1979).

Table 8.15 Typical polyphenols in tobacco.

	Virginia (mg/g)	Burley (mg/g)
Chlorogenic acids	34.71	12.83
Rutin	7.95	4.00
Scopoletin	0.13	0.06
Scopolin	0.94	0.35

Source: Sheen, *et al.* (1979).

The chlorogenic acids are primarily 3-O-caffeoylquinic acid with lesser amounts of the 5- and 6- linked isomers. Scopolin and scopoletin are the major coumarin compounds in tobacco.

(b) Lignin

Lignin comprises as much as 4 to 5% of tobacco weight and is the most abundant natural organic aromatic polymer found in the vascular plant kingdom. With cellulose and hemicellulose, lignin is one of the major cell wall components in leaf. Lignin is composed of a chain of polymeric phenolic constituents, such as

coniferyl alcohol, p-coumaryl- and synapyl alcohol. In many reviews on tobacco, lignin has been classified with 'carbohydrates' due to its association with, and being bound to hemicellulose. In woody plants, lignin acts as a cementing agent to help bind the matrix of cellulose fibers (Goheen & Hoyt, 1985).

Lignin is known to produce high levels of phenolic constituents on thermolysis, such as 2-propylphenol, vanillin, eugenol, guaiacol, pyrogallol and cresols, which are found in tobacco smoke.

(c) Other phenolics

Many more phenolics are found in tobacco smoke than in leaf, but the major isolated leaf phenols are shown in Table 8.16 (Wilson, *et al.*, 1982), albeit they are present in low quantities in leaf.

Table 8.16 Major phenolics isolated from steam distillation of tobacco types (parts per billion in leaf).

	Virginia	Burley	Oriental
Phenol	16	13	39
o-Cresol	9	4	11
m-Cresol	26	8	46
p-Cresol	31	4	18
Guaiacol	80	23	28
Dimethylphenols	9	3	15
4-Vinylphenol	54	2	161
2-Acetyl-3-methylphenol	—	2	2
Trimethylphenols	—	5	4
4-Ethylguaiacol	—	1	8
4-*t*-Butylphenol	1	1	8
Eugenol	0.5	—	—
4-Vinylguaiacol	14	3	8
4-Allyl-2,6-dimethylphenol	52	0.3	2

Source: Wilson, *et al.* (1982).

In addition, the vanilla-like phenolic, vanillin, has been isolated from the steam distillation of a tobacco blend (Green, *et al.*, 1980). It is probable that some of the phenolics isolated from tobacco by steam distillation may arise from lignin hydrolysis and, in fact, may be artifacts.

Phenolics are powerful aroma compounds and are considered to contribute significantly to smoke quality.

TOBACCO STEROLS

Sterols, steryl esters and esterified steryl glucosides comprise about 0.1 to 0.3% of tobacco weight. The distribution of sterols for both green and cured leaf has been studied and they can vary greatly within the tobacco plant. The major sterols are cholesterol, campesterol, stigmasterol and β-sistosterol. Table 8.17 provides the amounts of total individual sterols (after hydrolysis) reported in a sample of Virginia tobacco (Ellington, *et al.*, 1977) and in two lines of burley tobacco (Davis, 1976).

Table 8.17 Major sterols in tobacco.

		Burley lines	
	Virginia (mg/g)	L-1 (mg/g)	L-7 (mg/g)
Cholesterol	0.30	0.18	0.21
Campesterol	0.53	0.23	0.33
Stigmasterol	0.75	0.72	0.82
Sistosterol	0.88	0.38	0.94
Total sterols	2.46	1.51	2.30

Source: Davis (1976).

Flue-cured, burley and Maryland tobaccos have been reported to have higher sterol contents than Oriental, fire-cured and cigar leaf tobaccos (Johnstone & Plimmer, 1959).

Sterols in tobacco are considered to be a potential health negative in that the parent polycyclic structure may form polynuclear hydrocarbons on pyrolysis (e.g. stigmasterol forms benzo(a)pyrene at 750°C) (Johnstone & Plimmer, 1959; Stedman, 1968).

INORGANICS

Significant amounts of calcium, potassium, magnesium, chlorine, phosphorus and sulfur are found in the ash of tobacco (as well as sodium), as shown in Table 8.1. In addition, both free and complexed ammonia and nitrate are found in tobacco. The topic of ammonia in tobacco has previously been discussed in this section.

The tobacco content of these inorganics is in large part due to soil and fertilizer practices. Lower amounts of many other inorganic anions (e.g. zinc, aluminum titanium, strontium, silicon, rubidium, nickel, manganese, lithium, lead, iron, copper, chromium, cesium, boron, barium and arsenic) have been found in tobacco (Johnstone & Plimmer, 1959; Stedman, 1968). The presence of the major metallic anions certainly impacts (for example, magnesium and potassium accelerate) the burning characteristics of tobacco leaf and the ash-

holding properties of cigarettes. Phosphorous and chloride tend to slow the burning process. In fact, high chloride levels provide both negative burning and taste characteristics.

Tobaccos with high nitrate content are assisted in the burning process as nitrate is itself a combustible material. In air-cured tobaccos, the nitrate tends to accumulate in the stem portion and is considered a major reason why the smoking properties of burley stems are inferior to Virginia stems. For many years, major cigarette manufacturers 'washed' their burley stems to reduce the nitrate content. This served two purposes: improved the smoking properties and removed the nitrate oxidant which lowered the level of nitrosamines in smoke.

The trace elements (such as iron) in tobacco can have a profound effect on tobacco quality. For example, iron has been implicated in the speckling effects that develop in the so-called 'grey tobacco' deficiency of Virginia tobacco (Tso, 1972; Tso, 1990).

SUMMARY

Tobacco is a complex plant material from a chemical composition standpoint. No other plant material has been studied more extensively in the history of man. Yet even today, the quality of tobacco is judged largely by empirical experience and subjective sensorial evaluation. It is not possible to replicate the smoking properties of the tobacco leaf synthetically. Yet, through chemical knowledge and genetic advances, there is now a much better understanding of tobacco growth and important constituents that allows for improvements in quality using the best laboratory in the world – through optimization of the biology of tobacco itself in the tobacco growing field.

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