Tobacco Research 39 (2) : 93-100, 2013

NEUTRAL VOLATILE COMPOUNDS IN FCV TOBACCO CULTIVARS AND BREEDING LINES

C.V.N. SRIHARI*, K. SIVA RAJU, T.G.K. MURTHY, K. SARALA AND C.V. NARASIMHA RAO[1]

Central Tobacco Research Institute, Rajahmundry-533 105

(Received on 10th November, 2013)

Smoking quality of flue-cured Virginia (FCV) tobacco can be assessed by the relative abundance of the volatiles that vary with genotype and stalk position. Hence, gas chromatograph-mass spectrometry (GC-MS) analysis of neutral volatile compounds (NVCs) was undertaken in FCV tobacco ruling varieties and promising advanced breeding lines undergoing advanced varietal trials and pre-release bulk assessment in northern light soils (NLS) and traditional black soils (TBS) at CTRI Research Station, Jeelugumilli and CTRI Farm, Katheru, respectively. One hundred and thirty compounds were identified and their relative content in the neutral volatile fraction (NV) was calculated. The compounds were segregated into four groups *viz.*, nor-carotenoids, nor-isoprenoids, nor-thunberganoids and neophytadiene which accounted for 79 to 92% of the NV fraction in different samples. The remaining 8 to 21% of the NV fraction was composed of products of phenylalanine metabolism and Maillard reactions. Samples from TBS, had lower percentage of nor-isoprenoids and nor-carotenoids in the NV fraction than the samples from NLS and a similar trend was observed in the case of neophytadiene. However, the trend was reverse in the case of nor-thunberganoids. The qualitative and quantitative differences between the samples from NLS and TBS could be attributed to the genetic makeup of the samples and crop husbandry. Considering the proportion of the 25 most important compounds in the NV fraction, volatile profiles of different samples were prepared to help Plant Breeders in evaluating breeding material. Two somaclones, TOBIOS 6 and TOBIOS 7 and the advanced breeding lines, NLST 3 and NLST 5 had higher relative content of NVCs compared to the cultivar, Kanchan in NLS, thus exhibiting their potential for more flavour. In TBS, the inter-specific cross derivative TBST 2 had a volatile profile superior to the ruling variety, Siri. Higher relative content of neophytadiene, geranyl acetone, isomers of megastigmatrienone, 3-hydroxy solavetivone, benzyl alcohol, phenylethyl alcohol was recorded in the T-position in the samples from NLS when compared to L-position samples.

*Ph.D Scholar

[1]Retired Principal Scientist, CTRI, Rajahmundry

INTRODUCTION

A survey of the literature on aroma-bearing constituents reveals that the 300-odd volatile compounds, identified so far do not fall into a single class of compounds but represent a wide array of chemical entities like degradation products of thunberganoids, carotenoids and labdanoids, basic nitrogenous compounds, acidic substances, simple carbonyls and several types of neutral compounds. Wahlberg and Enzell (1987) reported that two major classes of diterpenoids *viz.*, monocyclic cembranoids and the bicyclic labdanoids are found in tobacco. Virginia and Burley tobaccos contain only the cembranoids, while Oriental and Cigar tobaccos contain both the labdanoids and cembranoids. It is reported that about 41 compounds are found in substantial quantities to enable the smoker to perceive their impact on smoke flavour. The compounds are broadly classified as acids, alcohols, aldehydes, amides, anhydrides, esters, ethers, hydrocarbons, ketones and lactones. As the aroma-bearing constituents are basically volatile in nature, an objective evaluation involves either of the three basic approaches: (i) steam distillation, subsequent extraction and fractionation, (ii) direct extraction with solvents like chloroform and fractionation and (iii) head-space vapour collection fraction. A micro-analytical technique like gas chromatograph-mass spectrometry (GC-MS) is generally employed, as the individual component of the isolates is in minute quantity. Sakaki *et al.* (1986) observed that smoking quality of flue-cured tobacco can be evaluated by the relative abundance of the volatiles that are related to tobacco variety and stalk position.

As a part of comprehensive investigations on the chemical constituents responsible for smoke flavour in Indian tobacco, particularly FCV tobacco, the present study was taken up with the objective of assessing the status of neutral volatile compounds (NVCs) in FCV tobacco ruling varieties and breeding material.

MATERIALS AND METHODS

FCV tobacco samples representing northern light soils (NLS) and traditional black soils (TBS) were collected from Plant Breeding experiments conducted during the 2012-13 crop season from CTRI Research Station, Jeelugumilli, Andhra Pradesh (AP) and CTRI Farm, Katheru, AP, respectively. At both the centres, the experimental crop was raised as per the recommended package of practices. Cured leaf samples were collected from the Bulk Assessment Trial and Advanced Varietal Trial 2 conducted at Jeelugumilli with genotypes *viz.,* Kanchan (cultivar), TOBIOS 2, TOBIOS 6 and TOBIOS 7 (somaclones), NLST 3, NLST 4, NLST 5 and NLST 6 (advanced breeding lines) and NLSH 1 (hybrid). Leaf samples of TOBIOS 6, TOBIOS 7, NLST 5, NLST 6 and Kanchan were collected from the leaf (L) and tip (T) positions of the plant. Similarly, leaf samples of the ruling cultivar, Siri and the stabilized advanced inter-specific cross derivative, TBST 2 were collected from Rajahmudry. Mid-ribs were removed from the leaf and the lamina portion was dried at 60°C in an oven. The dried leaf lamina was powdered in a Wiley mill to pass through a 0.1 mm sieve, 10 g of powder was subjected to steam distillation, the distillate was extracted with dichloromethane and concentrated to 1 ml (Wu *et al.*, 1992). The GC-MS analysis was carried out using a QP 2010 Plus GC-MS system equipped with AOC - 20i auto sampler (Single quadrupole, Shimadzu Corporation, Kyoto, Japan). A ZB-5 MS (5% Phenyl, 95% Dimethyl polysiloxane) (Zebron™ – Phenomenex, USA) capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness was used. The column oven temperature was programmed to rise from an initial temperature of 60°C (held for 1 min) to 140°C (held for 5 min) @ 6°C/min, from 140°C to 180°C (held for 5 min) @ 6°C/min and to a final temperature of 210°C @ 6°C/min, the final temperature was held for 14 min with a total run time of 50 min. Helium was used as the carrier gas with a flow rate of 1 ml/min. The inlet and interface temperatures were kept at 250°C. The Electron Ionisation (EI) source was operated at 200°C and the quadrupole temperature was 150°C. All the samples were analysed in scan mode with a mass range of 50 to 500 units. One micro liter (µl) of the sample was injected in split-less mode by the auto-sampler. The obtained peaks were identified using US National Institute of Standards and Technology (NIST) standard mass spectral library database. As authentic standards of the compounds are not available for quantification, the area normalization method was adopted and the per cent of a particular compound in the total neutral volatile (NV) fraction was calculated.

RESULTS AND DISCUSSION

One hundred and thirty compounds were identified and their relative content in the NV fraction was calculated. Also, the relative content of the major classes *viz.,* nor-carotenoids (dihydroactinidiolide, isophorone, megastigmatrienones, 3-hydroxy-β-damascone, β - and â-ionone, 3-oxo-β-ionol etc.), nor-isoprenoids (3-hydroxy solavetivone, solavetivone, geranyl acetone, geraniol, caryophyllene, linalool, citronellol, α-terpineol, α-cedrene, α-elemene etc.), nor-thunberganoids (solanone, norsolanadione, duvatrienediol, thunbergol etc.) and neophytadiene was computed by grouping the listed compounds falling under the respective class (Figs. 1-4). It is inferred from the data that the four classes accounted for 79 to 92% of the NV fraction in the samples analysed. The remaining 8 to 21% of the NV fraction was composed of the following compounds: benzyl alcohol, benzaldehyde, phenylethyl alcohol and phenylacetaldehyde (products of phenylalanine metabolism), furfural, 2-acetylpyrrole and 2-acetylfuran (products of Maillard reactions), globulol, epiglobulol, phytol, nootkatane etc. Huang *et al.* (2006) reported that 102 volatile compounds among 138 separated peaks were identified and quantified accounting for about 88.9% of the total content. Zhu *et al.* (2009) have identified and quantified 39 volatile components of the tobacco flavour samples accounting for 86.54% of the total content. In the FCV tobacco samples from NLS, KLS and SLS, the identified NVCs accounted for 83.4, 93.2 and 89.4% of the total fraction, respectively (Srihari *et al.*, 2013).

Higher relative content (12.1%) of nor-isoprenoids was recorded in the cultivar Kanchan, followed by TOBIOS 7, NLST 5, Siri and TBST 2. In case of TBS samples, the value was ~8.5%, the principal compounds being, 3-hydroxy solavetivone, geranyl acetone and farnesyl acetate in this group. The per cent of nor-carotenoids was high (12.4%) in TOBIOS 6, followed by TOBIOS 7, NLST 3, NLST 5 and Kanchan. The isomers of megastigmatrienone were the principal constituents in the group. Generally, the degradation products of carotenoids were low (~6.0%) in black soil grown genotypes, TBST 2 and Siri. Among all the 11 samples, the proportion of thunberganoids in the NV fraction was very high (29.1 and 39.0%) in TBS samples. The thunberganoids proportion was comparatively low in NLS samples (13.9 – 23.3%). Duvatrienediol and thunbergol were the important nor-thunberganoids. The relative content of neophytadiene was high (53.9%) in NLST 6 followed by NLSH 1, TOBIOS 2 and Kanchan while the value was low (38.3%) in Siri.

From the foregoing it can be inferred that the samples from black soils, Siri and TBST 2 had lower percentage of nor-isoprenoids and nor-carotenoids in the NV fraction than the material from light soils and a similar trend was observed in the case of neophytadiene. However, the trend was reverse in the case of nor-thunberganoids. The qualitative and quantitative differences between the samples from NLS and TBS are evident from the analysis which could be attributed to the genetic makeup of the samples and crop husbandry. In NLS, production practices *viz.*, fertilization (N: 115 kg/ha; K_2O: 100 kg/ha), irrigation and bud topping at 24 laves contribute to higher levels of leaf nicotine and total nitrogen. Whereas in TBS, the crop is grown on conserved moisture, untopped and is applied with 45 kg N/ha, resulting in leaf with medium nicotine and nitrogen contents. Kameswara Rao *et al.* (1989) reported that light soil tobacco always showed higher total carotenoid levels and the per cent degradation was also higher, though not significant, than black soil tobacco grown in India. According to Court *et al.* (1984) nitrogen fertilization increased neophytadiene in flue-cured tobacco. Shi *et al.* (2009) observed that flavour quality of tobacco is highly correlated with nicotine and total nitrogen contents. In tobacco green leaf, â-carotene, lutein, violaxanthin and neoxanthin are the four major carotenoids and the total carotenoids decrease from 1000 to 100 ppm subsequent to maturity, senescence, curing and ageing. An inverse relationship was found between flavour and carotenoid content and flavour development is attributed to the extensive degradation of carotenoids in the aroma-rich leaves (Roberts *et al.*, 1973). Lutein which constitutes 40-60% of total carotenoids is considered as the precursor of megastigmatrienones, an important class of flavour compounds described as the heart of aromatics in the volatile fraction (Sheen and Calvert, 1969). The differences in nor-thunberganoids between the two sets of samples could be attributed to the differences in genetic makeup of TBS and NLS cultivars, as production of diterpenoids was postulated to be species-specific. The putative progenitors of *N. tabacum viz., N. sylvestris* and *N. tomentosiformis* produce diterpenoids of the thunbergane type and the labdane type, respectively (Enzell, 1977). Neophytadiene, the most predominant diterpene and the major constituent of the NV fraction, originates from phytol derived from chlorophyll which is destroyed during curing (Rowland, 1957). Chortyk *et al.* (1975) have reported that neophytadiene is associated with smoke quality as it is transferred directly into the smoke in appreciable quantities. Neophytadiene is associated with a soothing and smoothing effect upon smoke (Green, 1977).

After analyzing data on the relative content of the four major classes of compounds, attention was focused on the proportion of the 25 most important compounds in the NV fraction of different samples from L-position to prepare volatile profiles which can help tobacco breeders in evaluating breeding material (Table 1). In NLS, three samples were selected and arranged in the descending order of the proportion of NVCs in the L-position and the impact of the compounds on smoking quality is included (Table 2). Further, relative content of the fungi-toxic terpenoid, 3-hydroxy solavetivone was higher in the advanced breeding lines (NLST 5 > NLST 4 > NLST 6). In the samples from TBS, the proportion of neophytadiene, 3-hydroxy solavetivone, (1s,2E,4s,5R,7E,11E)-cembra-2,7,11-trien-4,5-diol, geranyl acetone, norsolanadione, dihydroactinodiolide, phenylacetaldehyde and benzaldehyde was high in TBST 2, while the relative content of megastigmatrienones, 3-oxo-α-ionol, thunbergol, duvatrienediol and valerenol was more in Siri. Wu *et al.* (1992) have studied the contribution of neutral volatiles to flavour intensity of tobacco during smoking by analyzing 20 compounds. Apart from the 25 compounds listed in Table 1, another set of 105 compounds were identified in some of the samples in the present study (Table 3) and their relative content ranged from 0.01 to 0.50%.

Leaf samples of TOBIOS 6, TOBIOS 7, NLST 5, NLST 6 and Kanchan were collected from the T-position also for the analysis of NVCs. It is inferred from the analysis results of NLS samples (Table 4) that in general, higher relative content of neophytadiene, geranyl acetone, megastigmatrienone isomers, 3-hydroxy solavetivone, benzyl alcohol, phenylethyl alcohol was recorded in the T-position when compared to L-position samples, whereas the trend was opposite in the case of duvatrienediol. Davis (1976) reported that leaves from the upper stalk position contain greater quantities of neophytadiene and also its content is more in flue-cured tobacco than air-cured tobacco. Crude lipid, chlorophyll and carotenoids were higher in top leaves than in bottom leaves (Grunwald *et al.*, 1977). Further, the ionones, megastigmatrienones, damascones and damascenones are considered to be the most important carotenoid derivatives found in cigarette smoke. According to Jing *et al.* (2010), most aroma compounds of mature tobacco leaves increased first with increasing leaf position from 8th up to 12th leaf and maximum compounds were found at the 10th leaf position.

It is concluded that the somaclones, TOBIOS 6 and TOBIOS 7, the advanced breeding lines, NLST 3 and NLST 5 had higher relative content of NVCs compared to the cultivar, Kanchan in NLS exhibiting their flavour potential, with higher proportion of NVCs in the samples from the T-position. In TBS, the inter-specific cross derivative TBST 2 had a volatile profile superior to the ruling variety, Siri.

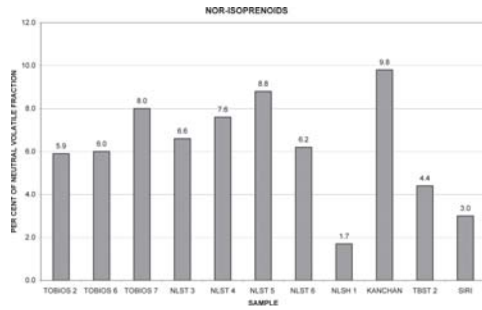

Fig. 1: Nor-isoprenoids in FCV tobacco L-position

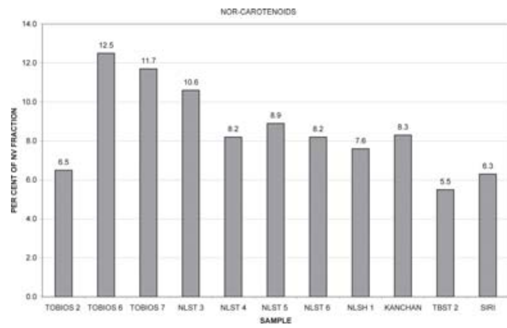

Fig. 2: Nor-carotenoids in FCV tobacco L-position

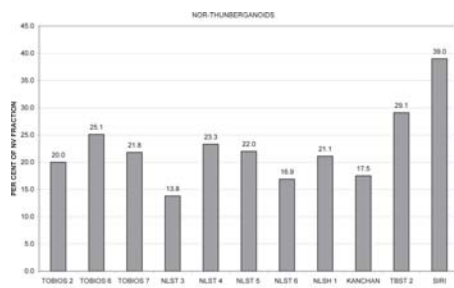

Fig. 3: Nor-thunberganoids in FCV tobacco L-position

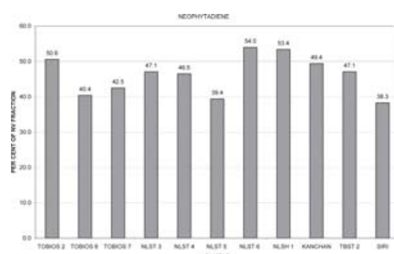

Fig. 4: Neophytadiene in FCV tobacco L-position

Table 1: Volatile profiles FCV tobacco (L position)

S.No.	Compound	TOBIOS 6	TOBIOS 7	NLST 5	NLST 6	TOBIOS 2	NLST 3	NLST 4	NLSH 1	KACHAN	Mean	TBST 2	SIRI	Mean
1	(1s,2E,4s,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	1.87	1.55	2.05	0.85	1.59	0.63	2.05	0.65	1.28	1.39	2.85	1.68	2.27
2	Furfural	0.18	0.32	0.17	0.15	0.17	0.24	0.17	0.17	0.17	0.19	0.10	0.10	0.10
3	Benzaldehyde	0.09	0.12	0.06	0.06	0.05	0.07	0.08	0.07	ND	0.08	0.30	0.18	0.24
4	Benzyl alcohol	0.75	0.54	0.56	0.49	0.56	0.87	0.60	0.51	0.47	0.59	0.32	0.51	0.42
5	Phenylacetaldehyde	0.27	0.33	0.17	0.24	0.23	0.33	0.19	0.26	0.39	0.27	0.70	0.50	0.60
6	2-Acetylpyrrole	0.15	0.12	0.12	0.05	0.12	0.12	0.12	0.19	0.21	0.14	0.09	0.13	0.11
7	Phenethyl alcohol	0.70	0.48	0.55	0.42	0.43	0.70	0.46	ND	0.40	0.52	0.37	0.40	0.39
8	Indole	0.16	0.14	0.14	0.08	ND	0.13	0.13	0.05	0.14	0.12	0.13	0.11	0.12
9	Geranyl acetone	1.14	1.15	0.84	0.71	1.22	1.15	0.80	0.92	0.98	0.99	3.55	2.53	3.04
10	Farnesyl acetate 2	1.69	2.19	1.95	1.24	1.23	1.47	ND	1.47	1.69	1.62	4.16	2.94	3.55
11	Neophytadiene	40.36	42.47	39.38	53.95	50.58	47.06	46.49	53.42	49.40	47.01	47.09	38.28	42.69
12	Nootkatane ±	2.46	2.39	3.22	2.98	3.20	1.80	3.84	1.97	3.20	2.78	0.16	0.09	0.13
13	Nootkatone	0.38	0.42	0.51	0.39	0.40	0.26	0.53	0.24	0.71	0.43	ND	ND	ND
14	Epiglobulol	1.84	1.93	2.16	2.16	1.67	1.36	2.43	1.94	2.57	2.01	2.07	3.92	3.00
15	Phytol	0.78	0.66	0.69	0.48	ND	0.64	0.74	1.09	0.72	0.73	ND	ND	ND
16	Isophytol	0.13	0.24	0.23	0.25	0.04	0.12	0.14	0.04	0.20	0.15	0.18	0.14	0.16
17	Valerenol	0.65	0.59	0.11	0.09	0.36	0.10	0.44	0.04	0.42	0.31	ND	0.60	0.60
18	α-Elemene	0.76	1.83	1.69	1.30	0.71	1.38	1.58	0.47	1.20	1.21	ND	ND	ND
19	3-Hydroxysolavetivone	3.34	3.29	5.60	3.70	2.75	2.93	4.61	1.86	4.76	3.65	0.24	0.21	0.23
20	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	1.94	1.86	1.30	1.41	0.27	1.79	1.24	0.88	0.75	1.27	ND	0.17	0.17
21	Dihydroactinidiolide	0.57	0.52	0.49	0.35	0.44	0.44	0.35	0.29	0.50	0.44	0.67	0.63	0.65
22	Megastigmatrienone isomers	9.01	7.76	6.58	5.91	5.04	7.37	5.60	5.11	6.75	6.57	4.00	4.89	4.45
23	3-Oxo-α-ionol	0.72	0.57	0.61	0.43	0.52	0.52	0.61	0.36	0.59	0.55	0.30	0.43	0.37
24	Duvatriendiol	14.59	13.30	13.92	9.82	13.08	8.42	10.88	10.54	13.12	11.96	17.55	19.32	18.44
25	Thunbergol	9.91	8.47	7.76	3.35	6.33	5.44	6.87	6.24	6.96	6.81	10.32	14.39	12.36

Table 2: NLS samples selected on the basis of relative content (%) of important neutral volatile compounds and their impact on smoking quality

Compound	Impact	Samples in the descending order
Neophytadiene	Soothing, smoothing*	NLST 6 > NLSH 1 > TOBIOS 2
Isomers of megastigmatrienone	Spicy, peppery, add body**	TOBIOS 6 > TOBIOS 7 > NLST 3
Solanone	Smooth, ketonic**	NLST 6 > NLST 5 > TOBIOS 7
Geranyl acetone	Green, adds body**	TOBIOS 2 > TOBIOS 7 > TOBIOS 6
3-Oxo-α-ionol	Sweet, adds flue-cured body**	TOBIOS 6 > NLST 5 = NLST 4
Dihydroactinidiolide	Slight cooling**	TOBIOS 6 > TOBIOS 7 > NLST 5
Benzyl alcohol	Weak, floral, smoothing**	NLST 3 > TOBIOS 6 > NLST 4
Benzldehyde	Almond, cherry	TOBIOS 7 > TOBIOS 6 > NLST 4
Phenylethyl alcohol	Floral, rose**	TOBIOS 6 = NLST 3 > NLST 5
Phenylacetaldehyde	Intense floral**	TOBIOS 7 = NLST 3 >TOBIOS 6
Furfural	Sweet, buttery**	TOBIOS 7 > NLST 3 > TOBIOS 6
2-Acetylpyrrole	Floral, green, winey, adds body**	NLSH 1 > TOBIOS 6 > TOBIOS 7
α-Elemene	Floral	TOBIOS 7 > NLST 5 > NLST 3
Duvatrienediol	Oxidation products (solanone, oxysolanone, branched chain volatile acids) contribute to smoke flavour***	TOBIOS 6 > TOBIOS 7 > NLST 5
Indole	Smoothing, floral**	TOBIOS 6 > TOBIOS 7 = NLST 5

References: *Green (1977); **Leffingwell (1972); ***Demole and Dietrich (1977)

Table 3: Other neutral volatile compounds in FCV tobacco

S.No.	Compound	S.No.	Compound
1	Abienol	25	cis- Tetrahydro-3,4-furandiol
2	2-Acetylfuran	26	Ethyl linoleolate
3	Safranal	27	Hexahydrothunbergol
4	α-Cyclocitral	28	4,5,9,10-Dehydro-isolongifolene
5	Methylethylmaleimide	29	5-Methyl furfural
6	Cembrene	30	Rishitin
7	Nerol	31	2,5-Dimethyl-2,6-heptadiene
8	Globulol	32	3a,4,5,6-Tetrahydro-3a,6,6-trimethyl-2 (3H)-benzofuranone
9	Isolongifolol	33	trans-Geraniol
10	Longifolenaldehyde	34	Geranial
11	Cycloisolongifol-5-ol	35	Geranyl isobutyrate
12	Sativene	36	Geranyl isovalerate
13	1-Heptadecanol	37	Geranyl linalool isomer B
14	5-Methyl-2(5H)-furanone	38	4-Oxo-α-isodamascol
15	5-Methyl-2-furfural	39	Isoborneol
16	2-Methyl-2-hepten-6-one	40	Longiborneol
17	3-(4,8,12-Trimethyltridecyl) furan	41	α-Tumerone
18	2,5-Dihydro-2,5-dimethyl furan	42	Isobutyl phthalate
19	2,3-dihydro-4-(1-methylpropyl)-furan	43	Carvone
20	2,5-Dimethyl furan	44	E-Citral
21	2,3-Dihydro- benzofuran	45	cis-Limonene oxide
22	3,6-Dimethyl-2,3,3A,4,5,7A-hexahydrobenzofuran	46	Coumaran
23	3-Furaldehyde	47	Clovane
24	1-(2-Furanyl)but-3-ene-1,2-diol	48	Dehydrolinalool

Contd.

S.No.	Compound
49	Farnesol
50	DL-6,7-Dihydro-2-cis-farnesol
51	trans-Farnesol
52	Hexahydrofarnesol
53	Farnesol isomer B
54	(E,E)-Farnesyl acetone
55	Farnesyl acetone A
56	Farnesyl acetone B
57	Farnesyl bromide
58	Geraniol
59	Iso Valleral
60	Methyl hexadecanoate
61	Nerolidol isomer
62	D-Nerolidol
63	Nerolidol epoxyacetate
64	Nerolidyl acetate
65	9,12,15-Octadecatrien-1-ol
66	Phytol isomer
67	Valencene
68	β-Terpineol
69	L-α-Terpineol
70	(Z)-Valerenyl acetate
71	β-Cedrene
72	Ethyl linalool
73	Dehydrolinalool
74	L- Linalool
75	Solavetivone
76	α-Isophorone
77	Isophorone

S.No.	Compound
78	cis-Caryophyllene
79	trans-Caryophyllene
80	Caryophellene oxide
81	(-)-Caryophyllene-(I1)
82	3,5,5-Trimethyl-2-cyclohexen-1-one
83	2,6,6-Trimethyl-2-cyclohexene-1,4-dione
84	(E,Z)-Pseudoionone
85	β-Ionone
86	3-Keto-β-ionone
87	ã-ionone
88	Dihydro-α-ionone
89	Dihydro-β-ionone
90	Methyl ionone
91	8-Methyl-á-ionone
92	(-)-(S)-2-Hydroxy-â-ionone
93	3-Oxo-7,8-dihydro-á-ionone
94	Tetrahydroionone
95	Dihydro-β-ionol
96	Dihydro-α-ionol
97	2,3-Dehydro-4-oxo-â-ionol
98	3-Hydroxy-7,8-dihydro-â-ionol
99	Citronellol
100	Citronellyl acetate
101	Citronellal
102	Valeraldehyde
103	Solanone
104	Norsolanadione
105	Viridiflorol

Table 4: Neutral volatile compounds in leaf (L) and tip (T) positions of FCV tobacco

S. No.	Compound	TOBIOS 6 L	TOBIOS 6 T	TOBIOS 7 L	TOBIOS 7 T	NLST 5 L	NLST 5 T	NLST 6 L	NLST 6 T	KANCHAN L	KANCHAN T
1	Benzyl alcohol	0.8	0.9	0.5	0.7	0.6	0.7	0.5	0.8	0.5	0.8
2	Phenylethyl alcohol	0.7	0.9	0.5	0.7	0.6	0.7	0.4	0.7	0.4	0.7
3	Geranyl acetone	1.1	1.0	1.2	0.8	0.8	1.0	0.7	0.9	1.0	0.9
4	Neophytadiene	40.4	54.1	42.5	51.3	39.4	47.3	54.0	45.0	49.4	54.2
5	3-Hydroxysolavetivone	3.3	4.8	3.3	1.6	5.6	3.5	3.7	2.9	4.8	4.3
6	Megastigmatrienone isomers	9.0	8.1	7.8	8.0	6.6	9.7	5.9	6.9	6.8	8.5
7	Duvatriendiol	14.6	8.5	13.3	9.1	13.9	13.1	9.8	12.9	13.1	8.6

ACKNOWLEDGEMENTS

The first author expresses profound thanks to the Director, Central Tobacco Research Institute, Rajahmundry for according permission and providing facilities for carrying out the investigations. The support extended by the Head, Division of Crop Chemistry and Soil Science is gratefully acknowledged. Also, the help rendered by Sri N. Johnson, Technician TI in the GC-MS analysis is sincerely acknowledged.

REFERENCES

Chortyk, O.T., R.F. Severson and H.C. Higman. 1975. Chromatographic determination of hydrocarbon waxes in tobacco leaf and smoke. **Beitr. Tabakforsch. Int.** 8: 204-10.

Court, W.A., J.M. Elliot and J.G. Hendel. 1984. Influence of applied nitrogen fertilization on certain lipids, terpenes and other characteristics of flue-cured tobacco. **Tob. Sci.** 28: 69-72.

Davis, D.L. 1976. Waxes and lipids in leaf and their relationship to smoking quality and aroma. **Rec. Adv. Tob. Sci.** 2: 80-106.

Demole, E. and P. Dietrich. 1977. The chemistry of burley flavor. **Proc. of American Chemical Society Symp. Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke**. The 173rd ACS Meeting, New Orleans, Louisiana. pp. 1-36.

Enzell, C.R. 1977. Recent progress in the chemistry of sun-cured tobacco. **Proc. of American Chemical Society Symp. Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke**. The 173rd ACS Meeting, New Orleans, Louisiana. pp. 37-77.

Green, C. 1977. Relationship of leaf composition to smoke composition. **Proc. of American Chemical Society Symp. Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke**. The 173rd ACS Meeting, New Orleans, Louisiana. pp. 426-71.

Grunwald, C., L. Simsi and S.J. Sheen. 1977. Effects of nitrogen fertilization and stalk position on chlorophyll, carotenoids and certain lipids of three tobacco genotypes. **Can. J. Plant Sci.** 57(2): 525-35.

Huang, L.F., K.J. Zhong, X.J. Sun, M.J. Wu, K.L. Huang, Y.Z. Liang, F.Q. Guo and Y.W. Li. 2006. Comparative analysis of gas chromatography-mass spectrometry (GC-MS) and combined chemometric methods. **Analytica Chimica Acta** 575: 236-45.

Jing, Y.Q., H.L. Zhang, Y.X. Yang, Q. Wu, L. Jin, C.L. Liu and Y.F. Tian. 2010. Analysis of neutral aroma component in different leaf positions of flue-cured tobacco. **J. Hunan Agril. University** 36(2): 151-4.

Kameswara Rao, B.V., S.R. Prabhu and V. Ushasri. 1989. Total carotenoids levels of Indian FCV tobacco. **Tob. Res.** 15: 59-64.

Leffingwell, J.C. 1972. *Tobacco Flavoring for Smoking Products*. R.J. Reynolds Tobacco Company, Winston-Salem, NC, USA. pp. 11-60.

Roberts, D.L., C.W. Miller and R.A. Lloyd. 1973. Tobacco carotenoids. 27th Tobacco Chemists Research Conference, Winston-Salem, NC. October 3-5.

Rowland, R.L. 1957. Flue-cured tobacco. II. Neophytadiene. **J. Amer. Chem. Soc.** 79: 5007-10.

Sakaki, T., K. Niino, H. Sakima and S. Sugawara. 1986. Relationship between tobacco headspace volatiles and their smoking quality. **Agric. Biol. Chem.** 50(2): 317-23.

Shi, H.Z., H.H. Di, X.D. Zhao, G.S. Liu, Y.J. Ma, W.C. Wang and J. Duan. 2009. Relationship of nicotine and total nitrogen contents with neutral aroma components in flue-cured tobacco in Central area of Henan Province. **Acta Agronomica Sinica** 35(7): 1299-305.

Sheen, S.J. and J. Calvert. 1969. Studies on polyphenol content activities and isozymes, polyphenol oxidase, peroxidase during air-curing in three tobacco types. **Plant Physiol.** 44: 199-204.

Srihari, C.V.N., C.V. Narasimha Rao and N. Johnson. 2013. Neutral volatile compounds in FCV tobacco. **Tob. Res.** 39: 35-40.

Wahlberg, I. and C.R. Enzell. 1987. Tobacco isoprenoids. **Natural Products Reporter** 4(3): 237-76.

Wu, Z., W.W. Weeks and R.C. Long. 1992. Contribution of neutral volatiles to flavour intensity of tobacco during smoking. **J. Agri. Food Chem.** 37: 1038-45.

Zhu, X.L., Y. Gao, Z.Y. Chen and Q.D. Su. 2009. Development of a chromatographic fingerprint of tobacco flavoring use of GC and GC-MS. **Chromatographia** 69: 735-42.