Tobacco Research 39 (2) : 76-85, 2013

ORGANIC AND SMOKE CONSTITUENTS IN HDBRG TOBACCO

K.V. SATYANARAYANA*, K. SIVA RAJU AND C.V. NARASIMHA RAO[1]

Central Tobacco Research Institute, Rajahmundry -533 105

(Received on 22nd November, 2013)

In order to generate information on a broad spectrum of leaf chemical constituents and smoke constituents in HDBRG tobacco, leaf samples were collected during 2009-10 and 2010-11 seasons from bulk crop grown at CTRI Research Station, Guntur, Andhra Pradesh for analysis. Carbonyls ranged from 590.8 to 770.5 mg/100 g with an overall mean of 678.3 mg/100 g. The mean carotenoid content was in the range of 0.55 to 0.68 mg/g. The levels of chlorogenic acid (5.66 mg/g) and rutin (6.01 mg/g) were on a par and the total polyphenol content was ~1.2%. Total volatile bases (TVB) content was higher (1.05%) and comparatively total volatile acids (TVA) content was lower (0.68%). Malic acid (2.75%) was the important non-volatile acid followed by oxalic (2.30%) and citric (1.87%) acids. Among the volatile acids, isovaleric acid (47.66%) and β-methyl valeric acid (24.92%) were the major acids. Palmitic, linolenic and linoleic acids accounted for 81% of the fatty acid fraction. The proportion of saturated and unsaturated fatty acids was 55 and 45%, respectively. In the smoke, tar, nicotine and carbon monoxide values were 19.42, 3.05 and 15.02 mg/cig, respectively. Higher carbonyl content compared to other non-FCV tobacco types, higher proportion of isovaleric acid and β-methyl valeric acid and lower citric acid level in the non-volatile acid fraction can be considered as the positive attributes of HDBRG tobacco smoking quality.

INTRODUCTION

A wide array of tobacco types viz., flue-cured Virginia (FCV), Burley, Oriental, *Bidi*, *Natu*, *Lanka*, *Hookah*, Chewing and HDBRG are produced in varied agro-ecological conditions in India. These tobacco types have distinct physical, chemical and organoleptic characteristics which are primarily governed by the soil, climate, variety and crop husbandry. The important chemical constituents are alkaloids, carbohydrates, nitrogenous compounds, acids, bases and lipids influencing the leaf quality. Some of the compounds have a positive impact on the leaf aroma and smoke flavour, while the others have negative impact. Scientists of Central Tobacco Research Institute, All India Network Research Project on Tobacco and R & D Centres of tobacco industry have made comprehensive studies on the chemistry of FCV, *Bidi*, *Natu*, *Lanka* and Chewing tobaccos. However, except for the data on chemical quality parameters *viz.*, nicotine, reducing sugars and chlorides, there is paucity of information on the chemistry of HDBRG tobacco. Hence, comprehensive investigations were taken up to generate information on a broad spectrum of leaf chemical constituents and smoke constituents. In this paper data on polyphenols, carbonyls, carotenoids, total volatile acids & bases, volatile & non-volatile acids, fatty acids and smoke constituents are presented.

MATERIALS AND METHODS

Sample collection

Nine HDBRG tobacco leaf (~1 kg) samples each from the mid-stalk position were collected during 2009-10 and 2010-11 seasons from bulk crop grown at CTRI Research Station, Guntur, Andhra Pradesh. 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 0.1mm sieve for the analysis of different chemical constituents *viz.*, total polyphenols, total carbonyls, carotenoids, total volatile bases (TVB), total volatile acids (TVA), non-volatile acids, volatile acids and fatty acids. polyphenols, carbonyls and carotenoids were analysed in all the nine samples each from the two seasons. A composite sample was prepared by thoroughly mixing the nine samples and dividing into three parts for analysis of TVB, TVA, non-volatile and fatty acids.

*Ph.D Scholar

[1]Retired Principal Scientist, CTRI, Rajahmundry

Carbonyls, Carotenoids, Polyphenols, TVB and TVA

Carbonyl compounds in the samples were extracted by methanol with simultaneous carbon clean up and determined by measuring the absorbance of 2, 4 – dinitrophenylhydrazine (DNPH) derivatives at 480 nm using acetone as standard (Chakraborty and Prabhu, 1974). Carotenoids were extracted with dimethyl sulfoxide at 60°C for 3 h, cooled to room temperature and the absorbance was recorded at 510 and 480 nm (Hiscox and Iscrelston, 1979). Polyphenols (chlorogenic acid and rutin) were determined by the spectrophotometric method (Sheen, 1971) involving extraction, colour development with aluminum chloride and Arnow's reagent and recording the absorbance at 416 and 510 nm, respectively. The UV–Visible recording Spectrophotometer (Model: 160A Shimadzu, Japan) was used in all the above estimations. Total volatile bases and total volatile acids (Bacot, 1960) were estimated volumetrically by treating tobacco samples with tri sodium orthophosphate & sodium hydroxide and tartaric acid, respectively followed by steam distillation.

Non-volatile acids

Non-volatile organic acids viz., malic, citric, oxalic, fumaric and succinic were estimated as the methyl esters using methanol + sulphuric acid as the extraction and esterification solution (Harvey *et al.*, 1970). The method was modified by finally dissolving the methyl esters of standard acids and tobacco in hexane instead of chloroform. The Hewlett Packard 5890 Series II gas chromatograph (GC) with a flame ionization detector (FID) was used for the analysis. The column was a 6ft 1/8" length of stainless steel packed with 10% DEGA and 2.3% H_3PO_4 on Chromosorb W/HP (80/100 mesh), Chromato-Pak, Mumbai. Separation of the acids was achieved through an optimized oven temperature programme starting from 125°C (held for 4 min), ramped @ 20°C /min to 210°C (held for 9 min) with a total run time of 17.25 min. The inlet temperature was 250°C and the carrier gas (helium) flow was maintained at 30 ml/min. The responses in peak heights (mm) were plotted versus mg/ml concentrations for the individual acids and standard curves were obtained.

Volatile acids

For GC-MS analysis, volatile acids were recovered from the tobacco by modification of method suggested by Bacot (1960). Tobacco powder (50 g) was taken in a 1 l Kjeldahl flask, 200 ml of 10% H_2SO_4 was added, thoroughly mixed for 30 min, 100 ml distilled water was added and steam distilled to collect 500 ml of distillate. The distillate was saturated with sodium chloride and extracted with diethyl ether. The diethyl ether fraction contained all the neutral and acidic volatile constituents of tobacco. The volatile acid fraction was separated from the mixture by scrubbing with dilute NaOH solution, further liberating the volatile acids from the sodium salts by pH adjustment with dilute HCl and then extracting the free acids with diethyl ether. The ether layer was made free of mineral acid by repeated water washings. It was dried over anhydrous sodium sulphate and finally reduced to 2ml at room temperature for GC-MS analysis. In this study, an approach to directly quantify volatile acids in the complex mixtures without sample clean up and derivatization was explored (Ai, 1997). In order to improve GC peak shapes, a polar polyethylene bonded phase column (DB -WAX from J&W) was used.

The GC-MS analysis was performed using a QP 2010 Plus GC-MS system equipped with AOC-20i auto sampler (Single quadrupole, Shimadzu Corporation, Kyoto, Japan). A ZB-Wax (Polyethylene glycol) (Zebron™ – Phenomenex, USA) capillary column of 30 m length, 0.32 mm internal diameter and 0.25 µm film thickness was used. The oven was programmed from an initial temperature of 50°C (held for 3 min) to the final temperature of 240°C (held for 5 min) @ 10°C/min. Helium was used as the carrier gas with a flow rate of 1.4 ml/min. The inlet and interface temperatures were kept at 220°C. The EI source was operated at 230°C and all the samples were analysed in the scan mode with a mass range of 40 to 800 units. Sample (2 µl) was injected in split mode with the ratio of 1:20 by the auto sampler. The peaks obtained were identified using US National Institute of Standards and Technology (NIST) standard mass spectral library database. The area normalization method was adopted and the proportion of a particular volatile acid in the fraction was calculated.

Fatty acids

Hexane extractives (100 mg) were weighed into a 150 ml Erlenmeyer flask, 20 ml of the extraction and esterification solution (10% H_2SO_4 in absolute methanol) was added by pipette, the flask was stoppered, shaken for 4 hrs on a mechanical shaker and then allowed to stand overnight at room temperature. Solids were removed by filtering through glass wool, esterified extractives were transferred to a 125 ml separating funnel, 20 ml of distilled water was added and the methyl esters were extracted by two successive extractions with 10 ml portions of hexane. The extract thus obtained was passed through anhydrous sodium sulphate for removal of residual moisture and the hexane extractives were made up to 25 ml for GC-MS analysis.

The GC-MS analysis was performed 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 oven was programmed from an initial temperature 200°C (held for 3 min) to the final temperature of 220°C at the rate of 5°C/min. The final temperature was held for 23 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 EI source was operated at 225°C and all the samples were analyzed in scan mode with a mass range of 50 to 500 units. One microliter of the sample was injected in split mode with the ratio of 1:50 by the auto sampler. The peaks obtained were identified using US National Institute of Standards and Technology (NIST) standard mass spectral library database. The area normalization method was adopted and the proportion of a particular compound in the total fatty acid fraction was calculated.

Cigarette making and smoke analysis

Plain cigarettes of 70 mm length were made from uniform shreds of two HDBRG tobacco bulk samples using Laboratory model cigarette making machine (Model, Hauni Baby, Heinr-Borgwaldt, Germany) without any additives. The sample cigarettes were conditioned in a humidity cabinet at 25°C and 60% RH (ISO, 1999a). Cigarettes falling within ±20 mg of the average weight were selected using a cigarette weighing machine (Model: Cerulean QTM 8, UK). Pressure drop of the cigarettes was measured on a Pressure Drop and Ventilation apparatus (Model: Cerulean QTM 5, UK) and cigarettes of pressure drop within ±5 mm WG of the mean value were selected. The circumference of the cigarettes was measured employing a Circumference apparatus (Model: Cerulean QTM 3, UK). The cigarettes selected on weight, circumference and pressure drop and ventilation basis were smoked on a 20–port harmonized cigarette smoking machine (Model: SM 450, Cerulean, UK) following the standard parameters as per the ISO method 4387 (ISO, 2000a). Moisture (ISO, 1999b) and nicotine (ISO, 2000b) in the total particulate matter (TPM) were analyzed using GC (Model 5890 Series II, Agilent, USA). Carbon monoxide (CO) was estimated by the NDIR method (ISO, 2007) using COA205 Analyser (Cerulean, UK). Solanesol in TPM was estimated adopting the external standard method using a SIGMA standard (Narasimha Rao *et al.,* 2000).

RESULTS AND DISCUSSION

Results are presented in the tables (1-6) and discussed taking into consideration, the mean values of all the samples analysed in the two seasons.

Carbonyls

Carbonyl content of tobacco can be regarded important, as carbonyls are known to contribute to the organoleptic properties of leaf and smoke. Carbonyls are chemical constituents containing aldehyde or ketone functional group and levels of carbonyl compounds are positively correlated with the aroma of tobacco (Prabhu and Chakraborty, 1983). Carbonyls ranged from 552.3 to 809.0 mg/100 g and 625.0 to 757.6 mg/100 g during 2009-10 and 2010-11 crop seasons, respectively with an overall mean of 678.3 mg/100 g (Table 1). It is observed that though the carbonyl content was less compared to FCV tobacco, it was higher compared to other tobacco types and it is an index of the smoking quality of HDBRG tobacco.

Chakraborty and Prabhu (1974) surveyed the volatile and non-volatile carbonyl content of Indian tobaccos and observed that flue-cured tobacco (1040.4 mg/100 g) was maximum in carbonyl content followed by *bidi* (595.9 mg/100 g), burley (489.0 mg/100 g), *natu* (300.9 mg/100 g) and cigar filler (312.8 mg/100 g) types. Siva Raju (2013) reported that carbonyl compounds content varied from 189.66 to 633.43 mg/100 g among the chewing tobacco varieties grown in Tamil Nadu under different curing methods.

Carotenoids

Among the carotenoids reported in tobacco, β- carotene, lutein, violaxanthin and neoxanthin are important in green leaf. Volatile compounds viz., ionones, megastigmatrienones, damascones and damascenones are identified to be the most important carotenoid derivatives found in cigarette smoke (Roberts, 1988). The mean carotenoid content was in the range of 0.55 to 0.68 mg/g (Table 1). Siva Raju (2013) reported that carotenoid content varied from 0.56 to 0.78 mg/g among the sun-cured varieties of chewing tobacco from Tamil Nadu and the levels were higher compared to smoke-cured and pit-cured, sun-cured tobacco samples.

Polyphenols

Polyphenols are important tobacco constituents that affect the final properties and quality of the cured leaf, particularly leaf colour and are reported to be precursors of smoke phenols. It is observed from the data (Table 1) that the levels of chlorogenic acid (5.66 mg/g) and rutin (6.01 mg/g) were on a par, the total polyphenol content was ~1.2% with a ratio of ~ 1. It is reported that the total phenolic content was about ~ 7% in flue-cured tobacco, while it varied from 0 to 0.5% in air-cured tobaccos (Kameswara Rao *et al.*, 1977). The difference in phenolic composition among various types of tobacco reflects the combined effect of genetic, cultural and curing practices (Penn and Weybrew, 1968; Sheen and Calvert, 1969; Anderson *et al.,* 1970). Studying the quantitative variation of polyphenols in different types of Indian tobacco, Kameswara Rao *et al.* (1977) reported the following trend: flue-cured (chlorogenic acid: 3.52%, rutin: 1.52%) > *bidi* (chlorogenic acid: 1.39%, rutin: 0.45%) > *natu* (chlorogenic acid: 0.98%, rutin: 0.53%) > cigar filler (chlorogenic acid: 0.17%, rutin: 1.20%) > cigar wrapper (chlorogenic acid: 0.33%, rutin: 0.99%) > burley (chlorogenic acid: 0.60%, rutin: 0.40%) > chewing tobacco (chlorogenic acid: 0.15%, rutin: 0.79%). The differences in chlorogenic acid and rutin content in air-cured (lower) and flue-cured (higher) tobaccos could be attributed to the inactivation of oxidizing enzymes at the higher temperatures attained during flue-curing process giving scope for accumulation of polyphenols.

TVB and TVA

In the samples analysed (Table 2), TVB (expressed as ammonia) content was higher (1.05%) and comparatively TVA (expressed as acetic acid) content was lower (0.68%). It is reported that higher nicotine (~ 3.70%), higher nitrogen (~ 3.40%) and lower reducing sugars (~ 0.70%) in HDBRG tobacco (Satyanrayana and Narasimha Rao, 2013) and also the higher dose of nitrogen (150 kg/ha) applied to the crop are indicators of higher TVB and lower TVA contents. In the studies on chemical quality parameters of Indian *natu* tobacco, Gopalakrishna and Hanumantha Rao (1980) found an inverse relationship between TVB and total sugars, corroborating the present finding. During pyrolysis of Virginia tobacco, generation of formic acid was more than in the case of burley tobacco and significantly more ammonia was generated during the pyrolysis of burley tobacco compared to Virginia tobacco (Fenner, 1988). According to Leffingwell (1999), among the tobacco types, burley tobacco had higher TVB (0.62%) compared to FCV (0.28%), Maryland (0.37%) and Oriental (0.29%).

Non-volatile acids

It is reported that the major carboxylic acids in tobacco are citric, malic, oxalic and malonic which in total can comprise 14-18% in burley and cigar tobacco, 7-10% in Maryland, 6-8% in Oriental and 5-10% 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 (Kalianos,1976). It is inferred from the data (Table 3) that based on the mean values of all the samples

analysed, total organic acids content varied from 6.03 to 9.24% and malic acid (2.76%) was the major acid followed by oxalic (2.29%), citric (1.86%), fumaric (0.11%) and succinic acid (0.06%). Higher variation was observed in the levels of oxalic and citric acids in 2009-10 samples and in the case of malic acid in 2010-11 samples. The non-volatile acid composition of HDBRG revealed the positive attributes of HDBRG tobacco smoking quality. Studies on the relation between the acids and tobacco quality indicated that the smoking quality was directly proportional to reducing sugars and inversely proportional to citric acid (Phillips and Bacot, 1953). Kalianos (1976) also reported an inverse relationship to the smoking quality of Virginia tobacco and the quantity of citric and oxalic acids, although this is probably just an indicator and is not due to the absolute amounts of these acids present in leaf. GC analysis of burley tobacco non–volatile acid fraction (Harvey *et al.*, 1979) revealed the presence of malic acid (2.7%), citric acid (7.1%) and oxalic acid (4.7%).

Volatile acids

Tobacco leaf contains significant quantity of fatty acids, both volatile and non-volatile. The volatile acids (C_2 – C_8) are known to be important aroma compounds in many fruits, foodstuffs and tobacco. Schmeltz *et al.* (1963) identified the following acids: formic, acetic, propionic, isobutyric, n- butyric, isovaleric, valeric, β-methyl valeric, isocaproic, n-caproic, n-heptylic and n-caprylic. Among the 19 volatile acids identified in HDBRG tobacco, isovaleric acid (47.66%) and β-methyl valeric acid (24.92%) were the major acids, followed by acetic acid (5.81%) and 2-methyl propionic acid (5.57%) in the fraction, which can be considered as a positive attribute of HDBRG tobacco (Table 4). Stedman and Stills (1965) observed that a mixture of isovaleric acid and β–methylvaleric acid can effectively substitute Turkish tobacco used as a blend in cigarettes. In *natu* tobacco, an indigenous type grown in India, Nagaraj and Chakraborty (1979) reported the following acids by adopting GC technique: acetic (3.0%), propionic (4.0%), n-butyric (7.9%), isovaleric (14.4%), n-valeric (5.1%) β- methyl valeric (41.0%), n-hexanoic (8.9%) and n-octanoic (15.6%) acids. Kameswara Rao (1983) reported relative percentages of volatile acids in *lanka* tobacco *viz.*, formic and acetic (6.72%), propionic and isobutyric (8.54%), isovaleric (16.76%), n-valeric (6.95%), â-methylvaleric (4.45%), n-hexanoic (18.74%) and n-octanoic (9.05%) by using GC-FID.

Fatty acids

Based on the relative content, palmitic acid (34.07%) was the major fatty acid followed by linolenic (32.05%) and linoleic (14.86%) acids in the fraction (Table 5). The proportion of saturated fatty acids (C14:0 + C15:0 + C16:0 + C17:0 + C18:0) was 55.44%, while that of unsaturated fatty acids (C18:2 + C18:3) was 44.56%. The major higher fatty acids are the saturated and unsaturated C16 and C18 acids along with 15 – 25 other minor components. Air-cured and fire-cured tobaccos contain lesser of these non-volatile acids than flue-cured and Turkish tobaccos (Stedman, 1968). Nagaraj and Chakraborty (1979) analysed fatty acids in *natu* tobacco by GC and reported the presence of decanoic (6.08%), lauric (8.51%), myristic (14.13%), myristoleic (17.02%), palmitic (10.94%), palmitoleic (6.38%), stearic (9.12%), oleic (7.98%), linoleic (1.60%) and linolenic (18.24%) acids. The saturated acids constituted about 48% of the total whereas 52% was accounted for by unsaturated fatty acids. Kameswara Rao (1983) reported the following major fatty acids in *Lanka* tobacco leaf, C18:3, C16:0, C18:2 and C18:1, the unsaturated acids accounting for 47%.

Smoke constituents

Higher levels of smoke total particulate matter (TPM), nicotine, solanesol, tar and carbon monoxide (CO) were observed in the 2010-11 sample (Table 6). This finding is in consonance with the published report on leaf nicotine, solanesol, potassium and hexane solubles of the particular sample (Satyanarayana and Narasimha Rao, 2013) wherein higher nicotine (4.20%), solanesol (2.46%), hexane solubles (10.50%) and lower potassium (0.92%) compared to the 2009-10 sample with lower nicotine (3.19%), solanesol (1.97%), hexane solubles (9.72%) and higher potassium (1.28%). Gangadhar *et al.* (2011) have reported a significant positive correlation between smoke TPM and leaf nicotine, solanesol and petroleum ether extractives/hexane solubles, while leaf potassium was negatively correlated to TPM.

Table 1: Carotenoids, carbonyls and polyphenols in HDBRG tobacco

	Carotenoids (mg/g)			Carbonyls (mg/100g)			Chlorogenic acid (mg/g)			Rutin (mg/g)		
	2009-10	2010-11	Mean	2009-10	2010-11	Mean	2009-10	2010-11	Mean	2009-10	2010-11	Mean
1	0.54	0.65	0.59	633.6	697.7	665.65	6.28	5.95	6.12	6.13	6.64	6.38
2	0.52	0.68	0.60	637.8	697.7	667.75	6.28	6.68	6.48	5.51	6.50	6.01
3	0.53	0.67	0.60	680.6	646.4	663.50	6.49	6.04	6.27	6.32	7.37	6.85
4	0.48	0.68	0.58	552.3	629.3	590.80	4.69	5.54	5.12	4.18	6.08	5.13
5	0.50	0.69	0.59	595.1	680.6	637.85	4.91	5.04	4.98	4.70	6.46	5.58
6	0.56	0.69	0.63	612.2	625.0	618.60	5.26	4.90	5.08	4.39	6.21	5.30
7	0.65	0.69	0.67	809.0	732.0	770.50	5.99	6.06	6.03	4.57	7.27	5.92
8	0.61	0.69	0.65	753.4	719.1	736.25	5.49	6.36	5.93	5.39	7.63	6.51
9	0.61	0.70	0.65	749.1	757.6	753.35	5.47	4.48	4.98	5.19	7.63	6.41
Mean	**0.55**	**0.68**	**0.62**	**669.23**	**687.27**	**678.25**	**5.65**	**5.67**	**5.66**	**5.15**	**6.87**	**6.01**
SD	**0.06**	**0.01**	**0.03**	**85.00**	**46.27**	**62.09**	**0.64**	**0.73**	**0.61**	**0.76**	**0.61**	**0.58**
CV (%)	**10.40**	**2.16**	**5.36**	**12.70**	**6.73**	**9.15**	**11.36**	**12.90**	**10.85**	**14.68**	**8.89**	**9.70**
SEm±	**0.019**	**0.005**	**0.011**	**28.33**	**15.42**	**20.696**	**0.21**	**0.24**	**0.205**	**0.25**	**0.20**	**0.194**

Table 2: Total volatile bases (TVB) and Total volatile acids (TVA) in HDBRG tobacco

Sample	TVB (%)			TVA (%)		
	2009-10	2010-11	Mean	2009-10	2010-11	Mean
1	0.86	1.00	0.93	0.82	0.73	0.78
2	0.79	1.38	1.09	0.71	0.62	0.67
3	1.19	1.07	1.13	0.66	0.50	0.58
Mean	**0.95**	**1.15**	**1.05**	**0.73**	**0.62**	**0.68**
SD	**0.21**	**0.20**	**0.11**	**0.08**	**0.12**	**0.10**
CV (%)	**22.57**	**17.59**	**10.08**	**11.21**	**18.65**	**14.80**
SEm±	**0.12**	**0.12**	**0.06**	**0.05**	**0.07**	**0.06**

Table 3: Non-volatile organic acids in HDBRG tobacco

Sample	Oxalic acid			Malic acid			Citric acid			Fumaric acid			Succinic acid		
	2009-10	2010-11	Mean	2009-10	2010-11	Mean	2009-10	2010-11	Mean	2009-10	2010-11	Mean	2009-10	2010-11	Mean
1	3.28	2.19	2.74	3.66	3.24	3.45	2.09	1.92	2.01	0.13	0.12	0.13	0.08	0.07	0.08
2	1.76	2.39	2.08	2.79	2.06	2.43	2.05	1.83	1.94	0.11	0.10	0.11	0.06	0.07	0.07
3	2.06	2.08	2.07	2.77	2.01	2.39	1.05	2.24	1.65	0.10	0.10	0.10	0.05	0.07	0.06
Mean	**2.37**	**2.22**	**2.29**	**3.07**	**2.44**	**2.76**	**1.73**	**2.00**	**1.86**	**0.11**	**0.11**	**0.11**	**0.06**	**0.07**	**0.07**
SD	**0.81**	**0.16**	**0.38**	**0.51**	**0.70**	**0.60**	**0.59**	**0.22**	**0.19**	**0.02**	**0.01**	**0.01**	**0.02**	**0.00**	**0.01**
CV (%)	**34.02**	**7.08**	**16.68**	**16.53**	**28.57**	**21.86**	**34.06**	**10.79**	**10.30**	**13.48**	**10.83**	**12.03**	**24.12**	**0.00**	**11.46**
SEm±	**0.46**	**0.09**	**0.22**	**0.29**	**0.40**	**0.35**	**0.34**	**0.12**	**0.11**	**0.01**	**0.01**	**0.01**	**0.01**	**0.000**	**0.004**

Table 4: Composition of volatile organic acids in HDBRG tobacco

S.NO	Acid	R. Time (min)	Proportion (%)		
			1	2	Mean
1	Acetic acid	9.44	5.81	5.81	5.81
2	Propionic acid	10.59	0.39	0.36	0.38
3	2- Methyl propionic acid (Isobutyric acid)	10.94	4.42	6.71	5.57
4	Butanoic acid	11.69	0.74	0.75	0.75
5	Acrylic acid (2-Propenoic acid)	11.88	0.16	0.15	0.16
6	3-Methyl butanoic acid (Isovaleric acid)	12.15	48.29	47.02	47.66
7	Pentanoic acid (Valeric acid)	12.96	0.74	0.71	0.73
8	Crotanoic acid (2- Butenoic acid)	13.39	0.15	0.14	0.15
9	3- Methyl pentanoic acid (β - Methylvaleric acid)	13.56	25.38	24.45	24.92
10	3- Methyl - 2 - butenoic acid	13.62	0.40	0.40	0.40
11	4- Methylpentanoic acid (Isocaproic acid)	13.68	1.78	1.76	1.77
12	Hexanoic acid (Caproic acid)	14.13	1.00	0.89	0.95
13	2- Methyl crotanoic acid	14.17	2.54	2.49	2.52
14	2- Methyl - 2 – pentenoic acid	14.73	0.43	0.44	0.44
15	4- Methyl hexanoic acid	14.98	1.16	1.17	1.17
16	Heptanoic acid (Enanthic acid)	15.24	0.18	0.16	0.17
17	Octanoic acid (Caprylic acid)	16.31	0.62	0.64	0.63
18	Benzoic acid	19.83	2.07	2.13	2.10
19	Benzene acetic acid	20.81	3.73	3.83	3.78

Table 5: Composition of fatty acids in HDBRG tobacco

S.No.		Relative content (%)								Mean
		2009-10				2010-11				of two
	Fatty acid methyl ester	1	2	3	Mean	1	2	3	Mean	seasons
1	Tetradecanoic acid (Myristic Acid) C14:0	5.73	4.76	4.48	4.99	4.43	4.34	4.27	4.35	4.67
2	Pentadecanoic acid C15:0	3.67	3.40	2.71	3.26	1.96	2.16	1.82	1.98	2.62
3	Hexadecanoic acid (Palmitic Acid) C16:0	35.68	36.77	33.27	35.24	32.90	33.39	32.42	32.90	34.07
4	Heptadecanoic acid (Margaric acid) C17:0	2.70	2.72	1.43	2.28	2.43	1.81	2.07	2.10	2.19
5	9,12-Octadecadienoic acid (Linoleic Acid) C18:2	15.02	14.43	14.85	14.77	14.41	15.42	15.02	14.95	14.86
6	9,12,15-Octadecatrienoic acid (Linolenic Acid) C18:3	27.27	28.52	33.59	29.79	34.47	33.49	34.94	34.30	32.05
7	Octadecanoic acid C18:0 (Stearic acid)	9.93	9.40	9.67	9.67	9.40	9.39	9.47	9.42	9.54

Table 6: Smoke constituents in HDBRG tobacco

Smoke constituent/Parameter	2009-10	2010-11	Mean
TPM (mg/cig)	22.73	27.59	25.16
Moisture (mg/cig)	2.62	2.77	2.70
Nicotine (mg/cig)	2.09	4.00	3.05
Solanesol (mg/cig)	0.65	0.89	0.77
TAR (mg/cig)	18.03	20.81	19.42
Carbon monoxide (mg/cig)	12.35	17.69	15.02
Puff count	7.1	10.6	8.9

Based on the results HDBRG tobacco is typified by higher carbonyl content compared to other non-FCV tobacco types, higher proportion of isovaleric acid and β-methyl valeric acid and lower citric acid level in the non-volatile acid fraction, which are considered as some of the positive attributes of tobacco smoking quality. It is also concluded that higher nicotine, nitrogen and lower reducing sugars in HDBRG tobacco and also the higher dose of nitrogen applied to the crop are indicators of higher TVB and lower TVA contents. Higher leaf nicotine, solanesol and hexane solubles and lower potassium content have contributed to higher levels of smoke TPM, nicotine, tar, solanesol and CO recorded.

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, Technical Assistant in the GC-MS analysis is sincerely acknowledged.

REFERENCES

Ai, J. 1997. Direct method for the quantification of free saturated fatty acids in tobacco. **J. Agric. Food Chem.** 45: 1278-82.

Anderson, R.A., J.F. Chaplin, R.E. Currin and Z.T. Ford. 1970. Plant phenols in flue-cured tobacco fertilized at different rates. **Agron. J.** 62: 415-7.

Bacot, A.M. 1960. The chemical composition of representative grades of the 1952 and 1954 crops of flue-cured tobacco including chemical methods. **Tech. Bull. No.1225**, U.S. Dept. Agric., Washington. pp.120-1.

Chakraborty, M.K. and S.R. Prabhu. 1974. Spectrophotometric determination of carbonyl content of tobacco. **Tob. Sci.** 18: 139-42.

Fenner, R.A. 1988. Thermo-analytical characterization of tobacco constituents. **Rec. Adv. Tob. Sci.** 14: 82-113.

Gangadhar, V., C.V. Narasimha Rao and V. Ranga Rao. 2011. Studies on relation between leaf and smoke constituents of FCV tobacco. **Tob. Res.** 37: 60-3.

Gopalakrishna, C.V.S.S.V. and A. Hanumantha Rao. 1980. Studies on some chemical quality parameters of *natu* tobacco. **Tob. Res.** 6: 94-7.

Hiscox, J.D. and C.P. Israelstam. 1979. A method for extraction of chlorophyll from leaf tissues without maceration. **Can. J. Botany** 57: 1332-4.

Harvey, W.R., R.W. Hale and R.M. Ikeda. 1970. The determination of organic acids in plants and food products. **Tob. Sci.** 14: 141–4.

ISO. 1999a. International Standard – ISO 3402: Tobacco and tobacco products: Atmosphere for conditioning and testing. International Organisation for Standardisation, Geneva, Switzerland. pp. 4.

ISO. 1999b. International Standard – ISO 10362-1: Cigarettes – Determination of water in smoke condensates– Part1–Gas-chromatographic method. International Organisation for Standardization, Geneva, Switzerland. pp. 7.

ISO. 2000a. International Standard – ISO 4387: Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine. International Organization for Standardization, Geneva, Switzerland. pp. 17.

ISO. 2000b. International Standard – ISO 10315: Cigarettes – Determination of nicotine in smoke condensates – Gas-chromatographic method. International Organization for Standardization, Geneva, Switzerland. pp. 8.

ISO. 2007. International Standard – ISO 8454: Cigarettes – Determination of carbon monoxide in the vapour phase of cigarette smoke – NDIR method. International Organisation for Standardisation, Geneva, Switzerland. pp. 7.

Kalianos, A.G. 1976. Phenolics and acids in leaf and their relationship to smoking quality and aroma. **Rec. Adv. Tob. Sci.** 2: 61-79.

Kameswara Rao, B.V., J.A.V. Prasad Rao and A. Hanumantha Rao. 1977. Effect of fertilizer combinations on chlorogenic acid and rutin content of different types of Indian tobacco. **Tob. Res.** 3: 112-5.

Kameswara Rao, B.V. 1983. Chemical studies on *lanka* tobacco and its smoke. Ph.D. Thesis, Andhra University, Waltair, India.

Leffingwell, J.C. 1999. Basic chemical constituents of tobacco leaf and differences among tobacco types. In *Tobacco: Production, Chemistry and Technology* (D.L. Davis and M.T. Nielsen, Eds.). Blackwell Science, Oxford, UK. pp. 265-84.

Nagaraj, G. and M.K. Chakraborty. 1979. Alkaloids and volatile acids of *natu* tobacco (*N. tabacum*). **Indian J. Chem.** 17:648-9.

Nagaraj, G. and M.K. Chakraborty. 1979. Lipophilic constituents of *natu* tobacco. III. Fatty acids. **Tob. Res.** 5: 125-8.

Narasimha Rao, C.V., S.R. Prabhu, K. Mahendra, Shefali Srivastava, Kanwal Raj and A.P. Bhaduri. 2000. Reverse phase HPLC analysis of solanesol. Paper presented at the **National Symp. on Tobacco**, 13-16 December, 2000, Rajahmundry. Abstr. No. C 19, p. 50.

Penn, P.T. and J.A. Weybrew. 1958. Some factors affecting the content of the principal polyphenols in tobacco leaves. **Tob. Sci.** 2: 68-72.

Phillips, M. and A.M. Bacot. 1953. The chemical composition of certain grades of type II, American flue-cured tobacco. Relationships of composition to grade characteristics. **J. A. O. A. C.** 36: 504-23.

Prabhu, S.R. and M.K. Chakraborty. 1983. Investigations on smoke aroma-bearing constituents in flue-cured tobacco of different aroma attainments. **Tob. Res.** 9: 56-60.

Roberts, D.L. 1988. Natural tobacco flavor. **Rec. Adv. Tob. Sci.** 14: 49-81.

Satyanarayana, K.V. and C.V. Narasimha Rao. 2013. Chemical constituents in HDBRG tobacco. **Tob. Res.** 39: 28-34.

Sheen, S.J. 1971. The colorimetric determination of chlorogenic acid and rutin in tobacco leaves. **Tob. Sci.** 15: 116-20.

Schmeltz, I., R.L. Stedman and R.L. Miller. 1963. Composition studies on tobacco XVI: Steam volatile acids. **J.A.O.A.C.** 46: 779-84.

Sheen, S.J. and J. Calvert. 1969. Quantitative variation in polyphenol content in the green and air-cured leaves of tobacco. **Tob. Sci.** 13: 10-4.

Siva Raju, K. 2013. Effect of methods of curing on aroma compounds of chewing tobacco (*Nicotiana tabacum*) genotypes grown in Tamil Nadu. **Indian J. Plant Physiol.** 18(3): 295-300.

Stedman, R.L. and C.D. Stills. 1965. Turkish tobacco substitute. **West. Tob. J.** 92: 67.

Stedman, R.L. 1968. The chemical composition of tobacco and tobacco smoke. **Chem. Rev.** 68: 153-207.