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SHORT COMMUNICATION

Effect of methods of curing on aroma compounds of chewing tobacco (*Nicotiana tabacum*) genotypes grown in Tamil Nadu

K. Siva Raju

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Abstract An experiment was conducted to examine the effect of curing methods on quality characters of various chewing tobacco (*Nicotiana tabacum* L.) genotypes grown in Tamil Nadu. Seven varieties of chewing tobacco (I-64, Bhagyalakshmi, Abirami, Meenakshi, Maragdam, Thangam and Vairam) were grown under recommended cultural practices and subjected to different curing methods viz., sun-curing, pit-curing and smoke-curing. The sun-cured tobacco contained significantly higher levels of organic acids, protein and carotenoids, whereas significantly lower levels of petroleum ether extractives and free fatty acids compared to pit and smoke-cured tobacco. Pit-cured tobacco showed significantly higher content of petroleum ether extractives and free fatty acids and lower levels of carotenoids, organic acids and carbonyl compounds. Smoke-cured tobacco showed significantly higher levels of carbonyl compounds and lower levels of protein. The free fatty acid content varied from 8.66 to 68.63 μmol g^{-1} among the varieties under different curings. The variety Vairam showed significantly higher content of free fatty acids, while variety Abirami showed significantly higher content of carbonyl compounds, whereas the variety Bhagyalakshmi showed lower levels. The sun-cured variety Meenakshi showed 2.34 and 1.8 times more petroleum ether extractives when it was pit cured compared to sun and smoke-cured. There was a significant variation in the content of aroma compounds in the varieties when they were subjected to different curing methods. The results revealed that, in case of chewing tobacco, the method of curing significantly affects the biochemical constituents.

K. S. Raju (✉)
Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533105, India
e-mail: rajuks2002@yahoo.co.in

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Tobacco (*Nicotiana tabacum* L.) is an important commercial crop of India with an area of about 4 lakh ha and a production of about 700 million kg. Different types of tobaccos grown commercially are distinguished largely by region of production, distinct morphological features of the variety, chemical and physical characteristics and intended use. Chewing tobacco cultivation in Tamil Nadu is concentrated in an area of about 20,000 hectares, producing about 48.1 million tonnes of cured leaf annually. India stands second in the production and exports of chewing tobacco in the world, while Tamil Nadu stands first in India. Chewing tobacco is used in the manufacture of smoke-less tobacco products for internal consumption and some quantity is exported to gulf and other countries (Krishnamurthy and Deo Singh 2005).

Biochemical compounds viz., carotenoids, petroleum ether extractives, free fatty acids, carbonyl compounds, proteins and organic acids are some of the main constituents responsible for the aroma of tobacco, which are influenced by quantity and source of manures, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew 1981). The objectives of curing methods in tobacco are to maintain and enhance the potential quality embodied in the harvested leaf and to provide an environment conducive to the transformation of that leaf into a high quality cured tobacco product. This is achieved through control of the chemical and biochemical conversions and moisture removal that take place during curing. The chewing tobacco grown in Tamil Nadu is subjected to sun-curing, pit-curing and smoke-curing to get desired quality tobacco. Many of the constituents originally present in the green leaf,

under go enzymatic, microbial, photochemical and oxidative reactions based on the curing procedure employed (Enzell 1977). These processes lead to the development of typical tobacco aroma and most significantly contribute to the formation of various biochemical compounds, which influence the quality. During the curing process, the following changes may take place, viz., loss of volatile constituents, changes in the structure of compounds by oxidation, hydrolysis, degradation or transformation, polymerization of chemical components and leaf/stalk transformations (Enzell 1977). The objective of the present study was to examine the effect of curing methods and varietial variation on aroma bearing biochemical constituents vis-à-vis quality of chewing tobacco grown in Tamil Nadu.

Seven chewing tobacco varieties viz., I-64, Bhagyalakshmi, Abirami, Meenakshi (sun- cured), Maragdam, Thangam (smoke-cured) and Vairam (pit-cured) were grown at CTRI Research Station Farm, Vedasandur, Tamil Nadu during *rabi* season of (2008-09) with recommended cultural practices. The spacing was 75 cm between rows and 50 cm between the plants. Nitrogen@75 kg ha^{-1} and P_2O_5@100 kg ha^{-1} were applied and the crop was topped at bud initiation stage (Kumaresan et al. 2009). During the experimental season the mean maximum and minimum temperatures were 19.7 and 25 °C, respectively. The relative humidity was between 81 and 91 %. The experiment was replicated three times in a randomized block design. On maturity, the leaves were harvested and subjected to sun-curing, smoke-curing and pit-curing (Deo Singh and Prasada Rao 2005).

The harvested tobacco plants were tied and put on bamboo structures for sun-drying. The sun-drying is continued for 3 weeks. After sun-curing, leaves are arranged in the shed layer by layer in a rectangular bulk (length: 5 feet, breadth: 5 feet and height: 4 feet) with butt ends pointing out side. The bulk covered with date palm mat. Turnings were given once in 3–4 days depending upon the temperature of the bulk. After 4 or 5 turnings, the bottom leaves showed sign of separation from the stalk and the colour of the leaves turn to dark brown. The leaves were stripped individually from each stalk.

The harvested plants were allowed to wilt one day in the field. The leaves were cut individually from the wilted plants along with a portion of stalk (stem), tied and hanged on the nails provided in the smoke barn of 3.0 m × 2.7 m × 1.8 m (length, breadth and height) dimensions with opening of 0.9 m × 0.75 m. Coconut husks were lit in the barn, and immediately the fire was put off by sprinkling water so that only smoke is emitted from the coconut husks. Care was taken that smoke does not escape from the barn. Smoke-curing was done for 12 h. After smoke-curing, the leaves were taken out from the barn and arranged in a rectangular bulk and turnings were attended periodically for 15 days. The curing and bulking process is to be repeated three times. The cured leaves were then bundled weighing one kg each, and these after the leaves were dipped in sea water for 10 min and hanged on bamboo scaffolds. The excess sea water was drained out and the bundles were opened up. The leaves were spread out and rolled around the mid rib of each leaf like a tape and bulked for 1 month. The bulk was turned at fortnightly intervals.

In pit-curing method, the harvested plants were allowed to wilt overnight and heaped in a cement pit measuring 3.0 m diameter and 3.6 m depth. After complete heaping, the top portion of the pit was covered with palmyrah mats and mud plastered later. The temperature inside the pit was about 37–40 °C and relative humidity 92 %. After 2 weeks, the plants were removed from the pits and leaves were cut along with a portion of stem. These leaves were hanged on the bamboo scaffolds for 3 days for sun-drying. Later the cured leaves were bulked in the godown and the bulk was turned periodically at 4–5 days interval. Whitish incrustations and fruity odour can be observed in the cured leaf after bulking which is a characteristic feature of pit-cured produce.

The cured leaf samples were dried in the hot air oven at 60 °C for 6 h, powdered in iron mortar and sieved through 40 micron mesh. The powdered samples were analyzed for aroma bearing biochemical constituents. Carotenoids were extracted from 100 mg of leaf powder with 10 ml of dimethyl sulfoxide at 60 °C for 3 h (Hiscox and Iscrelston 1979). After 3 h, the tubes were cooled to room temperature and the absorbance was recorded at 510 and 480 nm. The amount of carotenoids was calculated by using the formulae and expressed as mg g^{-1} dry weight. Petroleum ether extractives were estimated by taking 5 g of leaf powder in Whatman thimble and extracted with 150 ml ether in soxhlet apparatus for 7 h (Andersen et al. 1977). After the extraction, the flasks were cooled and ether was removed using flash evaporator. The flasks were dried in the oven at 70 °C, cooled in the desiccator and weighed, and the values were expressed in per cent. Carbonyl compounds were estimated by taking 250 mg tobacco powder, 125 mg activated charcoal and 25 ml of carbonyl free methanol in 250 ml Erlenmeyer flask and kept for shaking (30 min). The extract was filtered through Whatman No. 1 filter paper. One ml of filtrate, 18.5 ml methanol, 0.5 ml saturated dinitrophenyl hydragene solution and 0.1 ml concentrated HCl were taken into 25 ml volumetric flask and heated in water bath at 60 °C for 15 min. The volumetric flask was cooled to room temperature and volume was made to 25 ml with methanolic KOH and absorbance was measured at 485 nm (Chakraborty and Prabhu 1974). Carbonyl content in the samples was quantified using acetone as standard. Free fatty acids were

estimated by dissolving 100 mg of petroleum ether extractives into 5 ml of benzene and adding 1 ml of cupric acetate—pyridine reagent (5 % copper acetate, adjusting the pH to 6.0–6.2 with pyridine). The contents were shaken in a cyclomixer for 2 min and centrifuged for 5 min to get the two layers separated. The upper benzene layer was collected and its absorbance was read at 715 nm (Chu et al. 1972). A standard curve was prepared by taking stearic acid in the range of 2–12 µmol. Organic acids were estimated by the method of Grunwald et al. (1977) by taking 1 g of tobacco powder in a small silica dish and was dried to constant weight at 75 °C, followed by heating in a muffle furnace for exactly 3 h at 600 °C, thereafter and allowed to cool in a disiccator and weighed. 10 ml of N/4 HCl is added to the silica dish containing the ash and warmed for 10 min on the steam bath. The contents of the dish were transferred to a beaker and made the volume to 25 ml. This solution was boiled for 2–3 min to expel CO_2 and the acid remaining after neutralization was titrated, while still hot, using phenolphthalein as indicator to end point with standardized N/10 NaOH. From the quantity of NaOH required to neutralize the excess of acid, total combined organic acids were calculated in m.eq. of NaOH per 100 g dry weight. Proteins were estimated as per Lowry et al. (1951) after extracting the protein by using phosphate buffer (0.1 M and pH 6.8). The data were statistically analyzed (Panse and Sukhatme 1957).

The free fatty acid (FFA) content varied from 8.66 to 68.63 µmol g^{-1} among the varieties under different curing methods (Table 1). There were significant variations in FFA content among the varieties. Pit-cured tobacco showed 1.9 and 5.6 times higher levels of FFA over smoke-cured and sun-cured tobacco, respectively. The pit-cured variety Vairam showed higher content of FFA (1.63–32.18 µmol g^{-1}) over all other varieties under pit-cured condition.

Petroleum ether extractives (PEE) content varied from 5.90 to 15.07 % among the varieties under different curing methods (Table 1). The varieties I-64 and Meenakshi showed significantly higher PEE content compared to other varieties. Pit-cured tobacco recorded 69.96 and 89.95 % higher PEE over smoke-cured and sun-cured tobacco, respectively. All varieties showed significantly high PEE content when pit-cured. The variety Meenakshi when pit-cured showed 2.34 and 1.8 times more PEE over sun and smoke-curing, respectively. The sun-cured varieties I-64, Bhagyalakshmi and Abirami were on par with respect to PEE when subjected to sun and smoke-curing. Higher levels of PEE have been reported to be positively correlated with aroma in FCV tobacco. Even in chewing tobacco higher levels of PEE are positively correlated with aroma as these extracts contains all lipids and fatty acids (Murthy and Gopalachari 1984). The differences among the flue-cured varieties in petroleum ether extractives (Chaplin 1967) and in the fatty acids and lipid residues among the varieties (Chu et al. 1972) have also been reported.

Carbonyl compounds content varied from 189.66 to 663.43 mg per 100 g among the varieties under different curing methods (Table 2). The variety Thangam showed higher content of carbonyl compounds over other varieties. The smoke-cured tobacco showed significantly higher content of carbonyl compounds ranging from 36.35 to 296.77 mg per 100 g over sun and pit-cured tobacco. The variety Bhagyalakshmi showed lower content of carbonyls in all curing methods. The varieties Thangam and Meenakshi showed higher content of carbonyls in sun curing and smoke curing, respectively. Carbonyls are chemical constituents containing aldehyde or ketone functional group which contributes to the aroma in tobacco (Prabhu and Chakraborty 1983).

The organic acid content varied from 264.6 to 296.3 µeq per 100 g among the varieties under different curing methods. There were no significant variations among the varieties, however the sun-cured samples showed significantly higher levels of organic acids over smoke-curing, while the lowest content were observed under pit-cured condition, with exception of Bhgyalakshmi and Maragadam. Malic, citric and oxalic acids account for up to 70 % of organic acids in tobacco. There is strong evidence that these compounds regulate the pH of tobacco and influence indirectly the taste and aroma of tobacco and smoke (Kallianos 1976).

The carotenoid content varied from 0.189 to 0.784 mg g^{-1} dry wt. among the varieties (Table 3). There were no significant variations in carotenoid content among the varieties Abirami, Maragadam, Thangam and Vairam but showed significantly higher content compared to the varieties I-64 and Bhagyalakshmi. Sun-cured tobacco showed significantly higher carotenoid content compared to smoke-cured and pit-cured samples. Among the sun cured varieties, variety Abirami showed higher quantity of carotenoids ranging from 0.021 to 0.091 mg g^{-1} over other sun cured varieties. Tobacco varieties differ in their ability to produce different quantities of carotenoid derivatives (Beatson et al. 1984). The carotenoids have been found in all types of tobacco and photo-oxidative degradation of carotenoids lead to the formation of many compounds, which influence the aroma of tobacco (Enzell 1977).

Protein content varied from 6.54 to 14.26 mg g^{-1} dry wt. among the varieties under different curing methods (Table 3). The variety I-64, Bhagyalakshmi, Abirami and Vairam showed significantly higher content of protein over other varieties. The sun-cured tobacco showed significantly higher protein content, i.e., 1.8 and 2.1 mg g^{-1} dry wt. over the pit and smoke-cured tobacco, respectively. The variety Bhagyalakshmi showed higher levels of protein over other sun-cured varieties. There were no significant variations in

Table 1 Effect of genotype and curing methods on free fatty acids and petroleum ether extractives in chewing tobacco grown in Tamil Nadu (dry wt. basis)

Variety	Free fatty acids (μmol g^{-1} dry wt.)				Petroleum ether extractives (%)			
	Sun-curing	Pit-curing	Smoke-curing	Mean	Sun- curing	Pit- curing	Smoke- curing	Mean
I-64	9.66	53.00	25.70	29.45	7.80	13.59	8.39	9.93
Bhagyalakshmi	9.50	36.42	25.64	23.86	7.06	12.06	8.12	9.09
Meenakshi	9.32	46.95	27.57	27.95	6.43	15.07	8.36	9.95
Abirami	10.08	67.18	20.25	32.50	7.23	13.67	6.62	9.17
Thangam	8.66	45.98	32.28	28.97	5.90	12.55	8.57	9.00
Maragadam	9.92	63.63	34.58	36.04	6.94	13.44	6.73	9.04
Vairam	10.40	68.81	34.66	37.95	7.43	12.31	7.73	9.16
Mean	9.65	54.57	28.67		6.97	13.24	7.79	
		SEm±	CD 5 %	CV %	SEm±	CD 5 %		CV %
Varieties		0.40	1.10	3.87	0.265	0.73		8.53
Curing methods		0.26	0.72		0.170	0.48		
Variety × curing methods		0.69	1.92		0.460	1.27		

Table 2 Effect of genotype and curing methods on carbonyl compounds and organic acids in chewing tobacco grown in Tamil Nadu (dry wt. basis)

Variety	Carbonyl compounds (mg (100 g)$^{-1}$ dry wt.)				Organic acids (μeq. NaOH (100 g)$^{-1}$ dry wt.)			
	Sun-curing	Pit-curing	Smoke-curing	Mean	Sun-curing	Pit-curing	Smoke-curing	Mean
I-64	328.93	321.33	307.70	319.32	294.3	264.6	281.6	280.2
Bhagyalakshmi	189.66	272.40	222.73	228.26	285.3	268.0	292.0	281.7
Meenakshi	400.80	431.73	633.43	488.65	294.0	273.6	286.3	284.6
Abirami	223.13	379.80	389.60	330.84	296.3	265.0	285.3	282.2
Thangam	631.76	461.06	482.26	525.03	294.0	267.6	285.6	282.4
Maragadam	505.50	372.76	383.73	420.66	287.6	268.3	290.3	282.1
Vairam	447.43	384.13	394.26	408.61	292.6	274.6	288.0	285.1
Mean	389.60	374.74	401.96		292.04	268.85	287.04	
		SEm±	CD 5 %	CV %	SEm±	CD 5 %		CV %
Varieties		6.39	17.73	4.93	2.47	NS		2.62
Curing methods		4.18	11.60		1.62	4.49		
Variety × curing methods		11.08	30.71		4.28	NS		

protein content among the pit-cured and smoke-cured tobacco. Significant variations were not observed in protein content in smoke cured varieties Thangam and Maragadam under different curing methods. Method of curing showed significant effect on soluble protein content. The data showed that degradation of protein was more in pit and smoke curing methods over sun curing. The rapidly sedimenting and high molecular weight fraction of soluble leaf protein (Fraction I) hydrolyses quickly during curing, whereas the remaining heterogeneous fraction (Fraction II) is relatively unchanged (Andersen et al.1977).

Samples of pit cured Vairam, showed higher levels of free fatty acids and petroleum ether extractives but lower levels of carbonyl compounds, organic acids, carotenoids and protein vis-a-vis lower levels of free fatty acids and petroleum ether extractives and higher levels of carbonyl compounds, organic acids, carotenoids and protein in the samples of same variety subjected to sun and smoke-curing. The smoke cured varieties Thangam and Maragadam showed lower levels of protein, carbonyl compounds and organic acids when compared to the samples of same varieties subjected to sun or pit-curing and higher levels of free fatty acids and petroleum ether extractives over sun-curing. The sun cured varieties I-64, Meenakshi, Bhagyalakshmi and Abirami showed higher levels or at-par protein, carotenoids, petroleum ether extractives, organic acids

Table 3 Effect of genotype and curing methods on carotenoids and protein in chewing tobacco grown in Tamil Nadu (dry wt. basis)

Variety	Carotenoids (mg g^{-1} dry wt.)				Protein (mg g^{-1} dry wt.)			
	Sun-curing	Pit-curing	Smoke-curing	Mean	Sun-curing	Pit-curing	Smoke-curing	Mean
I-64	0.561	0.189	0.395	0.382	10.41	8.34	8.43	9.06
Bhagyalakshmi	0.572	0.199	0.387	0.386	14.26	7.26	7.50	9.67
Meenakshi	0.631	0.256	0.353	0.413	6.54	7.40	7.86	7.27
Abirami	0.652	0.249	0.435	0.446	10.05	8.57	7.57	8.73
Thangam	0.700	0.223	0.386	0.436	7.73	8.27	7.88	7.96
Maragadam	0.784	0.224	0.434	0.480	8.83	8.36	6.99	8.06
Vairam	0.742	0.196	0.409	0.449	11.48	8.50	8.36	9.45
Mean	0.663	0.219	0.400		9.90	8.10	7.80	
		SEm±	CD 5 %	CV %	SEm±	CD 5 %		CV %
Varieties		0.016	0.046	11.65	0.43	1.19		15.05
Curing methods		0.01	0.03		0.28	0.78		
Variety × curing methods		0.028	0.079		0.74	2.07		

when subjected to sun-curing compared to the pit or smoke-curing. Thus there was a significant variation in the content of aroma compounds in the varieties when they were subjected to different curing methods. Varieties are subjected to specific curing methods viz., sun-curing (I-64, Bhagyalakshmi, Abirami, Meenakshi), smoke-curing (Maragdam, Thangam) or pit-curing (Vairam) in order to attain the desired chemical quality characters to meet the consumer preference.

Thus, the method of curing significantly affected the aroma bearing constituents in chewing tobacco. Pit-cured samples contained significantly higher levels of PEE and FFA, may be due to the leaching of water soluble compounds from the leaf and prevailing anaerobic conditions. The total mean of carbonyl compounds were significantly higher in smoke-cured samples but some of the genotypes have more carbonyl compounds under sun and pit curing methods. The generation of carbonyls takes place usually during thermal processing. During smoke-curing tobacco also under goes a mild heat treatment resulting in higher levels in smoke-cured samples. Prabhu et al. (1984) reported increase in carbonyl compounds during flue-curing and post curing operations. Carotenoid pigments are commonly found in all green plants but the diversity in tobacco is conspicuous in a way that is not seen in other plants. Six oxygenated carotenoid compounds having important flavour properties providing floral feelings to tobacco have been reported (Kaneko 1980). Murthy and Gopalachari (1984) reported that protein nitrogen positively contribute to the chewing taste. The varieties are subjected to specific curing based on the consumers preferences even though some of the varieties meant for particular curing showed very good aroma when they were subjected to other curing. The genotype and post-harvest processing, i.e., curing have a pronounced influence on the aroma bearing constituents of chewing tobacco cultivated in Tamil Nadu.

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