CHEMICAL CHARACTERIZATION OF WHITISH INCRUSTATIONS FORMED DURING FERMENTATION OF CHEWING TOBACCO

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Whitish incrustations formed on the leaf surface of chewing tobacco is an important quality criterion. Incrustations collected from chewing tobacco of Bihar and Tamilnadu States showed 49.5% and 84.5% total ash, respectively. They contained predominantly SiO_2, K_2O, Cl and CO_3 with small quantities of CaO, MgO, P_2O_5 and SO_4.

Incrustations collected from Bihar were rich in malic acid whereas those from Tamilnadu State contained citric and malic acids along with petroleum ether extractables and non-protein nitrogenous constituents. The chemical nature and possible mechanisms of formation of incrustations is discussed.

INTRODUCTION

Chewing tobacco (N. *tabacum)* grown in India is generally stalk-cut, sun-cured, stalk-fermented, stripped and strip-fermented. Regardless of the curing method all chewing tobacco is subjected to fermentation. Formation of whitish incrustations is conspicuous and they appear toward the end of fermentation. Their appearance signifies successful fermentation.

Since whitish incrustations occur in both Bihar and Tamilnadu States and are indicative of quality, they were collected from both regions and subjected to chemical analysis. The probable mechanism of formation of incrustation is suggested in this paper.

MATERIALS AND METHODS

Fermented chewing tobacco leaf belonging to top grade from six locations from Bihar and Tamilnadu States were collected. Whitish incrustations collected from the six locations of Bihar State were pooled together to obtain one sample weighing 100 g representing the entire tract. Similar procedure was adopted for Tamilnadu State for obtaining 100 g of incrustation. The whitish incrustations on the leaf surface was scraped with a fine brush in a closed room. Small pieces of lamina contaminants were removed by sieving and the whitish powder was stored in polyethylene containers. Composite leaf sample of the top grade was taken for comparison.

The incrustations were analyzed for total ash, sand and silica by I.S.I. method (1), total nitrogen (2), potassium (3), protein-nitrogen and petroleum ether extractables (4), nicotine (5), calcium (7), phosphorus (8), sulphate and magnesium (9), chloride (10), and carbonate (12).

Organic acids in incrustations and tobacco leaves were determined by gas chromatography following the extraction procedure of Harvey *et al* (6), using a thermal conductivity detector. The carrier gas was hydrogen with a flow rate of 60 ml/min. A silicone column SE.30-5% of 61 cm length with a column temperature at 160°C was used to separate the organic fraction. The work was done at the Regional Research laboratory, Hyderabad, Andhra Pradesh, India.

RESULTS AND DISCUSSION

In the order of decreasing prevalence, the major inorganic con-

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stituents in Bihar incrustations were carbonate, silica and calcium. Those in Tamilnadu incrustations were silica, chloride, potassium, carbonate and calcium. The total ash content in the Tamilnadu sample was 84.5% whereas that in the Bihar sample was only 49.5%. Differences in contents of chloride and potassium accounted for most of the differences in ash contents.

Since incrustations originate from the leaf and larger quantities of incrustation are associated with better leaf grades, the chemical composition of the best grade leaf and of associated incrustations are compared in **Table 1.** The major organic constituents in the Bihar incrustation are malic acid, along with smaller quantities of nitrogenous and petroleum ether extractables. Those in the Tamilnadu sample were citric acid, malic acid, and petroleum ether extractables whereas, the nitrogeneous constituents were present in smaller quantities. The content of total organic solids of the Bihar sample was 50.5%, whereas those of the Tamilnadu sample was only 15.5% **(Table 1).**

Since the carbonate determined in the ash is the result of the combustion of organic acids (12) and silica may have been influenced by extraneous sources during curing, bulking, fermentation etc., they are not included in further discussion. It appears that Bihar tobacco incrustations are composed mainly of calcium malate and those of Tamilnadu mostly of chloride, malate and citrate salts of potassium and calcium.

During fermentation, gradual breakdown of cell membranes within leaves occurs. Cell wall ruptures are induced under active respiratory activity by alternating swelling and shrinking of leaf tissues. The breakdown of cell walls may occur to a limited extent under the influence of leaf enzymes but it is enhanced considerably by the proper types of micro-organisms. Toward the end of fermentation with the death and disorganization of protoplasm and weakening of the cell wall due to degradation of pectin, the heat liberated during fermentation might have induced diffusion of the vacuolar and protoplasmic constituents to the surface and the others may have been formed in situ from glandular hairs. The diffusion of the salts to the leaf surface reaches a maximum at the end of fermentation.

The composition of incrustation on chewing tobacco is similar to that on the grain of cigar leaf (11). The latter contained crystalline aggregates of oxalates, malates and citrates of calcium, magnesium and potassium. The mechanism of their formation may, however, differ considerably, as in the former case there is a deposit of these substances as whitish incrustations all over the leaf surface whereas in the latter they form into minute pimples embedded in the leaf surface entirely.

Table 1. Chemical composition of whitish incrustations and leaf lamina of fermented chewing tobacco from Bihar and Tamilnadu (expressed as % oven dry basis).

	Bihar		Tamilnadu	
	Incrustation	Leaf Lamina Var. HPS-20	Incrustation	Leaf Lamina Var. I-64
SiO_2	11.31	-	24.40	-
Potassium (K_2O)	0.82	2.05	16.55	3.30
Calcium (CaO)	5.95	4.97	2.36	5.20
Magnesium (MgO)	0.75	-	0.46	-
Phosphorus(P_2O_5)	0.32	-	0.22	-
Sulphate (SO_4)	0.09	-	0.03	-
Carbonate (CO_3)	15.99	-	6.41	-
Chloride (Cl)	0.67	0.95	20.81	4.77
Ash	49.54	-	84.40	-
Nicotine	0.19	-	0.53	-
Total nitrogen	1.54	-	1.57	-
Protein nitrogen	1.20	1.69	0.80	1.56
Petroleum ether extractables	1.50	6.54	2.52	7.29
Malic acid	32.30	12.85	2.38	8.01
Citric acid	-	6.01	5.22	10.43
Organic solids	50.46	-	15.54	-

CONCLUSIONS

During active fermentation, cell wall breakdown occurs in leaf tissue due to alternating swelling and shrinking caused by vigorous respiratory activity. Salts diffuse to the leaf surfaces leaving a whitish coating which is adjudged to be an indicator of quality. These incrustations on the chewing tobacco give pleasure to the chewer and are responsible for good saliva secretion. Since chewing tobacco with whitish incrustations is valued by consumers as well as the tobacco trade, it is surmised that the salts formed by calcium and potassium with malic and citric acids contribute positively to the chewing quality of the leaf.

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