Note

Changes in Volatile Fatty Acids and Free Amino Acids during Air-tight Fermentation of Tobacco Stalks

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In a previous paper,¹⁾ we reported utilization of tobacco stalks as a raw material of cigarettes. As a result, it was clarified that an air-tight pile fermentation of tobacco stalks was effective for improvement of aroma and taste, and the fermented stalks was evaluated to be a useful tobacco material. Since no analytical data have been obtained, we have planned to examine the changes of chemical components during the fermentation, focussing on volatile materials and free amino acids which would concern to aroma and taste of tobacco.

Preparation of the experimental materials. Stalks of tobacco plants (*Nicotiana tabacum*, Florida 15) were obtained from tobacco fields just after harvesting the leaves. The stalks were cut to small pieces (less than 10 mm length) by a Yamamoto stalk chopper machine. To 75 kg of the chopped stalks containing 72.4% water were added 5 kg of refined sugar and mixed thoroughly. The mixture was put into a 100 litre vinyl bag (Fig. 1) and fermented under air-tight condition for 2 months at room temperature. The bag was going to expand in a few hours by emitted CO₂ and was fully expanded in a day. The expanded bag was kept throughout the fermentation process at room temperature. The fermented product was brownish yellow and smelled sweet and a little sour.

Chemical contents of the treated materials. As the control sample, the dried up stalks without the fermentation process were prepared. Simultaneous determination of volatile fatty acids and lactic acid by a gas chromatography by Kageyama's analytical method²⁾ was done for the water-extracts of fermented products and control sample. The results are shown in Table I. The contents of acetic acid, iso-butyric acid and lactic

FIG. 1. Vinyl Bag for Air-tight Pile Fermentation.

TABLE I. CONTENTS OF VOLATILE FATTY ACIDS AND LACTIC ACID IN THE FERMENTED PRODUCTS AND THE CONTROL

Components	Fermented Products	Control
	(dry wt basis of %)	
Acetic acid	0.94	0.02
Propionic acid	0.16	tr
Isobutyric acid	0.49	tr
Butyric acid	0.08	tr
Lactic acid	5.41	0.16

Gas chromatography conditions; column, 0.3 × 150 cm glass column packed with 10% terephthalic acid, polyethylene glycol 6000 on shimalite 60~80 mesh; column temp., 120°C 4°C/min. 180°C (hold); carrier gas, He 0.8 kg/cm².

TABLE II. CONTENTS OF FREE AMINO ACID IN THE FERMENTED PRODUCTS AND THE CONTROL

Components	Fermented Products	Control
	(μ mol/g dry wt)	
Aspartic acid	16.0	8.4
Threonine	2.4	3.2
Serine	11.8	5.9
Proline	29.0	14.5
Glutamic acid	1.4	0.9
Glycine	57.4	14.2
Alanine	72.4	2.0
Valine	1.0	1.0
Methionine	3.2	1.0
Isoleucine	1.2	1.8
Leucine	22.0	11.8
Tyrosine	19.6	3.4
Phenylalanine	12.4	7.8
Lysine	1.0	1.0
Histidine	0.4	0.8
Arginine	0.4	0.2

acid in the fermented products were more than those of the control sample. Kaburaki³⁾ reported that contents

FIG. 2. Gas Chromatogram of the Particulate Phase of the Smokes (TMS derivatives).

Gas chromatography conditions; 0.25 mm $\times$ 60 m glass capillary column coated with silicone OV-101; column temp; 50°C 2°C/min, 200°C, carrier gas; He 0.8 kg/cm². 6, lactic acid; 7, glycolic acid; 11, cresol; 25, 5-hydroxymethyl-2-furaldehyde; 26, glycerol; 33, hydroxyquinone; 42, 4-vinylcatechol; 61, γ -phytadiene.

of total volatile fatty acids in flue-cured tobacco leaves were at most 0.1~0.2%, and Japanese domestic tobacco leaves were 0.01~0.05% on dry matter weight basis. Comparing to the values, the contents of volatile fatty acids in the fermented products are considerably higher than those of the tobacco leaves and control sample. The contents of lactic acid in the fermented products showed a similar tendency to the contents of volatile fatty acids.

To determine amino acids, samples were powdered after drying at 70°C for 3 hr. The analytical data of free amino acids are shown in Table II. Glycine, alanine and tyrosine were increased during the fermenta-

tion. On the contrary, the contents of basic amino acids such as histidine and arginine were decreased. We are interested in the phenomenon of increase of alanine content by fermentation process, since some species of microorganisms such as *Bacillus subtilis* may participate to this phenomenon. Noguchi⁴⁾ reported that alanine content of tobacco plant was comparably lower than those which in fermented products ranging 1~10 μ mol per dry basis.

Aroma and taste of the fermented products. In order to evaluate aroma and taste of cigarette smoke of the fermented products, the fermented products were ex-

tracted with hot water at 80°C for 120 min. The residue was grained to make paper sheets by a disc refiner (Kumagai Industrial Co.) at 100 μ m slit and was submitted to paper making process. The extract was concentrated in vacuo at 610 mmHg. After painting the condensate on the paper sheets directly, the paper was dried up and then cut into 0.8 mm shred. Smoke aroma of the cigarettes which was made from the fermented products was more aromatic and tastable than that of the control cigarettes. Smoke taste of the control cigarettes was bitter, irritant and had a papery sensation, whereas smoke taste of the cigarettes which was made from the fermented products was mild and sweet. To detect dominant compounds of the fermented products, a gas chromatographic analysis was carried out. The gas chromatogram of trimethylsilylated particulate phase of the cigarettes smoke are shown in Fig. 2. The quantities of lactic acid, 5-hydroxymethyl-2-furaldehyde, γ -phytadiene and glycerol of the cigarette smoke from the fermented products were more than those of the control cigarettes and tobacco leaves. A few additional peaks which could not be detected in the gas chromatogram of cured-tobacco cigarettes smoke were appeared. Sugawara *et al.*⁵⁾ reported that a gas chromatogram of the particulate phase of the smoke of a flue-cured tobacco had 100 peaks, and that these peaks occurred by pyrolytic

degradation of the main tobacco constituents, *i.e.*, alkaloids, sugar, resinous substances, *etc.* Even in these 100 peaks, these peaks specifically occurred during fermentation were not detected. These results may show concerning of these compounds to aroma and taste of the fermented products. On the other hand, a decrease of water soluble carbohydrates and a formation of a large quantity of lactic acid was observed during the fermentation. This phenomena may implicate participation of *lactobacilli* during the anaerobic fermentation process. The authors thank Dr.M. Noma for his co-operation in amino acid analysis.

REFERENCES

- 1) Y. Miyake, H. Ishizaki, T. Akiya and K. Nishida, *Research Report of Utsunomiya Tobacco Experiment Station (J.T.S.C.)*, **28**, 64 (1976).
- 2) K. Kageyama, H. Mori, and K. Satoh, *Jap. J. Zootech. Soc.*, **44**, 465 (1973).
- 3) Y. Kaburaki and Y. Satoh, *Nippon Nôgei Kagaku Kaishi*, **36**, 865 (1962).
- 4) M. Noguchi and E. Tamaki, *Agric. Biol. Chem.*, **26**, 689 (1962).
- 5) S. Sugawara, U. Kobashi and H. Sakurai, *ibid.*, **35**, 596 (1971).