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Changes of Organic Acids, Polyphenols, Pigments and Fiber Concentration with a Different Stalk Position and Grade of Korean Flue-cured Leaf Tobacco

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ABSTRACT : This study was carried out to analyze the organic acids, polyphenols, pigments and fiber materials concentration with a different stalk position and grade of korean leaf tobaccos. Eight kinds of flue-cured leaf tobaccos which were different stalk position and grade were used for this study. Three kinds of major organic acids(citric, malic and oxalic), 2 kinds of polyphenols(chlorogenic acid and rutin), 3 kinds of pigments(β -carotene, chlorophyll-a and chlorophyll-b), and 2 kinds of fiber components(pectin and lignin) were analyzed. All of these chemical components were changed with a different stalk position. When the citric acid, malic acid, β -carotene, chlorophyll-a, and lignin concentration were low in the middle stalk position and high in both bottom and upper position, oxalic acid and chlorogenic acid show the highest concentration in the middle stalk position. All of these chemical components also changed with a different grade of leaf tobaccos. As the citric acid, malic acid, β -carotene, chlorophyll-b, and lignin concentration decreased as the grade ascended, the oxalic acid and chlorogenic acid concentration increased as the grade ascended. This results assumed that the quality of korean leaf tobacco was directly proportional to oxalic acid and chlorogenic acid concentration but it was inversely proportional to citric acid, malic acid, β -carotene, chlorophyll-b and lignin concentration.

Key words **: organic acid, pigment, polyphenol, fiber materials, stalk position, grade of leaf tobacco**

Tobacco quality represents a balance of chemical compounds in a product which meets the preference of a special group of consumers at a given time and location. Many chemical and physical characteristics are being used to judge quality. The best quality of a given variety can only be obtained under balanced condition of leaf constituents. Chemical composition of leaf tobacco varies with genetic makeup, environmental conditions, and every step of production and handling. Smoke delivery and smoke composition depend on the characteristics of leaf tobacco. The major organic acids in tobacco are citric, malic, oxalic and malonic which in total can comprise 8% to 10% in flue-cured tobacco leaf after curing. A substantial portion on such acids are complexed as salts with nicotine, ammonia and inorganic anions of calcium, potassium, and

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sodium(Leffingwell, 1999). Polyphenols and pigments are among the major components which influence leaf quality and usability. Phenolic compounds are known to play an important role in leaf color, quality, and physiological strength of smoke. Major polyphenolics found in tobacco include chlorogenic acid, rutin, scopoletin and scopolin(Penn & Weybrew, 1958). Pigment is often considered as an indicator of the physiological state of growth, and cured leaf color may reflect value in the marketplace. The tobacco pectins are the "glue" that holds tobacco leaf together. Pectin comprises 6 to 12% of tobacco weight and as such contributes importantly both to the structural stability of leaf and to pyrolysis products that contribute to the smoke chemistry. Lignin comprises as much as 4 to 5% of tobacco weight and is the most abundant natural organic aromatic polymer found in the vascular plant kingdom(Goheen & Hoyt, 1985). There are major and important differences in chemical composition among tobacco types, such as varieties, grades or stalk position. The effects of stalk position and grade are clearly illustrated in the smoke constituents. Stalk position is an important indicator of certain physical and chemical properties. Also, the grade is another indicator of certain chemical properties of leaf tobacco. A change on any of these factors can markedly alter the chemical composition of leaf and thus affect smoking quality(Tso, 1990). This study was conducted to find out the differences of chemical components of leaf tobacco by different stalk position and garde.

Materials and Methods

1. Sample preparation

Tobacco leaf samples, do farming and harvested in 2001, were collected at the Kimcheon leaf tobacco processing factory by the different grade and stalk position from March to July in 2002. The three grades and four stalk positions of flue-cured tobacco were A_2OR, A_3OR, B_1O, B_2O, C_1L, C_2L, D_2L, D_3OR. Leaf tobacco samples were cut by 0.9 mm by using cutting machine and cigarette were made by using cigarette machine.

2. Determination of organic acid

For the determination of organic acids, capillary electrophoresis was used.
Tobacco samples were extracted with water in an ultrasonic batch and then separate by using buffer solution(chromate; pH=8; 10% acetonitril). Qantitative analysis was done by using the external calibration method.

3. Determination of polyphenols

To determine the chlorogenic acid concentration, an amount of 5 g tobacco is weighed in a beaker; 200 ml of hot water is added and given in a boilng water bath for 20 minutes. After cooling, water is added to the suspension to final volume of 250 ml and filtrated. Give 1 ml of the filtrated in two 50 ml graduated flasks. One of the flask is filled up with phosphate buffer; one is filled up with borate-phosphate buffer. Both solutions are measured at 357 nm by means of a UV. From the difference of absorption at the two wavelengths is determined the amount of chlorogenic acid.

For the determination of rutin concentration, 50 g of tobacco is weighted in a 500 ml graduated flask; 35 ml of hot water is added and the flask is given in a boiling water bath for 20 minutes. After cooling, water is added to the suspension to final volume of 50 ml and filtrated, whereby the first 10 ml are discharged. Give 10 ml of the filtrate in two 50 ml graduated flasks, add 5 ml of phosphate buffer each two flasks, respectively. 2 ml of the flavognost solution is added to one of the flasks and fill both flask

with water to the final volume of 50 ml. Both solutions are measured at 381 nm and 448 nm by means of a UV. From the difference of absrorption at the two wavelengths, rutin concentration is determined.

4. Determination of pigments

An amount of 1 g tobacco is grounded by a lab mill and given in a mortar. The extraction is done by mixing the tobacco sample with portions of 10 ml of acetone till no yellow color is resulting in the acetone solution. Each portion of acetone is filtered from the tobacco sample and given to a glass beaker. Weigh the beaker before adding acetone for a determination of the acetone used for the extraction. By weighing the beaker with the acetone solution, the exact amount of acetone can be determined, which is needed for further calculations. Chlorophyll is determined at a wavelength of 665 + 604 nm and carotene is measured at wavelengths of 454, 481, 413 nm. Quantitative analysis is done with external standard method.

5. Determination of fiber materials

To determine pectin concentration, 3 g of tobacco sample is washed with hot water(75℃) for 15 minutes and then washed tobacco is filtered by using a glass fiber filter. The residue is mixed with sodium polyphosphate solution for 60 minutes at 75℃. The filtra te is cooled to 10℃ and add 1M HNO_3. The pectin is filtered from solution and then washed with ethanol. Finally the precipitate is filtered over a glass fiber filter and dried at 55℃ for 16 hours and weighed.

For the determination of lignin, 1 g of dry tobacco sample is added 15 ml of 72% H_2SO_4 solution at 2℃ and keep in a water bath at 20℃ for 2 hours. Give 300 ml of H_2O in a flask and add the tobacco suspension, and then reflux the suspension for 4 hours. Afterwards let the suspension stay at room temperature over night. Filter the precipitate over a glass fiber filter, and dry at 105℃ before weigh the residue.

Results and Discussion

Organic acids

Table 1 show the changes of major organic acid concentration with a different stalk positions of korean flue-cured tobacco leaves. The concentration of citric, malic, and oxalic acid in tobacco leaves ranged from 0.61 to 2.87%, 5.6 to 10.3% and 0.060 to 0.454%, respectively. All of these organic acid concentration changed by stalk position. When the citric and malic acid were low in the middle stalk position and high in both bottom and upper position, oxalic acid show the highest concentration in the middle stalk position. The difference in chemical composition among leaves from various stalk position is well known. Court et al.(1982) also reported that leaves of the lower stalk position had the highest concentration of each organic acid. Oxalic acid mainly occurs as calcium salt which was reported to affect the availability of Ca to plant growth and therefore caused apparent calcium deficiency(Brumagen and Hiatt, 1966). There is an inverse relationship between smoking quality of Virginia tobacco and the quantity of citric and oxalic acid, although this is probably just an indicator, and is not due to the absolute amounts of these acids present in leaf (Kalianos, 1976). Malic acid was considered as the key compound in organic acid synthesis. Formic acid was converted to malic acid and acetic acid, malic acid to citric acid, and citric acid to malic acid formed from glycoloic acid was higher in the dark than in the light(Zbinovsky and Burris, 1952).

The citric acid and malic acid concentration were decreased as the grade ascended. The oxalic acid concentration was the reverse. The acid concentration found in the leaf generally reflects

Table 1. Changes of major organic acid concentration with a different grade and stalk position of flue-cured tobacco leaves

Sample	Citric acid (%)	D,L-Malic acid(%)	Oxalic acid(%)
A3OR	1.69	6.7	0.061
A2OR	1.27	6.6	0.057
B2O	0.68	5.6	0.075
B1O	0.61	5.9	0.082
C1L	0.57	5.7	0.060
C2L	1.04	7.9	0.060
D2L	2.63	10.3	0.059
D3OR	2.87	9.5	0.045
Average	1.42	6.47	0.060

the state of maturity of the leaf, and this phenomenon is almost always reflected in the quality of the leaf. This results assumed that the oxalic acid was the favorable factor to leaf quality, and the citric and malic acid were reverse. Studies by Phillps and Bacot(1953) also indicated that the quality of smoking tobacco was inversely proportional to citric acid. The addition of malic acid to tobacco has much impact on smoke strength due to high nicotine. Because malic acid mellow the taste and reduce the impact of high nicotine.

Polyphenols

A number of simple phenolic compounds exist in tobacco in small quantities but chlorogenic acid, rutin and scopletin are the predominant phenolic compounds found in tobacco. Some phenolic compounds can produce volatile compounds that add flavor to smoke by degradation and rearrangement reactions that occur during combustion(Kallianos, 1976). The concentration of polyphenols varies with stalk position and with grades of flue-cured tobacco, and in that respect, polyphenols are positively correlated with tobacco quality. Pyrolysis of free chlorogenic acid and rutin produces simple phenols and compounds which produce smoky aroma(Weeks, et. al., 1993). Figure 1 shows highest chlorogenic acid concentration in the middle stalk position and the lowest near the tip and lugs. The concentration of chlorogenic acid was 3-4 times higher than that of rutin. Rutin concentration shows no significant difference among stalk position. As the chlorogenic acid concentration decreased as the grade of leaf tobacco became deteriorated, the rutin concentration had no tendency as the grade change.

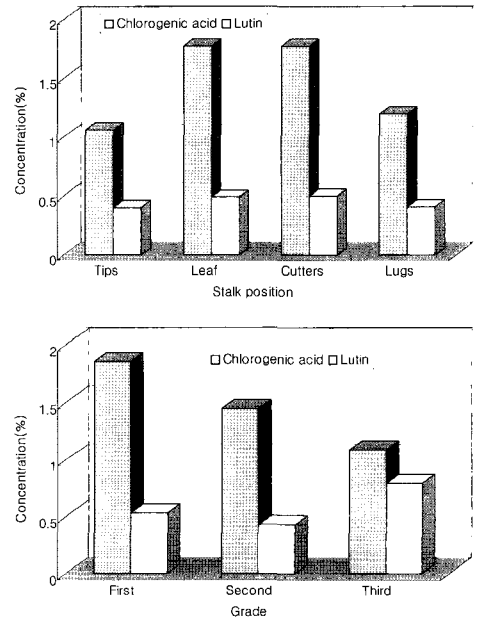

Fig. 1. Changes of chlorogenic acid and lutin concentration with a different stalk position and grade of flue-cured tobacco leaves.

Pigments

The major pigments of tobacco are the chlorophylls and carotenoid pigments, and the major green pigments are chlorophyll-a and

cholrophyll-b, both of which decrease as the tobacco reaches maturity, proceeds into senescence and continues to decrease during barn curing. Leaf color in the field as well as cured leaf is considered to be important in judging the quality of leaf and this has been extensively studied(Tso, 1990). The major carotenoid pigments in tobacco are lutein, β -carotene, neoxanthin and violaxanthin. The carotenoids are the precursors to many of the volatile aroma components of tobacco. Carotenoids decrease during maturation, senescence and curing. Carotene undergo about 65% degradation in burley and more extensive degradation has been reported for flue-cured tobacco (Court, Henel and Pocs, 1993). The degradation of β -carotene by both photo-oxidation and high pressure oxidation has been shown to generate many of the same non-cartenoid cmoponetns found in tobacco(Heij, Dort and Renes, 1992). The total amount and composition of pigments vary with tobacco variety, type stage of growth and handling practice. Table 2 shows the changes of major pigment concentration with different grade and stalk position. Total chlorophylls(chlorophyll a + chlorophyll b) of the samples vary from 20 to 40 ug/g of weight. The relative concentration and distribution of β -carotene in the tip and cutters amount to 112 ug/g and 48 ug/g respectively.

β -carotene has been found to be low in concentration in the cutter leaves, but rises to a maximum near the tip leaves. Chlorophyll-a, on the other hand, is highest in concentration in the lugs and the lowest in the leaf. The concentration of chlorophyll-b follows a complex curve, which being low in the leaf passing through a maximum in the tip leaves. Only small differences were observed in the chlorophyll-a to chlorophyll-b ratio among stalk position. The tips show the lower value of the ratio than those of other stalk position. The β -carotene concentration significantly increased as the grade became lowered. As chlorophyll-a had no significant concentration changes with a different grade, chlorophyll-b slightly increased with decreasing grade. This results indicated that the quality of leaf tobacco was directly proportional to carotene and chlorophyll-b concentration.

Table 2. Changes of major pigment concentration with a different stalk position and grade of flue-cured tobacco leaves

Sample	β -Carotene (ug/g)	Chlorophyll a (ug/g)	Chlorophyll b (ug/g)	Chlorophyll a / Chlorophyll b
A3OR	132.6	20.45	10.74	1.90
A2OR	91.4	24.74	8.73	2.83
B2O	77.7	20.11	6.38	3.15
B1O	92.1	17.82	5.10	3.49
C1L	80.1	30.67	8.67	3.54
C2L	86.5	29.08	7.80	3.73
D2L	92.8	35.59	8.20	4.34
D3OR	107.0	29.58	9.05	3.27
Average	95.0	26.00	8.08	3.22

Fibers

Cured leaf tobacco contains approximately 10% of cellulose and hemicellulose, which corresponds closely to the values obtained for a series of flue-cured varieties. Normally, high cellulose contents in a tobacco blend is a negative to smoking quality in that it tends to impart a sharp stinging harshness and a "burnt paper" odor to the smoke. the cellulose contents of stems plays an important part in the manufacture of reconstituted tobacco because the fiber provides structural strength(Green 1977). Lignin is one of the major cell wall components in leaf. Lignin is composed of a chain of polymeric phenolic constituents, such as coniferyl alcohol, p-coumaryl and synapyl alcohol. In woody plants, ligins acts as a cementing agent to

help bind the matrix of cellulose fibers(Goheen & Hoyt, 1985). Lignin, a high molecular weight polymer, contains 100 or more aromatic units high in methoxyl groups. Lignin is the source of such flavor compounds as vanillin, benzyl alcohol, 1-phenylethanol, 2-phenylethanol and other aromatic compounds produced and distilled into mainstream smoke(Green, 1977). It has been known that the pectin fractions of tobacco are modified chemically by curing procedures. Shmuk(1975) indicated tobacco quality was inversely proportional to pectin concentration. Fermentation of pectins produces as much as 1.0% to 1.5% acetic acid, which affects the taste of smoke by producing acrid taste and irritation(Green, 1977). Fig. 2 shows the changes of pectin and lignin concentration with different stalk position. Concentration of pectin was approximately the same at all stalk positions.

Lignin was the minimum concentration in the middle stalk position but rises to a maximum near the low stalk position. This figure also show the changes of pectin and lignin concentration with a different grade of tobacco leaves. Lignin concentration increased with decreasing the grade of leaf tobacco. Pectin was approximately the same at all grade of leaf tobacco. This result assumed that the quality of leaf tobacco was inversely proportional to lignin concentration.

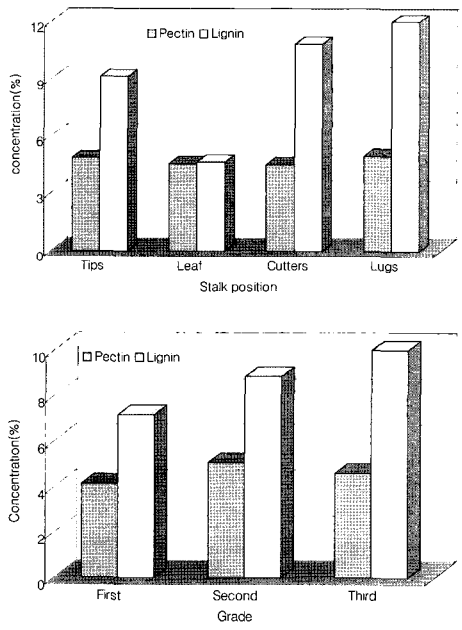

Fig. 2. Changes of pectin and lignin concentration with a different stalk position and grade of flue-cured tobacco leaves.

Conclusion

This study was conducted to analyze the organic acid, polyphenols, pigments and fiber materials concentration with a different stalk position and grade of korean leaf tobaccos. All of these chemical components changed with a different stalk position. When the citric acid, malic acid, β-carotene, chlorophyll-a, and lignin concentration were low in the middle stalk position and high in both bottom and upper position, oxalic acid and chlorogenic acid show the highest concentration in the middle stalk position. All of these chemical components also changed with a different grade of leaf tobaccos. As the citric acid, malic acid, β-carotene, chlorophyll-b, and lignin concentration decreased as the grade ascended, the oxalic acid and chlorogenic acid concentration increased as the grade ascended. This results assumed that the quality of korean flue-cured leaf tobacco was directly proportional to oxalic acid and chlorogenic acid concentration but it was inversely proportional to citric acid, malic acid, β-carotene, chlorophyll-b and lignin concentration.

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