Comparison of Lipoxygenase Activity and Lipid Composition in Various Harvests of Northeastern Indian Tea

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Study on the specific activity of tea shoot lipoxygenase in eight different genotypes at different pH levels from 6.5 to 9.5 revealed the presence of three lipoxygenase isoenzymes. However, all of the clones were assayed for activity in two separate peaks around pH 9.0 and 7.5. The role of lipoxygenase and lipid in different seasons and harvests of teas was studied to have some insights into its physical characteristics' control of volatile flavor compounds. With the maturation of tea shoot components, a rise in lipoxygenase activity and an increase of lipid content have been observed. The relative proportions of neutral and structural lipids such as phospho- and glycolipids also increase throughout maturation. Further, relative amounts of unsaturated fatty acids in different lipid fractions that may be crucial for the distribution of naturally occurring volatiles in the leafy shoots are discussed.

Keywords: *Tea; lipoxygenase; lipids; maturation*

INTRODUCTION

Northeastern India produces about 76% of the total Indian tea crop, and Assam alone produces about 50%. Fluctuations in the flushing behavior of tea during the plucking season from March to November is associated with growth and dormancy phases which are largely influenced by growing conditions in the field. At the beginning of a new cycle of flushing, the terminal bud, depending on agronomic practices, subsequently develops into numerous branches and sub-branches, facilitating higher productivity of the leafy crops (Barua, 1961; Baruah, 1970; Mahanta and Baruah, 1992; Wight, 1955).

The good quality and high-yielding cultivated tea crops consist of crosses between the two major races Assam and China. The distribution of chemical constituents such as total lipid and fatty acid components of fresh tea leaf shoots was maximum during the second flush period to give the superior and distinctive volatile flavor compounds (VFC) in made tea, compared to presecond flush, monsoon, and end-season flushing period. The difference in amounts of carotenoid and monoterpene alcohols such as linalool and geraniol indicates the differences between the Assam and China races of tea. However, the aroma of tea produced from the China variety in Darjeeling is found to be higher than that of the Assam race (Bhuyan *et al.*, 1991; Horita and Owuor, 1987; Mahanta *et al.*, 1993a,b; Venkatakrishna *et al.*, 1976). Postharvest quality of commercial black tea therefore depends on type of plant, seasons, maturity or physiological age of the shoot, and manufacturing conditions.

Lipoxygenase [(Lox) EC 1.13.11.12] catalyzes the peroxidation of 1,4-diene unsaturated fatty acids and carotene to produce carbonyl compounds responsible for the characteristic odor of fresh leaves. The volatile leaf alcohols and aldehydes responsible for characteristic green aromas, jasmonic acid, an inportant odoriferous principle, and a new group of senescence hormones are the best documented examples of the physiological role played by lipoxygenase in plants (Hatanaka *et al.*, 1987; Miersch *et al.*, 1989; Schewe *et al.*, 1986; Yamauchi *et al.*, 1985; Vick and Zimmerman, 1987). The various plants express lipoxygenase in multimolecular forms, which are known as isozymes. The different isozymes have different pH optima and differences in affinity among various polyenoic fatty acids and give rise to biosynthesis of a host of volatile compounds largely responsible for the characteristic odor of fresh leaves (Abbas *et al.*, 1989; Matoba *et al.*, 1989; Sanz *et al.*, 1993).

This information on lipid content and lipoxygenase activity as a function of volatile flavor development might be helpful in the identification of genetic characteristics of various clones and harvests and in the determination of their technological properties, being attempted in this paper.

MATERIALS AND METHODS

The young shoots consisting of two to three leaves and the apical bud were harvested from Tocklai clones TV1 and TV7 (China hybrid); TV2 and TV12 (Assam); and TV9, TV18, TV25, TV26 (Cambod hybrid) throughout the 1989–1991 plucking seasons. Tea flushes from different cultural practices were collected from the experimental garden of the research station: Borbhetta, Tocklai and Botanical plot.

Taking linoleic acid as substrate, lipoxygenase activity was determined spectrophotometrically by measuring the absorbance of conjugated diene hydroperoxide at 234 nm (Madison and Hughes, 1983; Van Den and Mendoza, 1982). Lipoxygenase isozyme activity varies with the acidity/alkalinity of the medium from pH 7 to 9. However, in all of our experiments enzyme levels were assayed at pH 7.5 and 9.0. The fatty acid contents (micrograms per gram) from various lipid fractions such as neutral lipid (NL), glycolipid (GL), and phospholipid (PL) were analyzed from different shoot components as well as in fine and coarse plucked shoots, as described by Mahanta *et al.* (1993a) and expressed as percent of dry weight.

RESULTS AND DISCUSSION

Lipoxygenase Activity and Seasonal Changes of Clonal Teas. Specific enzyme activity (units per milligram of protein) was measured by preparing the linoleic acid in different pH ranging from 6.5 to 9.5. Their activity profiles revealed the presence of three types of lipoxygenase at pH 7.0, 7.5, and 9.0 as shown

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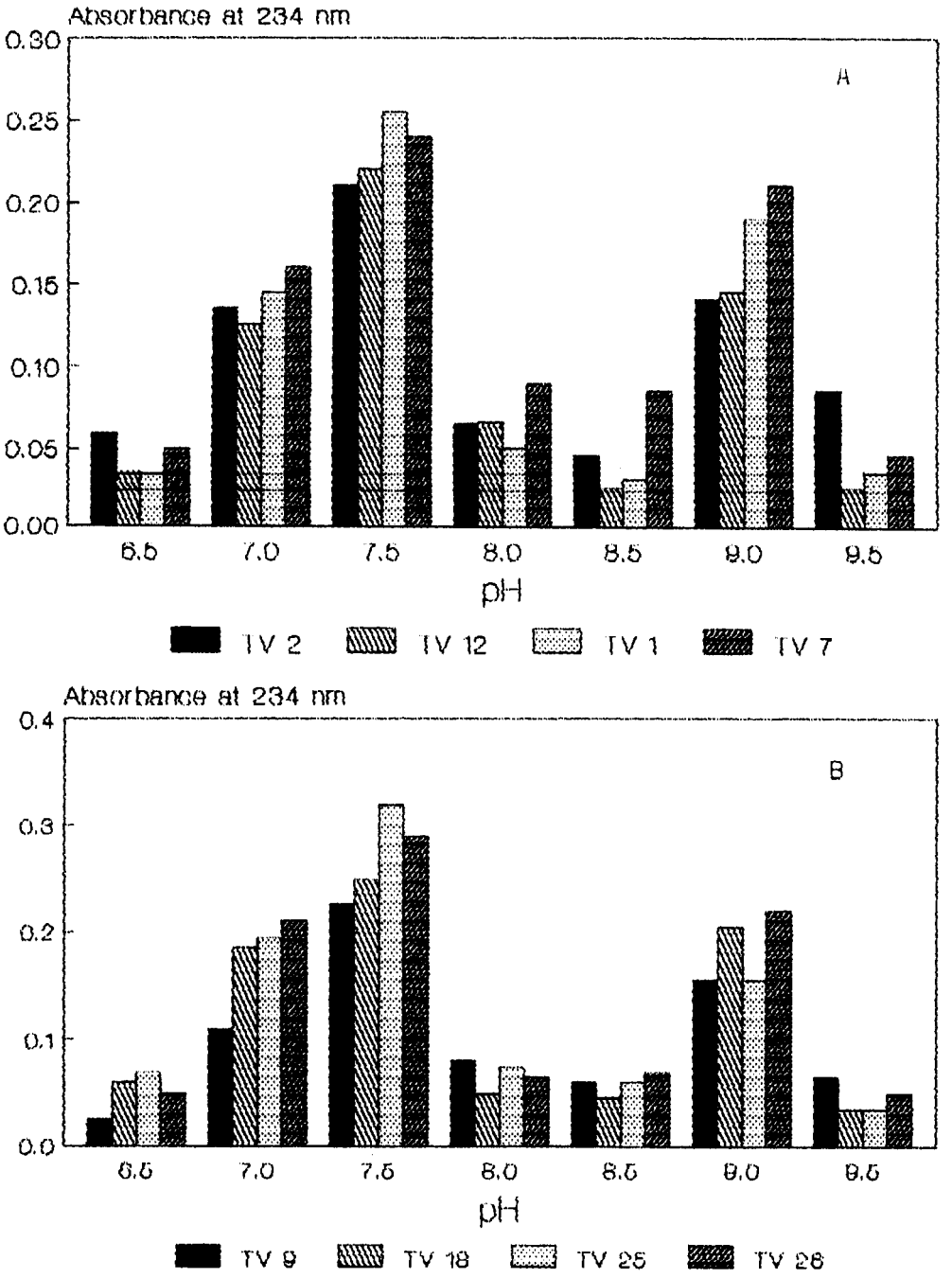

Figure 1. Effect of pH on lipoxygenase activity in tea of different cultivars.

in Figure 1. The highest activity, at pH 7.5, may be an indication of the presence of more isozymes in this pH range. There was a sharp rise in enzyme activity from pH 6.5 to 7.5 and thereafter a sudden fall at pH 8.5 followed by a rise at pH 9.0. The lipoxygenase that shows higher activity around pH 9.0 can be categorized as Lox-1, while Lox-2 shows a more acidic pH optimum (pH 5–7). Information on multiple forms of the enzyme seems to be important because they are responsible for the difference in carbonyl production during the various seasons (Matoba *et al.*, 1989; Sekiya *et al.*, 1984).

The specific activity of lipoxygenase among various clonal cultivars during plucking season from May to November is shown in Figures 2 and 3. Monthly changes in specific lipoxygenase activity were the same for all cultivars. It was highest in the month of August and lowest in the month of May. High lipoxygenase enzyme activity occurred during the heavy flushing period from July to September when flavor is lacking, and low enzyme activity occurred during May and June when flavorful teas are produced. Higher lipoxygenase activity during monsoon flushes (July–August) than in the early flushes may be an indication of production of a larger amount of unsaturated fatty acid degradation volatiles as compared to second flushes (May–June), during which monoterpene alcohols were more plentiful. With autumn flushes during October and November, when winter dormancy sets in, there was also a slight increase in lipoxygenase activity. Specific activity of 32.0 units/mg of protein was shown by TV25, while TV9 had 22.5 units/mg, and TV1, the most popular clone, was found to have 25.2 units/mg of protein lipoxygenase activity.

On the basis of agronomic trials tea cultivars such as TV1 and TV2 were categorized as quality clones and TV25 and TV26 as yield clones. The high-yielding

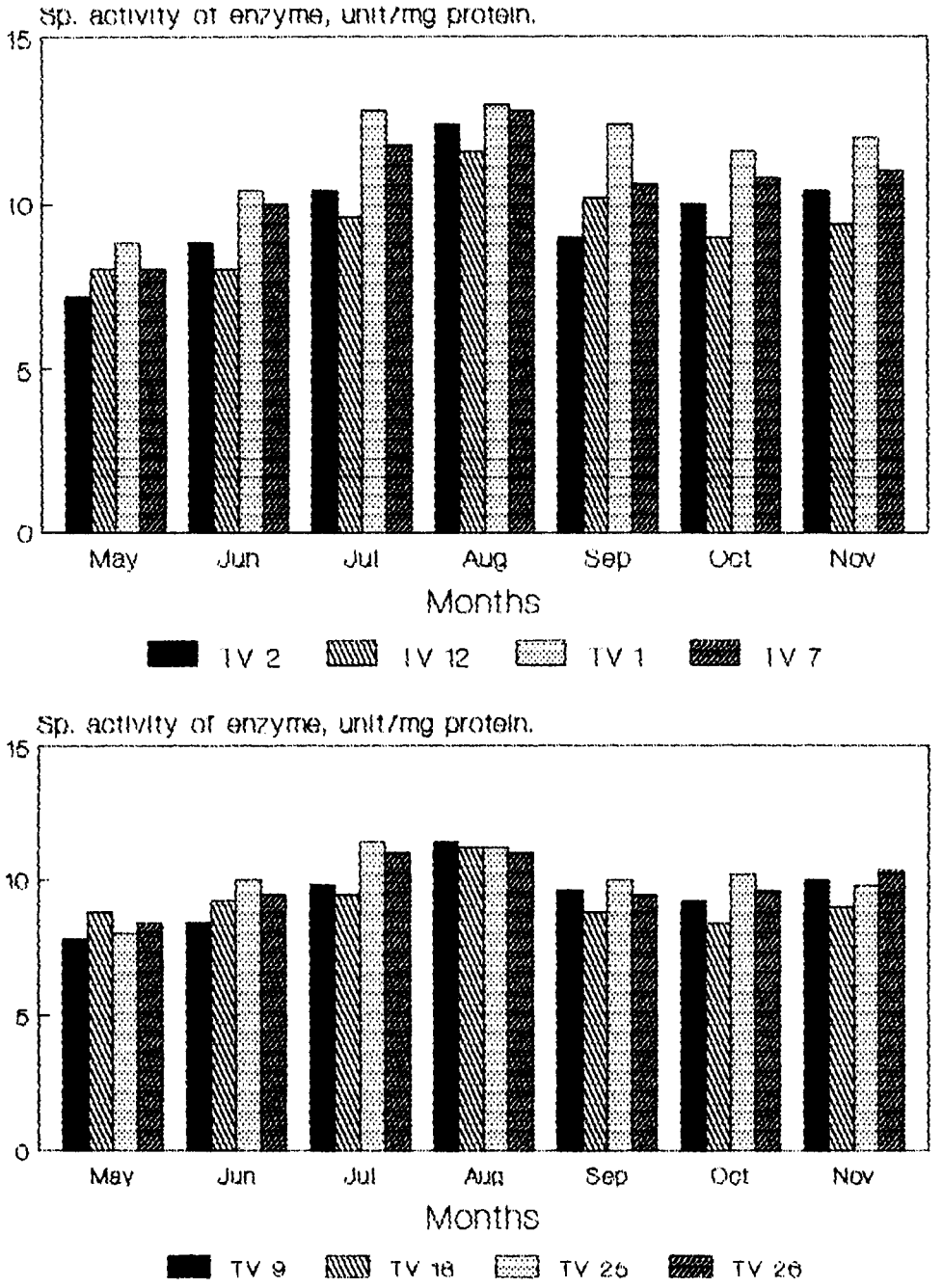

Figure 2. Monthly variation of lipoxygenase activity in various cultivars of tea at pH 9.0.

clones TV25 and TV26 showed the same levels of activity. Shoots of different cultivars synthesize different amounts of enzymes due to their genetic variations, as a result of which there are variations in their enzyme activity and seasonal changes of volatile compounds (Fernando and Roberts, 1984; Hazarika *et al.*, 1984; Sekhar and Reddy, 1982).

Lipoxygenase Activity and Shoot Maturation. Although shoots with two to three leaves and the terminal buds are used for black tea manufacture, coarser forms are sometimes harvested. Table 1 shows the changes in total lipid (percent of dry weight), total fatty acid (micrograms per gram) content, and specific lipoxygenase activities at pH 7.5 and 9.0 of various shoot components of clone TV1. There is a marked increase in the specific activity of lipoxygenase as well as lipid content with maturity of the shoot, while the stem portion was found to be comparable to the bud at both pH. However, the simple increase of lipid content as well as lipoxygenase activity does not produce good flavor in manufactured tea because of the harshness in such teas (Baruah *et al.*, 1986; Hatanaka and Harada, 1973; Mahanta *et al.*, 1985, 1988; Selvendran *et al.*, 1978; Takeo and Tsushida, 1980).

Lipid and Lipoxygenase in Pruned and Unpruned Leaves. Unpruned tea plants produce more dormant buds than growing buds. Therefore, total defoliation or pruning prior to harvest has been considered to have great effect on plant productivity and the quality of made tea. Table 2 shows the lipoxygenase activity of fresh tea shoots plucked from pruned and unpruned Assam and China bushes. The gradual attainment of maturity in shoot components can be attributed to a higher accumulation of lipid, and the high specific enzyme activity may result in desirable/undesirable flavor in manufactured tea (Bhuyan and Mahanta, 1989; Mazliak, 1987; Teranishi and Buttery, 1985).

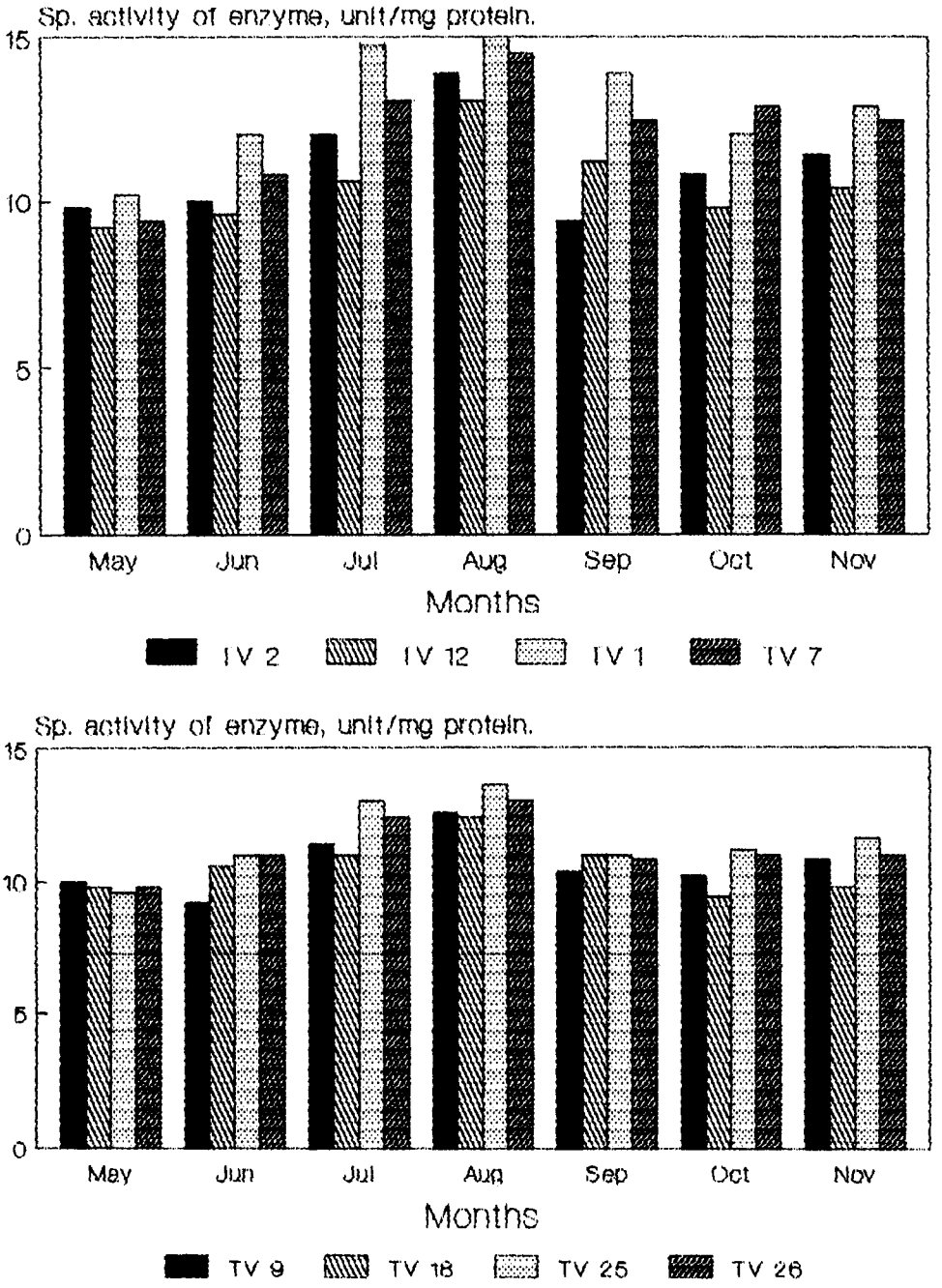

Figure 3. Monthly variation of lipoxygenase activity in various cultivars of tea at pH 7.5.

Table 1. Changes in Total Lipid, Total Fatty Acid Content, and Lipoxygenase Activity of Various Shoot Components

part of shoot component	total lipid (% dry wt)	total fatty acid (μg/g)	specific activity of enzyme (units/mg of protein)	
			pH 7.5	pH 9.0
bud	4.16	7666	11.2 ± 0.25	9.18 ± 0.31
first leaf	5.59	10184	12.1 ± 0.32	10.31 ± 0.29
second leaf	6.64	11552	13.8 ± 0.49	12.10 ± 0.62
third leaf	7.25	13490	15.5 ± 0.53	13.80 ± 0.52
fourth leaf	7.70	13664	16.1 ± 0.60	14.20 ± 0.43
stem portion	4.48	8288	10.82 ± 0.33	8.83 ± 0.29

As a result of interactions of plants and agronomical operations, changes in the various lipid fractions of tea shoots collected from both pruned (LP) and unpruned (UP) tea bushes are shown in Table 3. Lipid contents such as NL, GL, and PL were higher in pruned sections than in unpruned sections. The increase in the linolenic acid content may be related to chloroplast development,

Table 2. Specific Activity of Lipoxygenase in Pruned and Unpruned Bushes of Assam and China Plants

variety	pH	specific activity (units/mg of protein)	
		pruned	unpruned
Assam	7.5	14.20 ± 0.39	11.80 ± 0.40
	9.0	11.50 ± 0.48	9.52 ± 0.60
China	7.5	15.30 ± 0.61	12.41 ± 0.53
	9.0	11.81 ± 0.51	10.81 ± 0.61

as linolenic acid is one of the major constituents of chloroplast membrane lipids (Kirkpatrick *et al.*, 1983; Serghini-Caid *et al.*, 1988).

Maturational Variation in Relation to Lipid Content. The chemical distribution of lipid components of various harvests can be controlled at the garden level by "standard of plucking" (fine or coarse) and the "plucking interval" (the round) as shown in Tables 4 and 5. The highest amounts of unsaturated fatty acid, viz.

Table 3. Amount of Neutral Lipid (NL), Glycolipid (GL), and Phospholipid (PL) and Fatty Acid Compositions of Pruned and Unpruned Assam and China Races of Tea

			fatty acid composition (μg/g)				
lipid	%	fatty acid proportion	palmitic (16:0)	oleic (18:1)	linoleic (18:2)	linolenic (18:3)	CV[a] (%)
			Assam, Pruned				
NL	1.81	3235	660	469	993	796	2.32
GL	4.26	6164	1054	425	507	4043	3.19
PL	1.14	7458	2339	1051	2144	1685	2.98
			Assam, Unpruned				
NL	1.26	2707	658	290	910	640	2.32
GL	3.95	5249	850	363	425	3512	3.19
PL	0.94	6639	2051	770	1958	1573	2.98
			China, Pruned				
NL	2.03	3136	702	564	890	671	2.32
GL	4.36	5834	998	402	481	3827	3.19
PL	0.96	7451	2563	1051	2072	1535	2.98
			China, Unpruned				
NL	1.41	2521	645	338	729	575	2.32
GL	4.12	5041	817	348	408	3372	3.19
PL	0.78	6438	2053	747	1899	1461	2.98

[a] The differences between fatty acids are highly significant at $p < 0.01\%$.

Table 4. Changes in Fatty Acid Compositions of Tea from Various Lipid Fractions Such as Neutral Lipid (NL), Glycolipid (GL), and Phospholipid (PL) and from Different Shoot Components

				fatty acid composition (μg/g)			
shoot component	lipid	%	fatty acid proportion	palmitic (16:0)	oleic (18:1)	linoleic (18:2)	linolenic (18:3)
bud	NL	0.39	1637	355 ± 2.87	106 ± 0.82	630 ± 4.89	511 ± 4.11
	GL	3.17	2216	200 ± 1.70	111 ± 0.82	202 ± 1.63	1684 ± 14.29
	PL	0.60	3813	1140 ± 9.39	339 ± 2.87	953 ± 7.79	1265 ± 9.81
first leaf	NL	1.03	1947	551 ± 4.04	179 ± 1.25	504 ± 3.30	683 ± 4.55
	GL	3.68	3420	245 ± 2.05	155 ± 0.82	232 ± 1.25	2718 ± 17.63
	PL	0.88	4817	1542 ± 10.33	453 ± 2.87	819 ± 5.31	156 ± 9.48
second leaf	NL	1.55	2428	636 ± 18.28	282 ± 8.26	653 ± 18.67	709 ± 20.34
	GL	4.11	3705	311 ± 8.81	207 ± 5.91	280 ± 7.87	2852 ± 80.08
	PL	0.98	5419	1623 ± 48.12	650 ± 18.66	791 ± 22.69	1718 ± 49.40
third leaf	NL	1.77	3343	832 ± 18.12	357 ± 16.68	862 ± 9.07	1092 ± 23.90
	GL	4.38	4442	314 ± 8.06	212 ± 4.55	282 ± 6.16	3555 ± 7.76
	PL	1.10	5718	1555 ± 34.24	652 ± 14.45	749 ± 16.50	2283 ± 50.78
fourth leaf	NL	1.97	3376	841 ± 22.17	337 ± 7.13	871 ± 23.67	1104 ± 29.51
	GL	4.48	4505	316 ± 8.50	215 ± 5.89	287 ± 7.71	3359 ± 87.66
	PL	1.25	5783	1457 ± 34.50	659 ± 18.26	711 ± 19.07	2371 ± 63.28
stem portion	NL	0.49	1481	474 ± 9.46	124 ± 2.64	442 ± 7.85	405 ± 7.41
	GL	3.26	2069	136 ± 2.87	102 ± 2.16	141 ± 2.49	1614 ± 31.38
	PL	0.73	4738	1472 ± 31.48	327 ± 6.98	1354 ± 29.03	1326 ± 28.19

Table 5. Changes in Fatty Acid Composition from Various Lipid Fractions Such as Neutral Lipids (NL), Glycolipids (GL), and Phospholipids (PL) from Different Plucking Rounds and Plucking Standards

				fatty acid composition (μg/g)				
type of plucking	lipid	%	fatty acid proportion	palmitic (16:0)	oleic (18:1)	linoleic (18:2)	linolenic (18:3)	CV[a] (%)
(A) plucking round								
7 days	NL	1.61	1976	494	322	620	447	1.33
	GL	3.63	3828	471	239	256	2741	4.88
	PL	1.12	4040	1282	457	1159	960	1.90
9 days	NL	1.73	2524	578	399	824	644	1.33
	GL	3.73	3783	351	198	280	2769	4.88
	PL	1.17	4544	1370	500	1118	1212	1.90
11 days	NL	1.89	2738	641	364	871	673	1.33
	GL	3.77	3875	337	237	330	2774	4.88
	PL	1.19	4727	1430	458	1304	1314	1.90
(B) plucking standard								
fine	total	6.82	13555	3551	1319	3089	4834	3.50
coarse	total	7.68	13958	2860	1383	3521	5560	

[a] The differences between fatty acids are highly significant at $P < 0.01\%$.

linolenic acid (18:3), in the glycolipid portion followed by saturated palmitic acid (16:0) and linoleic acid (18:2) in the phospholipid portion as well as almost equal amounts (18:3 and 18:2) in the neutral portion were observed. Increases in the relative proportions of neutral and structural lipids such as phospho- and

glycolipids are associated with shoot maturity and are maximum in coarse harvested leaves. An increase of linolenic acid and a decrease in palmitic acid in the glycolipid fraction have been observed. Furthermore, palmitic acid content was found to be higher in the phospholipid fraction. From the data it is clear that *de novo* synthesis of linolenic acid is mostly taking place in the glycolipid fraction of the leaf chloroplast, in contrast to wheat and oats (De Man and Cauberghe, 1988; De Man and Bruyneel, 1987).

Conclusion. Flavor volatiles, which are synonymous with lipid, fatty acids, and lipoxygenase activity, can be a helpful tool to evaluate the quality of clonal cultivars as an objective measurement of organoleptic properties of black tea. It has been reported that all types of lipids and polyunsaturated fatty acids were major contributors to the development of desirable/undesirable flavor in manufactured tea. Therefore, it is very important to make clear the seasonal changes with respect to chemical constituents in the plant where agronomic practices have their special role to play.

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