JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 7691−7699 **7691**

Specific Fluctuations in the Composition of Lipoxygenase- and Glycosidase-Generated Flavors in Some Cultivated Teas of Assam

PORAGJYOTI SAIKIA

Tocklai Experimental Station, Jorhat 785 008, Assam, India

PRADIP K. MAHANTA*

Hindustan Lever Research Center, 64 Main Rd., Whitefield, Bangalore 560 066, Karnataka, India

Variations of fatty acid compositions, glycosides precursors, and lipoxygenase and glycosidase enzymatic activities were used simultaneously to differentiate for nine genetically different cultivated teas, four seasonal changes, and the affect of leaf maturity. The muscatel flavor of second-flush teas was associated with increased activities of glycosidase and several terpenes, phenolics, and aliphatic compounds bound to glycosides, whereas high levels of fatty acids and lipoxygenase activity biosynthesized more green volatiles in monsoon teas. Sequential hydrolysis of lipids and lipoxygenase-mediated reactions, during withering and rolling, showed a 3-fold increase of hexenol, hexenal, and related volatiles, but they decreased to the levels of fresh leaf during drying. However, a 4-fold increase of the floral bend of volatiles found in black tea developed due to the hydrolysis of the glycoside precursors throughout processing stages. About 45 key volatiles were monitored for flavor superiority among different clones. Various parameters affecting yield of volatiles were optimized and recommended.

KEYWORDS: Clonal cultivars; fatty acids; terpenes; glycoside aroma precursors; flavor index; tea processing; fermenting enzymes

INTRODUCTION

The pleasant aroma, brisk taste, and slight sweetness of Assam and Darjeeling teas, when objectively analyzed, was shown to be controlled by a combination of ∼30 aroma-active compounds (*1*, *2*). However, the introduction of several vigorously growing vegetatively propagated clonal varieties, in garden plantations, developed by cross-breeding of *Camellia cambodensis* (a subtype of var. assamica), was reported to produce poorer organoleptic quality compared to varieties derived from *Camellia assamica* or *Camellia sinensis* L. (China variety). Most of the research on conventional tea selection programs has mainly been directed toward obtaining desirable agronomic characteristics such as resistance to pests and diseases, better leafing-to-rooting ratios, also improved flavor quality in the brewed liquor (*3*, *4*). Another notable development in modern tea factories is that traditional orthodox processing has been overtaken by crush−tear−curl (CTC) and "Rotorvane" technologies in the recent decades.

More recently, aromatic cultivars meant for oolong or semifermented tea manufacturing were characterized for monoglycosidic (β-D-glucopyransides) aroma precursors with benzyl alcohol, (*Z*)-3-hexen-1-ol, benzaldehyde, linalool, and geraniol aglycons, as well as β-glucosidase activity (*5*−*8*). In addition, higher levels of disaccharide flavorless glycoside precursors composed of sugar residues β-D-xylopyranosyl-β-D-glucopyranosides to essential oils such as geraniol, linalool, 2-phenylethanol, benzyl alcohol, and both (*E*)- and (*Z*)-linalool 3,7-oxides, including 8-hydroxygeraniol, were isolated and characterized from fresh leaf (from different China species) (*9*−*11*). It has been possible to study the multiple hydrolytic properties of endogenous β-D-glycosidase and more particularly β-D-primevorosidase enzymes (Japanese tea plant Yabuchita, a hybrid of China−Assam), and their changes of glycoside precursors in incubation studies have provided the major aroma formation mechanism in fermented tea (*12*−*16*).

Another important mechanism of flavor generation is lipid metabolism, modulated through multiple lipoxygenase (LOX) action on linoleic acid and linolenic acid in the presence of oxygen (*17*, *18*). Typically during aging and mechanical injury, the hydrolysis of lipids by lipases and the formation of 9- and 13- hydroperoxide intermediates decisively formed "fresh-green" compounds such as (*Z*)-3-hexenol, (*E*)-2-hexenal, hexanal, and other related products have been confirmed (*19*−*21*). Also, characterization of the most odoriferous *epi*-methyl jasmonate in the seasonal flavors and in oolong tea has unequivocally proved their formation is influenced by environmental and processing conditions (*22*, *23*). Independently, these naturally

* Author to whom correspondence should be addressed (telephone 91-80-845 2959/845 3085; e-mail pk.mahanta@unilever.com).

10.1021/jf0257345 CCC: $22.00
Published on Web 11/22/2002

occurring plant pheromones were biologically synthesized from linolenic acid, lipoxygenase, cyclization of hydroperoxide, and β-oxidation (*24*).

Young vegetative parts, containing large numbers of secretary cells known as glandular trichomes, apart from transferring genetic character from parent to progenies, are also the site for the biosynthesis of a cornucopia of fragrance compounds, predominantly monoterpenoids, and the oil content varies with habitat and environmental conditions (*25*−*30*). The other most striking feature is the lack of consistency in the processed tea quality, because of imperial methods of manufacturing. Consequently, our objective is to examine the cultivar characteristics from glycosidic and lipid precursor analyses and to manipulate flavor attributes positively from hydrolytic and oxidative reactions, relevant to the two main categories of black teas, orthodox and CTC.

MATERIALS AND METHODS

Reagents and Reference Samples. Analytical reagent grade solvents were used. The fatty acids, aroma standards, and *p*-nitrophenyl-β-D-glucosidase (pNPG) as the substrate of glycosides in tea leaves were purchased from Sigma Chemical Co. (St. Louis, MO). Commercial Pectinex UPL and almond β-glucosidase were procured from Rhom. All other chemicals and reagents were purchased as described in our previous studies.

Plant Materials. Tocklai vegetatively propagated clonal cultivars (TV series), consisting of two to three leaves, the apical bud, and the included stem, maintaining 7-days plucking round, were used in each experiment during 1994−1996. TV-2, TV-12 (Assam type, large leaf), TV-7 (China type, small leaf), TV-1 and TV-17 (Assam−China hybrid), and TV-9, TV-19, TV-25, and TV-26 (Cambod hybrids, similar to Assam variety) as well as old seedling plants were obtained from the Tocklai model tea garden planted in 1981.

Manufacturing. After plucking, the fresh leaves (∼10−200 kg) were spread (5−8 cm) on withering troughs and allowed to undergo natural desiccation (12−20 h) depending upon type of manufacture: orthodox or CTC in the pilot factory. The hard withered leaves (moisture content of ∼65%) were subjected to "three crank roller" for ∼1 h for orthodox manufacture. Soft withered leaves (moisture content of ∼70%) were first subjected to a Rotorvane (a preconditioner), followed by three to four CTC macerations in tandem. After rolling, fermentation (aerial oxidation) was carried out for 40−50 min for CTC teas and for ∼2.5−3 h for orthodox. Finally, at the completion of fermentation, the material (dhool) was dried, using a tray dryer for orthodox tea and a fluid bed dryer (FBD) for CTC tea, at a temperature of 100−120 °C for 20−30 min, until the moisture contents were reduced to ∼2.5%. Samples during each processing stage, such as fresh leaves (FL), deactivated dried green leaf (DADGL), withered leaf (WL), fermented leaf (FL), and drier mouth samples (DMS), that is, black tea, were regularly collected for analyses.

Analysis of Lipids and Fatty Acids. Extraction of crude lipids, their fractionation into neutral lipids (NL), glycolipids (GL), and phospholipids (PL), hydrolysis of free fatty acids (FFA), and fatty acid methyl ester (FAME) preparations were performed according to Bhuyan et al. (*31*). FAMEs were measured in Netal gas chromatography (GC, model Mchro-9100), equipped with a flame ionization detector (FID), using DEGS-PS on Chromosorb WHP (80/100 mesh) packed on a stainless steel capillary column (2 m × $^1/_8$ in. o.d.), and N as the carrier gas (20 mL min^{-1}). The injector and detector temperatures were 220 and 200 °C, respectively, and programming of the column/oven temperature was from 120 to 180 °C at 4 °C min^{-1}. Comparison of their GC retention times with those of corresponding authentic standards was used to identify and quantify the separated fatty acid components.

Isolation of Glycosidic Aroma Precursors. The decaffeinated and volatile-free fresh tea samples (500 g) on extraction with hot water yielded organic solid (150 g of plant extract). Polyphenols were removed by adding polyvinylpolypyrrolidone (PVPP) to the crude solution, and on centrifugation a crude glycoside fraction (∼70 mg) was isolated. The crude was further purified through sequential application onto (1) Amberlite XAD-2 column and (2) silica gel column chromatography. Silica gel separations were obtained by eluting increasing amounts of MeOH in $CHCl_3$ and finally 40% MeOH in water. All of the fractions were further subjected to preparative HPLC analysis, and 12-glycoside fractions were detected as referred to in the literature (*9*−*11*).

Thin Layer Chromatography. Chromatographic mobility was determined on TLC silica gel plates in two systems: (1) EtoAc/HCOOH/HOAc/H20 (20:1:1:2) and (2) $CHCl_3$/MeOH/H_2O (16:9:2). Three fractions (spots) on the TLC plate responded positively for glycosides: (a) fluorescence using UV−vis scanning from 200 to 600 nm and (b) spraying anisaldehyde−sulfuric acid reagents (*32*, *33*).

Identification and Hydrolysis of Glycosides. The three major spots from the TLC plates and 12 fractions from preparative HPLC as well as from the crude glycosides were individually hydrolyzed to establish their aglycon composition. The hydrolyses were carried out by (1) incubation with endogenous [acetone-washed enzyme powder or soluble in ammonium sulfate, (30−100% cut)] and (2) exogenous enzymes, namely, pectinase and β-glucosidase. Typically a 0.1 mL substrate containing 20 mg, incubated with 0. 1 mL of crude enzyme preparation in a 50 mM citrate buffer (0.1 M pH 6.0) and a blank were kept at 40 °C overnight. After the completion of the reaction, the liberated aroma was injected into a GC, and the glycoside-bound precursors before and after hydrolysis were compared with HPLC peak areas (*15*, *16*).

Oil Distillation and Concentration. Fifty grams of black tea, 100 g of fresh, withered, rolled, and fermented leaf samples, and 1 mL of a 250 μg/mL solution of ethyl caproate (as the internal standard) were steam distilled under reduced pressure for 2 h at 50 °C as previously described (*1*). The concentrated aroma fractions were subjected GC analysis.

Oil Analysis. A Netal GC (model Mchro-9100) equipped with an FID detector and a 5% Carobowax x 20M on Chromosorb G (80−100 mesh) thick-film-packed column (5 m × 3 mm i.d.) was used for volatile analyses. The column temperature was programmed from 60 to 180 °C at a rate of 2 °C min^{-1}, after a 2 min isothermal period at 60 °C. The injector and detector temperatures were kept at 200 and 210 °C, respectively, and nitrogen was used as the carrier gas at a flow rate of 30 mL min^{-1}. The detector was connected to a recording data processor model 600.

Identification of the compounds was made by comparing the GC retention times with those of individual authentic volatiles. Further individual compounds were matched with the reported MS fragmentation pattern analyzed by the same program. The individual peak areas were expressed from the enhancement of peaks with pure substances as percent area as well as peak area ratio to the internal standard area.

Preparation of Chloroplasts. Ten grams fresh tea shoots was homogenized with 40 mL of prechilled McIlvine's buffer (0.1 M citric acid 0.2 M $NaHPO_4$), pH 6.3, containing 0.4 M sucrose and 10 g of polyclar AT. After the homogenate had been filtered through cheesecloth, the chloroplast fraction was precipitated by centrifuging at 100000*g*. The suspension was further washed twice with prechilled buffer without polyclar AT for lipoxygenase assay (*34*).

Polarographic Method of Lipoxygenase Assay. The activity of crude lipoxygenase extract was measured immediately using a Clark oxygen electrode (Gilson, model 5/6) on the assumption that there was 0.24 mM dissolved oxygen at 25 °C in the saturated air. The reaction mixture containing 0.30 mM substrate (10 mM linoleic acid, 99%) and 0.1 mL of the chloroplast suspension in 0.8 mL of McIlvine's buffer, pH 6.3, and sodium dithionate ($Na_2S_2O_6$) was used as standard. Oxygen consumption was recorded on a chart (speed = 0.1 mm/s) at 30 °C for 3 min, and the results were expressed as micromoles of O_2 per minute per gram of leaf (*35*).

Acetone-Washed Enzyme Powder. Fresh young shoots (50 g) were homogenized repeatedly with chilled acetone for 3 min in a dispersing mix; the solvent layer was removed by filtration. The dried powder (4 g) was suspended in a prechilled sodium citrate buffer at pH 5 (in 40 mL of 50 mM) with 0.5 g of polyclar AT and homogenized twice for 30 s at 0 °C. After 1 h of storage at 0 °C, the suspension was centrifuged for 20 min at 10000*g* at 4 °C. The clear supernatant was collected and used as a crude enzyme solution for the β-glycosidase assay.

Table 1. Lipids and Fatty Acid Composition in Different Shoot Components (Average of Three Years' Analysis, with Standard Deviation)

shoot component	lipid class	lipid amount, g/100 g of dry wt	fatty acid, μg/g of dry wt			
			palmitic, 16:0	oleic, 18:1	linoleic, 18:2	linolenic, 18:3
bud	NL	0.46	512 ± 41	152 ± 12	808 ± 71	737 ± 60
	GL	4.50	279 ± 24	155 ± 11	282 ± 23	2352 ± 196
	PL	0.90	1614 ± 127	480 ± 35	1249 ± 111	1791 ± 142
	total	6.00	2405	787	2339	4880
first leaf	NL	1.42	691 ± 52	209 ± 14	748 ± 64	582 ± 54
	GL	5.07	337 ± 27	195 ± 23	320 ± 17	3610 ± 230
	PL	1.21	2120 ± 205	595 ± 42	1115 ± 73	2640 ± 132
	total	7.70	3148	999	2183	7102
second leaf	NL	2.10	830 ± 210	375 ± 129	889 ± 93	914 ± 209
	GL	5.56	416 ± 42	182 ± 80	378 ± 81	3920 ± 109
	PL	1.31	1986 ± 137	869 ± 249	1036 ± 226	2577 ± 70
	total	8.97	3232	1424	2303	7411
third leaf	NL	2.50	1169 ± 253	575 ± 248	1159 ± 332	1535 ± 354
	GL	5.90	420 ± 105	295 ± 100	425 ± 125	4738 ± 450
	PL	1.30	1849 ± 469	840 ± 152	906 ± 220	3021 ± 288
	total	9.70	3438	1710	2490	9294
stem	NL	0.72	575 ± 82	211 ± 40	556 ± 124	525 ± 123
	GL	4.66	210 ± 39	138 ± 71	294 ± 83	2152 ± 526
	PL	1.00	1855 ± 458	450 ± 155	1857 ± 637	1775 ± 351
	total	6.38	2660	799	2707	4452

Table 2. Changes of Different Lipid Fractions (Grams per 100 g) and Fatty Acid Composition during Processing (Micrograms per Gram of Dry Weight) of Green Leaf [Three Years' Average (± Standard Deviation)]

sample	total lipids	neutral lipid	glycolipid	phospolipid	palmitic acid	oleic acid	linoleic acid	linolenic acid
green leaf	6.67 ± 0.37	1.63 ± 0.142 (24.44%)[a]	4.08 ± 0.28 (61.17%)	0.96 ± 0.01 (14.39%)	3158 ± 148	1963 ± 132	3487 ± 153	7287 ± 173
withered leaf	5.69 ± 0.33 [14.69%][b]	1.48 ± 0.01 [9.20]	3.44 ± 0.23 [15.69%]	0.77 ± 0.02 [19.79%]	2397**	1296**	2209**	4508**
rolled leaf	4.5 ± 0.13 [32.53%]	1.14 ± 0.05 [30.06%]	2.70 ± 0.17 [33.82%]	0.66 ± 0.02 [31.25%]	1753**	943**	1497**	1997**
fermented leaf	4.40 ± 0.21 [34.03%]	1.12 ± 0.01 [31.29%]	2.60 ± 0.10 [36.27%]	0.63 ± 0.01 [34.38]	1633**	898**	1384**	1802**
made tea (orthodox)	2.89 ± 0.17 [56.67%]	0.69 ± 0.06 [57.67%]	1.73 ± 0.15 [57.60%]	0.47 ± 0.02 [51.04%]	862**	547**	727**	1056**

[a] Percent of each fraction in comparison to green leaf is given in parentheses. [b] Percent degradation in comparison to green leaf is given in brackets.

***β*-Glycosidase Assays.** Glycosidase activities were measured from a 100 μL extract, using a 1 mM solution of pNPG as the substrate. The mixture was incubated at 40 °C for 10 min in 150 mL of a citrate buffer (pH 5.0 + 0.5 g of polyclar AT + 0.3 g of ascorbic acid). The reaction was stopped by adding 1 mL of a 1 M sodium carbonate solution, and the yellow color formed was measured at 400 nm in a Beckman spectrometer using a 1 cm quartz cell. The results expressed the amount of liberated *p*-nitrophenol, determined from the calibration curve. One unit of *β*-D-glucosidase activity was expressed in nanokatals per milliliter of enzymatic solution (*5*, *12*). Protein content in the enzyme solution was determined by using Lowry's method, and specific activity was expressed as units per milligram of protein.

RESULTS AND DISCUSSION

Leaf Maturity and Lipid Composition. The average results (three years' analyses) of various lipid fractions, namely, glycolipid (GL, 61%), phospholipid (PL, 14%), and neutral lipid (NL, 25%), and the fatty acid (FA) compositions of different parts of the shoot are shown in **Table 1**. In general, the levels of accumulation of various lipids and their saturated and unsaturated fatty acid contents found to increase with leaf maturity. Surprisingly, although the GL content was highest, the major fatty acids, palmitic acid (PA, C16:0), linoleic acid (LA, C18:2), and linolenic acid (LNA, C18:3), were predominately present in the PL followed by NL fractions. Ultimately, the LNA content determines (shoot parts or cultivar variations) the fresh green volatile contents that exist in the intact tissues or that develop during processing.

Table 2 shows the changes in the various fractions of lipids and fatty acids during withering, rolling, fermentation, and drying, compared to fresh leaf. The decrease in different lipid contents such as NL, GL, and PL and fatty acid contents such as palmitic, oleic, linoleic, and linolenic acids at each of the four stages of manufacturing is highly significant. **Figure 1** shows a typical profile of hydrolytic release of free fatty acids (FFA) and the subsequent breakdown of unsaturated FFA during withering by lipoxygenase. The continuous increase in FFA contents was because hydrolysis is greater than degradation in all four stages of manufacturing; therefore, FFA contents were found to be higher in black teas than in starting raw materials.

Enzymes versus Precursors. To evaluate the eventual role of LOX and *β*-glucosidase activities on the development of aroma precursors such as fatty acids and glycosides, their enzymatic activities in different parts of shoots were measured (**Table 3**). LOX activity was highest in the third leaf followed by the second leaf, first leaf, and stem and was lowest in the bud. The increased enzyme activity perhaps helped in the synthesis of membrane metabolites, especially palmitic, oleic, linoleic and linolenic acids, along with the shoot maturity (*36*,

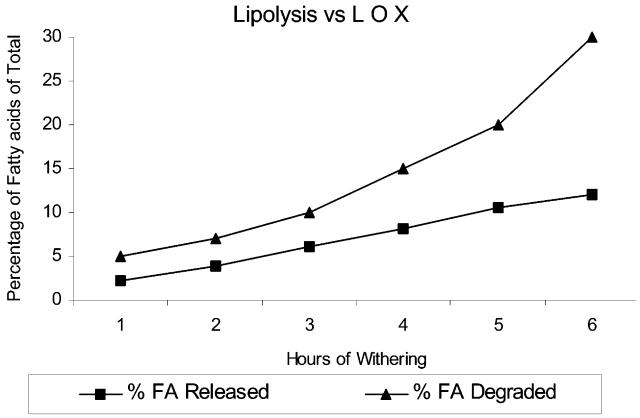

Figure 1. Percent of free fatty acid released and degraded during withering (average of three replications).

Table 3. Lipoxygenase and β-Glycosidase Activities at Different Stages of Leaf Maturity (Each Value Is the Replicated Mean of Three Analyses)

shoot part	LOX (μmol of O_2/min/g of dry wt)	β-glucosidase (specific activity, units/mg of protein)
bud	5.03	3.54
first leaf	7.21	4.13
second leaf	9.85	3.28
third leaf	11.51	1.17
stem	6.52	2.68

37). On the other hand, β-glycosidase specific activity was higher in younger leaves, which decreased as the leaf aged, and was exceptionally high in the stem. The lower specific activity of β-glycosidases (although reported major enzyme is β-primevorosidase), in the matured leaf and higher value for the younger ones, suggesting that glycosides be synthesized de novo rather than releasing by hydrolysis (*38*−*41*).

Floral and Green Volatiles. We then compared the volatile profiles in black teas manufactured from different parts of shoots (**Figure 2**). We categorized the aliphatic alcohols and aldehydes (derived from fatty acid oxidation), responsible for fresh green aromas, as group I volatiles, whereas several terpenes and benzenoids (hydrolysis of glycosides), responsible for sweet and flowery fragrances, were denoted group II volatiles. It was noticed that with leaf maturity, the C-6 to C-8 components were found to increase, because of higher polyunsaturated fatty acids and their degradation due to LOX (**Figure 2A**), whereas the terpenes and bezenoids showed a decreasing trend with leaf maturity, because they bind to sugars (**Figure 2B**).

The reason for the liberation of larger amounts of (*Z*)-3-hexenal and (*E*)-2-hexenal from the third leaf as compared to the tenderer parts may indicate that they are derived from glycopyranosidases and the β-glycosidase hydrolytic pathway (*6*). Another probable mechanism is due to enzyme alcohol dehydrogenase, wherein (*Z*)-3-hexenal reduces to (*Z*)-3-hexenol in coarse leaf (*42*, *43*). However, such a relationship does not exist in the stem, and the higher production of hexanol is assigned to a higher ratio of linoleic to linolenic acid than is found in the immature leaves (*44*).

Leaf Maturity: Glycosides and Glycosidases. To investigate the relationship that exists between volatile formation and their contents of glycoside aroma precursors and β-glycosidase enzyme, each shoot part was measured indirectly (clone TV-1). A crude aroma precursor was prepared from each part of steamed shoot (30 g) and incubated with crude β-glycosidase

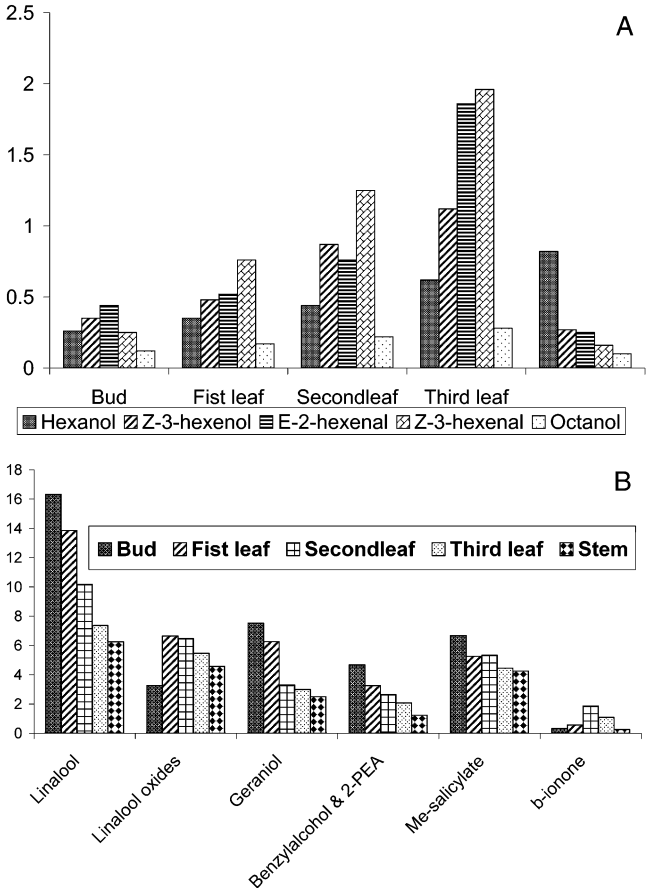

Figure 2. Green volatiles (A) and terpenes and phenolic aromatics (B) from different parts of a shoot (peak area ratios: average of three replications).

enzyme preparation (1 g, acetone-washed powder). The volatiles liberated were considered to be roughly proportional to the amounts of the corresponding aroma precursors. Similarly, β-glycosidase activity was determined by incubating crude enzyme prepared from each part of the shoot with a crude solution of aroma precursors. The hydrolytic activity was expressed in terms of the volatiles released.

It is interesting to note that a considerable amount of the characteristic major floral compounds (linalool, linalool oxides, geraniol, benzyl alcohol, 2-phenylethanol, including methyl salicylate) were found to be present in bound glycoside at the fresh leaf level. Their contents of free volatiles was found to decrease with leaf maturity in the order buds < first leaf < second leaf < third leaf and least in the stem (**Figure 3A**). With regard to β-glycosidase activity, a sharp maximum in extractable enzyme activity profile in the juvenile second leaf, which suddenly dropped from the thirrd leaf level, was noted (**Figure 3B**).

Although different sources showed variations in β-glycosidase activity, a multiple hydrolysis might take place simultaneously. The results also indicate that juvenile parts contain mainly free volatiles due to higher levels of β-glycosidase activity, and thereafter they are metabolized into glycosylated form via various β-glycosidases (mainly due to β-primevorosidase) with physiological age (*38*−*41*). This situation is similar to that of blooming flowers, where the β-glycosidase activity and headspace fragrance volatiles, attained maximum (*45*).

Species Variations and Flavor Index. As monoterpenoids contribute significantly to the varietal character, the terpene index (TI) (ratios of linalool plus its oxides divided by linalool

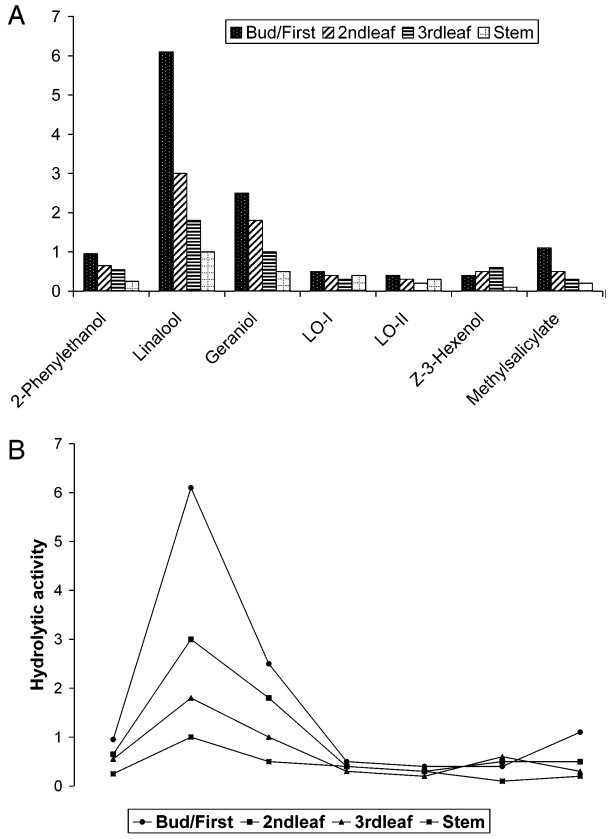

Figure 3. Aglycons (A) and (B) glycosidase enzyme activity (B) of different parts of fresh shoot (each value is a replicated mean value).

Table 4. Volatiles Variation and Flavor Index in Different Clonal CTC Black Teas (Peak Area Ratios; Each Value Is the Replicated Mean of Three Analyses)

VFC	TV1, Assam–China hybrid	TV2, Assam type	TV7, China hybrid	TV9, Cambod type	TV17, Assam–China hybrid
hexanal	0.05	0.07	0.04	007	0.06
hexanol	0.11	0.12	0.16	0.12	0.17
(*E*)-2-hexenal	0.54	0.79	0.43	0.80	0.59
(*Z*)-3-hexenol	0.18	0.16	0.19	0.16	0.19
hexenal	0.15	0.14	0.06	0.05	0.15
green total, group I	1.03	0.98	0.88	1.2	1.1
linalool oxide I	0.27	0.26	0.14	0.06	0.57
linalool oxide II	0.94	0.76	0.86	0.72	0.92
linalool	3.32	2.32	1.93	1.73	2.78
phenylacetaldehyde	0.54	0.27	0.98	0.68	0.47
methyl salicylate	0.84	0.74	0.82	0.90	0.92
geraniol	0.59	0.36	0.66	0.23	0.58
benzyl alcohol + 2PEA	0.78	0.55	0.72	0.59	0.85
α-ionone	0.56	0.16	0.70	0.18	0.36
β-ionone	0.35	0.16	0.56	0.17	0.26
floral total, group II	8.19	5.58	7.37	5.26	7.71
total area ratio	9.22	6.86	8.22	6.46	8.87
group II/group I	7.95	7	8.39	4.38	8.06
terpene index[a]	0.88	0.90	0.82	0.92	0.88
taster's flavor quality	better than Assam	good	best	poor	very good

[a] Area of linalool and its oxides/area of linalool, its oxides, and geraniol.

plus its oxides and geraniol) has been used to differentiate cultivar variations (*46*). **Table 4** illustrates the TIs in black teas manufactured from five genetically different clonal teas, the most popular in northeastern Indian plantations. Usually, the TI of pure *assamica* var. Assam is nearly 1.0 and that of *sinensis* var. China is close to 0.5. From the TI value, TV-2 is predominated by Assam character, TV-1 and TV-17 are

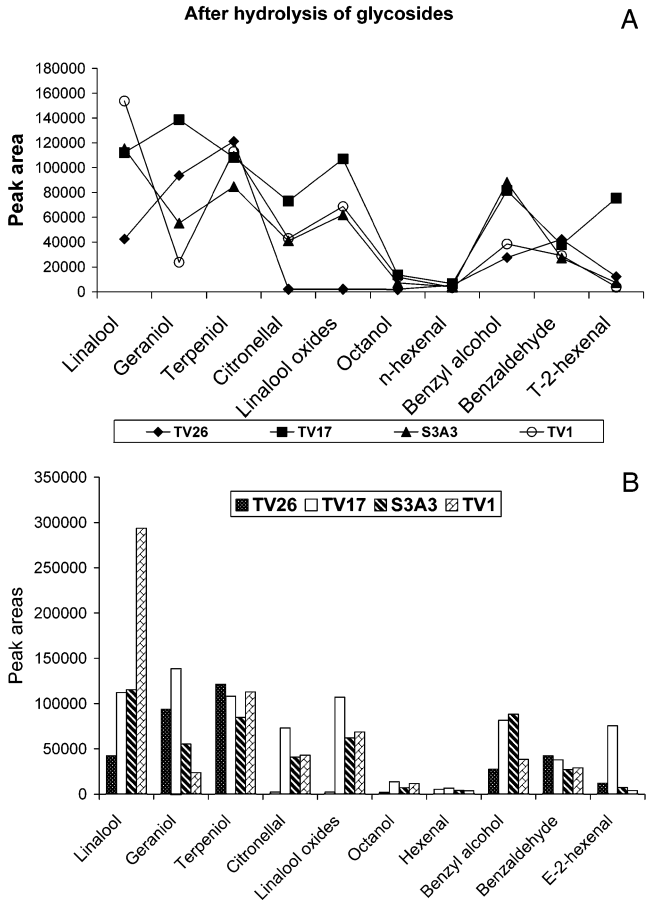

Figure 4. (A) Potential hydrolyzed products of individual clones (average of three replications); (B) clonal variations of volatile profiles in CTC made teas (each value is a replicated mean value).

Assam−China hybrids, TV-7 is a China hybrid, and TV-9 is a Cambod type. It is quite clear that levels of monoterpenoids play the key role in determining genetic variations as well as manufacturing potential of vegetatively propagated teas.

Furthermore, variability with other non-terpenoyl substances such as benzyl alcohol, 2-phenylethanol, benzaldehyde, norisoprenoids, and α- and β-ionones also exists among the various clones. We have developed a flavor index (FI) to evaluate objectively the variations of different black tea characters. The higher the FI, that is, the higher the sum of peak areas of the hydrolyzed volatiles (group II) divided by oxidized products (group I) ratios, the better is the flavor quality (*47*−*49*). From the sum of released volatiles, terpene index, flavor index, and taster's score, clonal superiority was found to be in the following order: China > Assam > Cambod varieties (**Table 4**).

To investigate their cultivar variability with respect to glycoside precursors TV-17, S3 A3, TV-1, and TV-26 (all from second flush) were isolated individually and subjected to enzymatic hydrolysis by acetone-washed enzyme powder and commercially available pectinase enzyme. **Figure 4A** shows their potential volatiles derived from glycoside precursors, whereas **Figure 4B** shows volatiles actually developed during manufacturing from the individual clones. From the result flavorist clone was found to be of the following order: TV-17 > S3 A3 > TV-1 > TV-26.

Quantitatively, however, free volatiles released during processing were much lower than the direct hydrolysis by enzyme preparations. TV-17, a cultivar well-known for its bright color and aroma (the taster's sensory evaluation), contained highest amount of essential oils and glycoside aroma precursors,

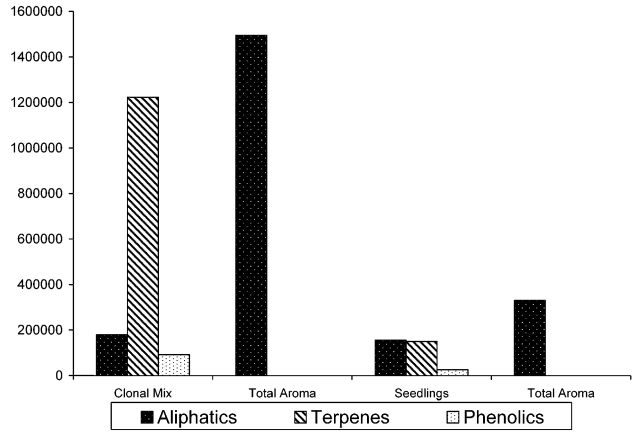

Figure 5. Fragrance variations of clonal Vs seedling teas (peak area ratios: average of three replications).

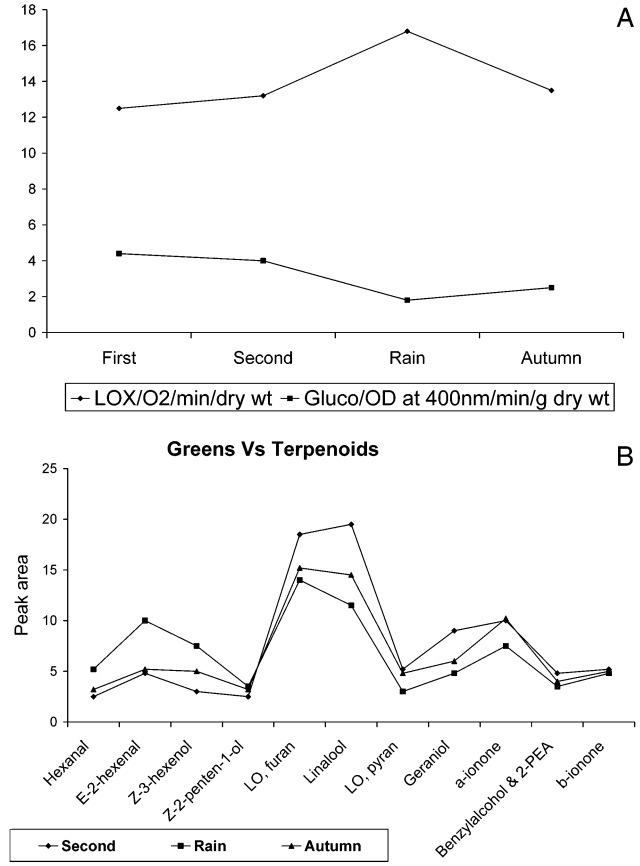

Figure 6. (A) Lipoxygenase and β-glucosidase activities in different flushes [April, May, July, and October (replicated mean value of six different clones]; (B) seasonal changes of aroma profiles [May, July, and October (each value is the replicated mean of six clonal teas)].

followed by S3 A3/TV-1, and the least was found in TV-26. **Figure 5** clearly demonstrates that tea manufactured from the clonal mixture produced 6−8 times higher terpenes, aliphatics, and phenolic aromatics than did tea from inherently inferior seedling shoots.

Seasonal Variations. Figure 6A shows the seasonal variations of lipoxygenase and β-glucosidase enzymatic activities of six clonal plants (same trend by individual clones) during the four flushes: first (March−April), second (May−June), third/monsoon (July−September), and autumn or fourth flush (October−November). Importantly, it can be seen that LOX exhibited highest activities during monsoon flushes, whereas

Table 5. Seasonal Changes of Fatty Acid Composition (Micrograms per Gram of Dry Weight) of Green Leaf (Three Years' Average)

fatty acid	second flush	rain flush	autumn flush
palmitic acid	1113 ± 52	3987 ± 123	2508 ± 96
oleic acid	694 ± 35	2599 ± 102	1504 ± 101
linoleic acid	1677 ± 79	4627 ± 212	2986 ± 150
linolenic acid	2418 ± 83	8676 ± 313	4892 ± 209

β-glucosidase enzyme activity was highest for the second-flush teas (**Figure 6A**). **Figure 6B** summarizes the characteristic volatile profiles in different flushes: highest amounts of linalool and its oxides, geraniol, phenolic aromatics, and ionones were found in second-flush teas, whereas higher levels of (*E*)-2-hexenal, (*Z*)-3-hexenol, and other aliphatic compounds predominated in rain-flush teas.

Apparently, yellow leaves found in second-flush teas can be characterized by higher levels of several terpenes and norisoprenoids: linalool, its oxides, geraniol, α-ionones, and β-ionones, formed either directly from degradation of carotenes or by their hydrolysis of glycoside precursors. Similarly, the prominent benzenoid components, namely, benzyl alcohol, 2-phenylethanol, and others are formed either from hydrolysis of fibers or from glycoside hydrolysis (enhancing group II volatiles in the second flush). On the other hand, the intense green leaf of the monsoon-flush teas better correlates with a higher amount of green volatiles and highest fatty acid content (**Table 5**) together with higher LOX activity.

In the course of these studies, we obtained that due to warm sunshine hours with cold nights and desiccating wind could stimulate maximum biogenesis of floral components in May-June than those of July-August. In the latter months, the vigorously flushing shoots in the heated and rainy days, and so they're endogenous enzymes progressively decline in reducing terpene biosynthesis to minimum. However, at the end of rainy seasons (October-November), flavor picks up, albeit with a different character, and is usually referred as autumn flavor (*50*−*52*).

ATTEMPTS AT PROCESS OPTIMIZATION

Enzyme Activity and Volatile Development. The stimulatory and/or inhibitory activities of lipoxygenase and β-glycosidase enzymes during withering, rolling, and fermentation stages are illustrated in **Figure 7A**. LOX activity increases steeply from 6 h onward up to 14 h (optimum time for withering) and the rise is ∼1.6 times, whereas β-glycosidase activity increases steeply as withering progresses and reaches ∼2.3 times that in the fresh leaves. Peak activity of both enzymes was observed during rolling (increased at 20−30 min) and fell steeply thereafter, which continues in the fermented leaf. The sharp decline in the level of β-glucosidase and LOX activities seems to be due to the precipitation or binding of the enzymes by the high polyphenol content in tea and their oxidation products (*53*, *54*). Their inhibitory action could also be due to stripping of cofactors during processing, especially divalent metal ions (*55*, *56*).

Effect of Temperature. Temperature shift experiments, during withering and fermentation (leaf heated at 40 °C), caused a remarkable reduction in the β-glycosidase enzyme activity compared to that at a cooled temperature of 25 °C (**Figure 7B**). Initially, however, at higher temperature, the activity increased up to 6 h of withering, which subsequently decreased, whereas at lower temperature, activities were enhanced. The fall in LOX activity (lower oxygen consumption), greater than that of

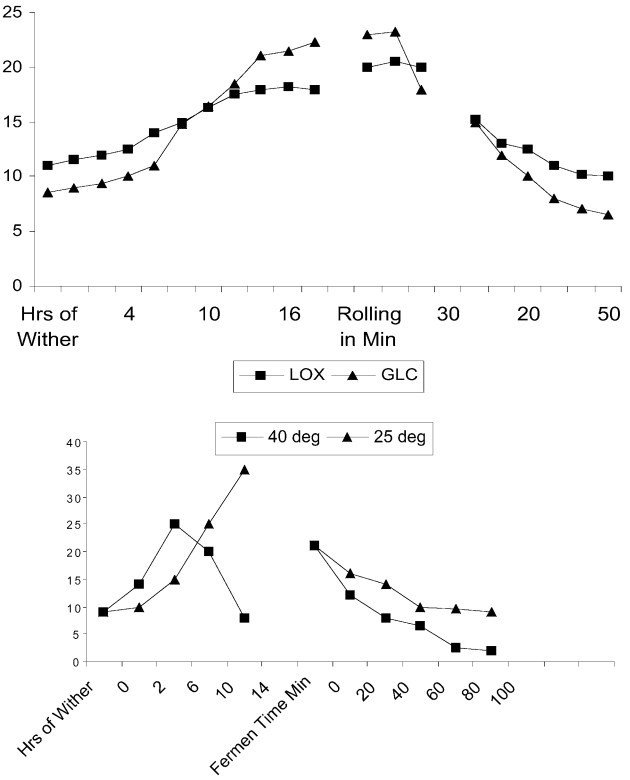

Figure 7. (A) β-Glycosidase and lipoxygenase activities at different stages of processing (each value is a replicated mean); (B) effect of hot and cold temperature on β-glycosidase activity during withering and fermentation (each value is a replicated mean).

β-glycosidase during later stages of withering (after 14 h), and in fermentation may be due to the relative stability of the enzymes. Furthermore, heat generated during withering and fermentation, at temperatures >30 °C, creates deleterious effects and deactivates the enzyme system, resulting in loss of flavor volatiles (data not shown).

Biotransformations. Table 6 shows the amount of aromatic compounds present in fresh leaves and developed during withering, rolling, and fermentation and in orthodox made tea. The amounts of group II volatiles**,** such as linalool, linalool oxides (all four isomers), geraniol, α-terpeniol, 2-phenylethanol, benzyl alcohol, and phenyl acetaldehyde, increased during withering and rolling, whereas the amounts of group I volatiles, such as pentenol, hexenol, and others, drastically decreased to the levels of fresh leaf in the dryer month black teas. By contrast, the enhanced characteristic volatile aglycones that are not originally present, imply that they are generated from the nonvolatile mono- or oligoglycoside aroma precursor due to β-glycosidase activities throughout processing of tea leaves. **Figure 8** summarizes how the amount of "green", "floral", and "spicy" volatile flavor compounds (VFC) increases with withering (maximum at 12 h) and fermentation time (optimum at 30 min after rolling) and thereafter declined.

The increased levels in the contents of fatty acid degradation products, for example, (*Z*)-2-pentenol, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-2-hexenal, (*Z*)-3-hexenal, and (*Z*)-3-hexenol, were due to their activities of enzyme isomerase and alcohol oxidoreductase (NADPH) during withering, rolling, and fermentation (*57*). The decrease in green volatiles in made teas, lower than the levels found in fresh leaf, is due to their high volatility; also, they may escape very easily on drying. The glycoside-derived volatiles, instead of evaporating, may be hydrolyzed, because of their better stability at higher

Table 6. Changes in Volatile Flavor Compounds and Flavor Index (Peak Area Ratios) during Different Stages of Processing (Each Value Is the Replicated Mean of Three Analyses)

VFC	green leaf	withered leaf	rolled leaf	fermented leaf	made teas
Oxidized Volatiles, Group I					
1. hexanal	tr[a]	0.35	0.93	2.21	2.15
2. (*Z*)-2-pentenol and (*Z*)-3-hexenyl actetate	tr	0.25	3.77	1.45	1.30
3. (*Z*)-2-hexenal	tr	0.66	0.23	2.75	1.25
4. (*E*)-3-hexenal	tr	1.25	3.35	5.17	3.45
5. (*Z*)-3-hexenol	7.25	8.30	11.39	2.46	2.21
6. hexenol	0.25	3.53	4.25	2.88	1.23
7. (*Z*)-3-hexenal	3.26	8.10	16.25	9.85	1.22
8. (*E*)-2-hexenol	0.25	2.92	8.26	5.28	0.97
Hydrolyzed Volatiles, Group II					
9. linalool oxide I	0.68	2.25	3.40	2.15	2.27
10. linalool oxide II	2.94	3.24	6.11	4.93	6.95
11. linalool	4.34	12.22	24.46	21.53	12.35
12. phenylacetaldehyde	1.29	3.28	7.42	14.00	6.75
13. α-terpeniol	0.65	0.95	2.96	1.20	2.47
14. linalool oxide III	0.18	0.07	0.16	0.15	0.22
15. linalool oxide IV	1.13	2.56	3.36	4.94	4.28
16. methyl salicylate	1.95	3.24	12.91	4.37	3.95
17. α-ionone	0.91	0.67	2.86	2.47	4.66
18. geraniol	0.86	3.79	4.53	5.75	4.24
19. benzyl alcohol + 2PEA	0.97	4.07	7.85	7.38	3.99
20. β-ionone	0.23	tr	tr	1.87	2.54
sum of fresh green, group I	19.03	25.36	48.43	32.05	13.78
sum of sweet, floral, group II	16.13	36.34	76.02	70.74	54.67
flavor index, group II/group I	0.85	1.43	1.57	2.21	3.97

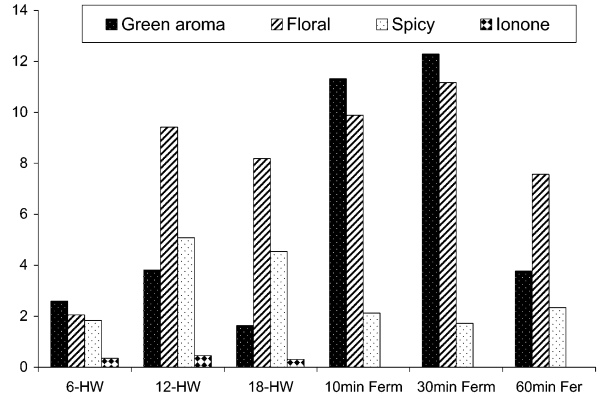

Figure 8. Summary of volatile compounds development during withering and roll fermentation (peak area ratios: average of three replications).

temperatures and increases in the contents of total odor-active volatiles and flavor ratios (group II/group I volatiles) in black tea by ~4 times (i.e., from 0.85 to 3.97) (**Table 6**).

Volatiles of Orthodox and CTC Teas. From time to time there has been a greater demand for conventional orthodox teas (large leaf grades), because of its high aromatic character compared to CTC teas (small particle size, for tea bags). This led to the development of "dual manufacture", where fine shoots (10%) are processed for orthodox teas with harder withering and rolled by three crank rollers and coarse and soft withered leaves (90%) are subjected to the Rotorvane and processed for CTC teas simultaneously. The distribution of flavor constituents between orthodox and CTC manufacture varied considerably, not only between classes of volatiles such as terpenes, benzenoids, and aliphatic carbonyls but also between the individual components.

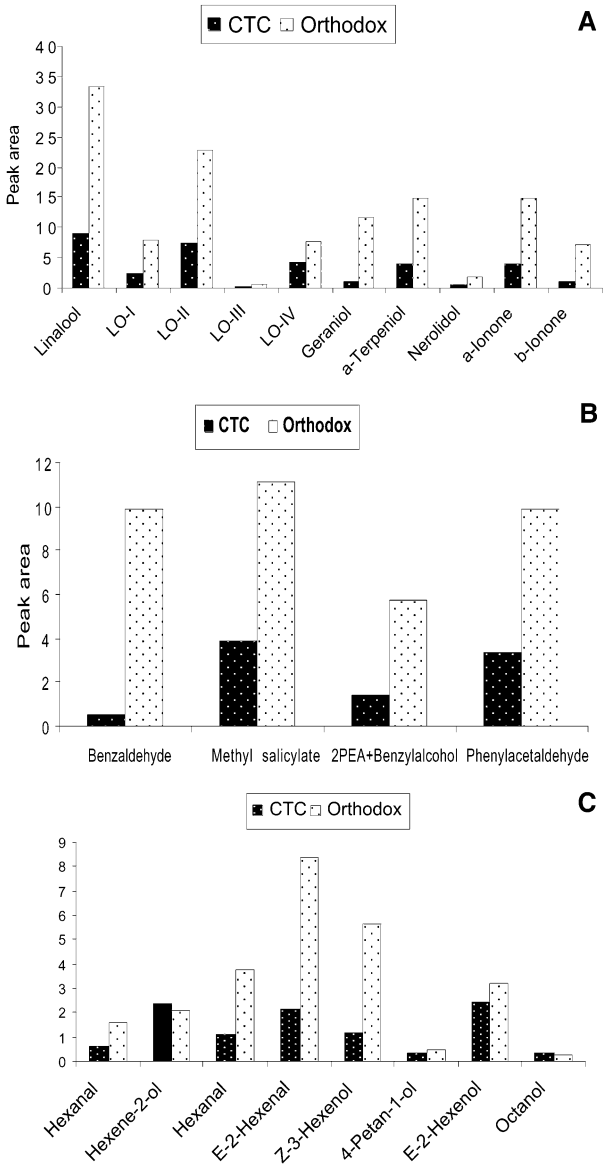

Figure 9. Variations in terpenoid contents (A), phenolic aromatic compounds (B), and green volatile compounds (C) of CTC and orthodox teas.

Figure 9A illustrates the higher levels of release of several terpenes such as linalool and its four oxides geraniol, α-terpeniol, nerolidol, and α- and β-ionones in orthodox teas compared with CTC teas. Similarly, levels of spicy/phenolic compounds such as benzaldehyde, 2-phenylethanol, methyl salicylate, and phenylacetaldehyde peaked during orthodox manufacture compared to CTC (**Figure 9B**). Furthermore, higher amounts of green volatiles such as aliphatic aldehydes and alcohols were also observed in orthodox compared to CTC teas (**Figure 9C**).

Our findings suggest that hard withering and mild prolonged rolling facilitate efficient hydrolysis and oxidation of precursors, producing more floral components. For example, 16% higher fatty acid degradation takes place in rolled orthodox teas, whereas certain glycoside aroma precursors remain unaffected in the 3-min cut CTC processing (results not shown). In addition, the higher rate of catechin oxidation products such as theaflavins and thearubigins, strongly inhibit β-glycosidase and lipoxygenase activities in cutting, crushing and curling (CTC) fermented teas (*58*). It is worth mentioning that teas manufactured under cooled withering and rolling by traditional roller were found to have enhanced yellow color and higher aroma, whereas hot withering and Rotorvane−CTC teas are redder and have poorer flavor (data not shown).

LITERATURE CITED

(1) Mahanta, P. K. Color and flavor characteristics of made tea. In *Modern Methods of Plant Analysis. Analysis of Nonalcoholic Beverages*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1988; Vol. 8, pp 221−295.

(2) Yamanishi, T. Tea flavor. In *Global Advances in Tea Science*; Jain, N. K., Ed.; Aravalli Books International: New Delhi, India, 1999; pp 707−722.

(3) Bezbaruah, H. P.; Dutta, A. C. Tea germplasm collection at Tocklai Experimental. *Two Bud* **1977**, *24*, 22−30.

(4) Barua, D. N. Shoot production in cultivated tea (*Camellia sinensis* L)—II. The branch system. *Phytomorphology* **1961**, *2*, 98−109.

(5) Yano, M.; Okada, K.; Kubota, K.; Kobayashi, A. Studies on the precursors of monoterpene alcohols in tea leaves. *Agric. Biol. Chem.* **1990**, *54*, 1023−1028.

(6) Kobayashi, A.; Kubota, K.; Joki, Y.; Wada, E.; Wakabayashi, W. Z-3-Hexenyl-β-D-glucopyranosides in fresh tea leaves as a precursor of green odor. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 592−593.

(7) Moon, J.-H.; Watanabe, N.; Ijima, Y.; Yagi, A.; Sakata, K. Cis and trans-Linalool 3,7-oxides and methyl salicylate glycosides and (Z)-3-hexenyl-β-D-glucopyranoside as aroma precursors for oolong tea. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1815−1819.

(8) Guo, W.; Sakai, N.; Fukuda, M.; Yagi, A.; Watanabe, N.; Sakata, K. Isolation of an aroma precursor of benzaldehyde from tea leaves (*Camellia sinensis* var. sinensis cv. Yabukuta). *Biosci., Biotechnol., Biochem.* **1998**, *58*, 1532−1534.

(9) Guo, W.; Hosoi, R.; Sakata, K.; Watanabe, N.; Ina, K.; Luo, S. (S) Linayl, 2-phenylethyl and benzyl disaccharide glycosides isolated as aroma precursors from oolong tea leaves. *Biosci., Biotechnol., Biochem.* **1994**, *62*, 2052−2054.

(10) Guo, W.; Sakata, K.; Watanabe, N.; Nakajima, R.; Yagi, A.; Ina, K.; Luo, S. (S) Geraniol 6-O-D-xylopyanosyl-β-D-glucopyranosides isolated as an aroma precursors from tea leaves for oolong tea. *Phytochemistry* **1993**, *33*, 1373−1375.

(11) Nishikitani, M.; Kubota, K.; Kobayashi, A.; Sugawara, F. Geranyl 6-α-L-arabinopyanosyl-β-D-glucopyranoside isolated as an aroma precursor from leaves of green tea cultivar. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 929−931.

(12) Ijima, Y.; Ogawa, K.; Watanabe, N.; Usui, T.; Kameyama, M.; Nagata, T.; Sakata, K. Characterization of β-primeverosidase, being concerned with alcoholic aroma formation in tea leaves to be processed into black tea and preliminary observations on its substrate specificity. *J. Agric. Food Chem.* **1998**, *46*, 1712−1718.

(13) Guo, W.; Ogawa, K.; Yamauchi, K.; Watnabe, N.; Usui, T.; Luo, S.; Sakata, K. Isolation and characterization of a β-primeverosidase concerned with alcoholic aroma formation in tea leaves. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1810−1814.

(14) Ogawa, K.; Moon, J.-H.; Guo, W.; Yagi, A.; Watanabe, N.; Sakata, K. A study on tea aroma formation mechanism: Alcoholic aroma precursor amounts and glycosidase activity in parts of the tea plant. *Z. Naturforsch.* **1995**, *50C*, 493−498.

(15) Wang, D.; Kurasawa, E.; Yamaguchi, Y.; Kubota, K.; Kobayashi, A. Analysis of glycosidically bound aroma precursors in tea leaves. 2. Changes in glycoside contents and glycosidase activities in tea leaves during black tea manufacturing process. *J. Agric. Food Chem.* **2001**, *49*, 1900−1903.

(16) Fischer, N.; Nitz, S.; Drawert, F. Free and bound aroma compounds from green and black tea (*Camellia sinensis*). *Z. Lebensm. Unters. Forsch.* **1987**, *185*, 195−201.

(17) Coggon, P.; Romanczyk, L. J.; Sanderson, G. W. Extraction, purification and partial characterization of a tea metalloprotein and its role in the formation of black tea aroma constituents. *J. Agric. Food Chem.* **1977**, *25*, 278−283.

(18) Hatanaka, A.; Kajiwara, T.; Sekia, J. Enzymic oxygenative-cleavage reaction of linolenic acid in leaves-chloroplastic lipoxygenase and fatty acid hydroperoxide lyase in tea leaves. In *The Metabolism, Structure, and Function of Plant Lipids*; Stumpf, P. K., et al., Eds.; Plenum: New York, 1987; pp 391−398.
(19) Sekiya, J.; Kajiwara, T.; Imoto, M.; Inuoye, S.; Hatanaka, A. Effect of lipolytic hydrolase on the activity for six carbon aldehyde formation in tea chloroplasts. *J. Food Sci. Agric*. **1982**, *30*, 183−185.
(20) Mahanta, P. K.; Tamuly, P.; Bhyuan, L. P. Comparison of lipoxygenase activities and lipid composition in various harvests of northeastern Indian tea. *J. Agric. Food Chem.* **1995**, *43*, 208−214.
(21) Hatanaka, A.; Harada, T. Formation of *cis*-3-hexenal, *trans*-2-hexenal and *cis*-3-hexenol in macerated *Thea sinensis* leaves. *Phytochemistry* **1973**, *12*, 2341−2346.
(22) Kobayashi, A.; Kawamura, M.; Yamamoto, Y.; Shimizu, K.; Kubuta, K.; Yamanishi, T. Methyl epijasmonate in the essential oil of tea. *Agric. Biol. Chem*. **1988**, *52*, 2299−2303.
(23) Wang, D.; Kubota, K.; Kobayashi, A. Optical isomers of methyl jasmonate in tea aroma. *Biosci., Biotechnol., Biochem*. **1996**, *60*, 508−510.
(24) Vick, B. A.; Zimmerman, D. C. The biosynthesis of jasmonic acid by several plant species. *Plant Physiol*. **1984**, *75*, 458−461.
(25) Ahmed, N.; Bezbaruah, H. P. Nature of hair in tender tea shoots: Seasonal variation in density of hair in some Tocklai released clones. *Two Bud* **1982**, *29*, 10−13.
(26) Koezuka, Y.; Honda, G.; Tabata, M. Essential oil types of the local varieties and their F1 hybrids of *Perilla frutescences*. *Shoyagaku Zasshi* **1984**, *38*, 238−242.
(27) Fujita, Y.; Fujita, S.-I.; Yoshikawa, H. Essential oils of *Camellia saquanqua* Thunb, *C. japonica* Linn, and *Thea sinensis* Linn. *Nippon Nogei Kagaku Kaishi* **1973**, *47*, 645−650 (in Japanese).
(28) Werker, E.; Putievsky, E.; Ravid, U.; Dudai, N.; Katzir, I. Glandular hairs and essential oil in developing leaves of *Oscimum basilicum* L (Lamiaceae). *Ann. Bot*. **1993**, *71*, 43−50.
(29) Wicremasinghe, R. L. The mechanism of operation of climatic factors in the biogenesis of tea flavor. *Phtochemistry* **1974**, *13*, 2057−2061.
(30) Bureau, S. M.; Razungles, A. J.; Baumes, R. L. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *J. Sci. Food Agric*. **2000**, *80*, 2012−2020.
(31) Bhuyan, L. P.; Tamuly, P.; Mahanta, P. K. Lipid content and fatty acid composition of tea shoot and manufactured tea. *J. Agric. Food Chem*. **1991**, *39*, 1159−1162.
(32) Kijima, H.; Ide, T.; Otsuka, H.; Ogimi, C.; Hirata, E.; Takushi, A.; Takeda, Y. Water-soluble phenolic glycosides from leaves of *Alangium premnifolium*. *Phytochemistry* **1997**, *44*, 1551−1557.
(33) Comte, G.; Allais, D. P.; Chulia, A. J. Pheoeniceoside, the first natural bis-furanone propane derivative from *Juniperus phaenicea* L. *Phytochemistry* **1996**, *37*, 2955−2958.
(34) Takeo, T.; Tsushida, T. Changes in lipoxygenase activity in relation to lipid degradation in plucked tea shoots. *Phytochemistry* **1980**, *19*, 2521−2522.
(35) Matheis, G.; Vitzthum, O. G.; Weder, J. K. P. On the polorographic determination of polyphenoloxidase activity in different teas. *Chem. Mikrobiol. Technol. Lebensm*. **1987**, *11*, 1−4.
(36) Moreau, P.; Juguelin, H.; Lessire, R.; Cassagne, C. Plasma membrane biogenesis in higher plants: In vivo transfer of lipids to the plasma membrane. *Phytochemistry* **1988**, *27*, 1631−1638.
(37) Kajiwara, T.; Yoshikawa, H.; Saruwatari, T.; Hatanaka, A.; Kawai, I.; Ishihara, M.; Tsuneya, T. Enzymatic formation of unsaturated long chain fatty aldehydes in *Uva pertusa*. *Phytochemistry* **1989**, *27*, 1643−1645.
(38) Pare, P. W.; Tumlinson, J. H. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol*. **1997**, *114*, 1161−1167.
(39) Tansakul, P.; De-Eknamkul, W. Geranylniol-18-hydroxylase: the last enzyme on the plaunotol biosynthetic pathway in *Croton sublyratus*. *Phytochemistry* **1998**, *47*, 1241−1246.
(40) Croteau, R.; Sood, V. K.; Renstom, B.; Bhushan, R. Metabolism of monoterpenes. Early steps in the metabolism of D-neomenthenyl-β-D-glucoside in peppermint (*Mentha piperita*) rhizomes. *Plant Physiol*. **1984**, *76*, 647−653.
(41) Sinnott, M. L. Catalytic mechanisms of enzymic glycosyl transfer. *Chem. Rev*. **1990**, *90*, 1171−1202.
(42) Gaillard, T.; Methiew, J. A. Lipoxygenase-mediated cleavage of fatty acids to carbonyl fragments in tomato fruits. *Phytochemistry* **1977**, *16*, 339−343.
(43) Hatanaka, A.; Kajiwara, T.; Tomihira, S.; Yamashita, H. Alcohol dehydrogenase from *Thea sinensis* seeds. *Phytochemistry* **1973**, *12*, 2341−2346.
(44) Selvendran, R. R.; Reynolds, J.; Galliard, T. Production of volatiles by degradation of lipids during manufacture of black tea. *Phytochemistry* **1978**, *17*, 233−236.
(45) Watanabe, N.; Watanabe, S.; Nakajima, R.; Moon, J.-H.; et al. Formation of flower fragrance compounds from their precursors by enzymatic action during flower opening. *Biosci., Biotechnol., Biochem*. **1993**, *57*, 1101−1106.
(46) Takeo, T. Effect of clonal specificity of monoterpene alcohol composition of tea shoots on black tea aroma. *J. Agric. Res. Q*. **1983**, *17*, 120−124.
(47) Yamanishi, T.; Botheju, S.; de Silva, J. An index for assessing the quality of Uva seasonal black tea. *S. L. J. Tea Sci*. **1989**, *58*, 40−49.
(48) Baruah, S.; Hazarika, M.; Mahanta, P. K.; Horita, H.; Toshinobo, M. Effect of plucking intervals on the chemical constituents of black teas. *Agric. Biol. Chem*. **1986**, *50*, 1039−1041.
(49) Owuor, P. O.; Obanda, M. The effects of blending clonal leaf on black tea quality. *Food Chem*. **1999**, *66*, 147−152.
(50) Mahanta, P. K.; Tamuly, P.; Bhyuan, L. P. Changes of fatty acid contents, lipoxygenase activities, and volatiles during black tea manufacture. *J. Agric. Food Chem*. **1993**, *41*, 1677−1683.
(51) Hazarika, M.; Mahanta, P. K. Compositional changes in chlorophylls and carotenoids during the four flushes of tea in North East India. *J. Sci. Food Agric*. **1984**, *35*, 298−303.
(52) Hazarika, M.; Mahanta, P. K.; Takeo, T. Studies on some volatile flavor constituents in orthodox black teas of various clones and flushes in North-east India. *J. Sci. Food Agric*. **1984**, *35*, 1208−1218.
(53) Sekiya, J.; Kajiwawa, T.; Monma, T.; Hatanaka, A. Interaction of tea catechins with proteins: Formation of protein precipitate. *Agric. Biol. Chem*. **1984**, *48*, 1963−1967.
(54) Guyot, S.; Pellerin, P.; Brillouet, J.-M.; Cheynier, V. Inhibition of β-glucosidase (Amygdalae dulces) by + catechin oxidation products and procyanidin dimers. *Biosci., Biotechnol., Biochem*. **1996**, *60*, 1131−1135.
(55) Callaghan, T.; Ross, P.; Weinberger-Ohhana, P.; Garden, G. β-glucosidase activators of Mung bean UDP-glucose: β-glucan synthase. *Plant Physiol*. **1988**, *86*, 1099−1103.
(56) Lecker, D. N.; Khan, A. Theoretical and experimental studies of the effects of heat, EDTA, and enzyme concentration on the inactivation rate of α-amylasefrom *Bracillus* sp. *Biotechnol. Prog*. **1996**, *12*, 713−717.
(57) Hatanaka, A.; Kajiwara, T.; Tomihira, S.; Yamashita, H. Alcohol dehydrogenase from *Thea sinensis* seeds. *Phytochemistry* **1973**, *12*, 2341−2346.
(58) Mahanta, P. K.; Hazarika, M. Chlorophylls and degradation products in orthodox and CTC black teas and their influence on shade of color and sensory quality in relation to thearubigins. *J. Sci. Food Agric*. **1985**, *36*, 1133−1139.

Received for review June 13, 2002. Revised manuscript received September 18, 2002. Accepted September 28, 2002. We are grateful to the Director, Tocklai Experimental Station of Tea Research Association, for a fellowship (P.S.) and help during this research.

JF0257345