Lipid Content and Fatty Acid Composition of Tea Shoot and Manufactured Tea

Lakshi Pd. Bhuyan, Pradip Tamuly, and Pradip Kr. Mahanta*

Department of Biochemistry, Tocklai Experimental Station, Tea Research Association, Jorhat 785 008, Assam, India

The contents of neutral lipid (NL), glycolipid (GL), and phospholipid (PL) and their fatty acid (FA) compositions in fresh and processed leaves of tea varieties at four stages of black tea manufacture were analyzed. Well-marked variation in FA compositions in different lipid contents of the three genotypes as well as in different manufacturing stages were shown. Glycolipid, which accounts for about 60% of the total lipids, contains a significantly higher amount of linolenic acid, which undergoes pronounced degradation during different stages of black tea manufacture. Palmitic (C16:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were found in considerably higher quantity than other FAs irrespective of cultivars. Palmitic acid occurred in higher proportion in PL, while myristic acid (C14:0) and lauric acid (C12:0) were abundant in NL. The quantity of major FA constituents of the GL fraction in curl, tear, and crush (CTC) black tea was higher than that in orthodox black tea.

Tea, being an important agronomic crop, is cultivated in more than 2.59 million ha in the world. Like any other agricultural product, the quality of tea is established in the field and technological aspects are adjusted so that desirable attributes are retained in the beverage. Tea plants of commerce are classified mainly into three varieties, *Camellia sinensis* L., China type; *C. assamica* (Masters), Assam type; and *C. assamica* ssp. *lasiocalyx* (Planch M.S), Cambod type. Before a cultivar is released to industry, extensive manufacturing trials under two traditional methods of black tea processing, orthodox and unorthodox [curl, tear, crush (CTC)], are tried, and the quality of the made tea is evaluated over a period of time. Thus, quality control forms a part of modern tea technology (FAO, 1989; Roberts, 1962). There have been many efforts to establish genotypical and process variables in lipid contents and fatty acid composition of plants (De Man and Cauberghe, 1988; Menon, 1981; Owuor, 1986; Serghini-Caid et al., 1988; Tunlid et al., 1989).

The process of cell rupturing in the orthodox roller and/or in the CTC machine cannot substitute for withering, allowing the plucked shoots to desiccate for about 12–24 h under natural conditions. Cell injury in the orthodox roller is milder in comparison with the CTC system of manufacture in which, besides brushing and distortion in the roller, the leaf shoots are cut into small fragments. In the latter process the rate of oxidation reaction is faster and the particle sizes are smaller than those of the corresponding orthodox teas. When the rolled or CTC injured leaf is exposed to air, rapid color and flavor development takes place, and this stage is known as fermentation (Mahanta, 1988; Roberts, 1962; Yamanishi, 1986). The process of fermentation is allowed to continue for approximately 1–2 h depending on the type of maceration, orthodox or CTC, and then the process is terminated by firing/drying. However, the appearance of dry leaf, the liquor character in terms of briskness and brightness, differs among China, Assam, and Cambod teas depending upon the method of processing (Cloughley et al., 1982; Hazarika et al., 1984).

Lipid emerges as an important area of biochemical study because of its being a structural and storage component of plant tissues. Neutral lipids and glyco- and phospholipids, which constitute the lamellae fractions or other cell membranes in leaf chloroplasts, are hydrolyzed by acylhydrolases to free fatty acids (Hatanaka et al., 1987; Selvendran et al., 1978; Wright and Fishwick, 1979). Although variation in fatty acids in different molecular species could play an important role in the evaluation of cultivars with improved tea-making potential, the study is limited in tea. Characteristic volatile constituents that have been identified so far are enzymatic and/or nonenzymatic degradation products of polyunsaturated fatty acids (Chan and Taniguchi, 1985; Hatanka and Harada; 1973). Grassy or green flavor is attributed to hexenal, and alcohols have characteristic odors which become more intense when fresh leaf is processed in black tea manufacturing (Gardner 1985; Mahanta and Baruah, 1989).

Table I. Percentage Fatty Acid (FA) Composition in Neutral Lipids and Glyco- and Phospholipids of Assam, China, and Cambod Varieties of Fresh Leaves[a]

cultivar type	lipid class	FA							ratio 18:2/18:3
		12:0	14:0	16:0	18:1	18:2	18:3	others	
Assam	neutral	4.6	2.7	18.3	13.1	23.0	18.8	19.5	1.2
	glyco	1.2	0.6	13.8	7.9	10.6	52.6	13.3	0.2
	phospho	1.2	0.8	19.7	15.8	27.0	16.5	18.9	1.6
China	neutral	4.6	3.1	11.8	10.3	18.5	16.8	35.0	1.1
	glyco	1.2	1.0	16.9	7.9	10.3	52.3	10.6	0.2
	phospho	1.1	0.9	19.0	12.5	28.4	16.4	21.7	1.7
Cambod	neutral	2.7	2.3	22.5	11.7	21.1	18.0	21.7	1.2
	glyco	1.5	0.8	14.1	5.0	6.9	55.3	16.6	0.1
	phospho	1.1	0.5	19.2	15.4	31.7	16.7	15.4	1.9

[a] ANOVA = 2.

Because of the variation in the degree of withering, type of rolling, and particle size in orthodox and CTC teas, it is expected that fatty acids would be affected differentially, hence, the flavor volatile in the end product (Saijo and Takeo, 1972, 1975; Mahanta and Singh, 1990). In our previous papers we have reported the relationship between total lipid content and flavor volatiles and fatty acid constituents in different cultivars and also in processed tea (Mahanta et al., 1985; Bhuyan and Mahanta, 1989). The present investigation was undertaken to study the fatty acid composition of neutral lipids and glyco- and phospholipids of Assam, China, and Cambod varieties of tea shoots and processed leaf at different stages of black tea manufacture which eventually would become an additional source of information on cultivar characteristics.

MATERIALS AND METHODS

Samples. Tea shoots comprising an apical bud with two or three leaves of Assam, China, and Cambod varieties and Tocklai vegetative clone, TV-1 (Assam × China hybrid), for different stages of black tea manufacture were collected from the Borb-

0021-8561/91/1439-1159$02.50/0

Table II. *F* Values and Level of Significance of ANOVA of Fatty Acid Composition in Neutral Lipids and Glyco- and Phospholipids of Assam, China, and Cambod Varieties of Fresh Leaves[a]

source	df	C12:0	C14:0	C16:0	C18:1	C18:2	C18:3
between obs	2	0.97	0.27	1.31	0.06	1.44	0.35
between varieties	2	8.95**	7.10**	57.33***	26.96***	7.71**	7.96**
between fractions	2	237.23***	173.55***	154.24***	350.40***	2101.35***	6261.33***
variety × fraction	4	13.81***	1.57 NS	138.61***	18.01***	50.58***	4.93**
error	16						
total	26						
mean		2.1	1.4	17.2	11.1	19.7	29.3
CV, %		14.51	18.43	3.11	5.62	3.30	2.71

[a] NS, not significant; *, significant at 5%; **, significant at 1%; ***, significant at 0.1%.

Table III. Fatty Acid Composition (Percent) of Neutral Lipids and Glyco- and Phospholipids at Different CTC Manufacturing Stages from Clone TV 1

process leaf	lipid class	FA C12:0	C14:0	C16:0	C18:1	C18:2	C18:3	others
DDGL	neutral	3.2	3.6	17.4	13.2	27.6	21.2	13.8
	glyco	1.0	1.1	16.3	10.4	9.3	57.5	4.4
	phospho	1.3	1.2	22.8	11.6	28.0	25.5	9.6
DDWL	neutral	2.4	3.1	20.0	11.6	30.6	22.9	9.4
	glyco	1.0	0.6	15.8	10.5	10.6	53.0	8.4
	phospho	0.5	0.3	20.9	11.1	34.9	21.5	10.8
DDRL	neutral	3.8	2.8	18.2	11.4	30.2	24.5	9.0
	glyco	1.9	1.2	18.5	9.0	10.9	46.9	11.5
	phospho	0.9	1.0	25.3	9.7	30.9	24.9	7.3
DDFL	neutral	4.5	4.5	21.1	12.1	23.9	19.8	14.0
	glyco	2.0	2.4	22.3	11.4	12.4	40.3	9.3
	phospho	2.1	1.0	22.2	11.6	27.9	24.8	10.5
black tea	neutral	4.2	5.6	22.8	15.1	19.4	11.9	21.0
	glyco	2.4	2.1	23.6	15.4	9.8	24.7	22.0
	phospho	1.2	1.0	24.4	12.2	27.8	22.0	11.5

hetta field plot of the Tocklai Experimental Station during the manufacturing season in 1987. Freshly harvested tea shoots were withered about 68% and 75% (w/w) for orthodox and CTC manufacturing, respectively. The leaves were rolled in three crank single-action rollers for 60 min for orthodox manufacture. A portion of the rolled leaves after 30 min was further passed through a pair of CTC rollers for CTC manufacture. The leaves were then allowed to ferment for about 30 min for CTC tea and 1 h for orthodox tea in a humidified room with a 2 °F hygrometric difference. The fermented leaves were fired in a batch-type dryer with inlet temperatures of 200–220 and 180–200 °F for orthodox and CTC tea, respectively. Samples drawn from different stages of black tea manufacture were deactivated under steam for 3 min and dried. The following samples were thus obtained from the different manufacturing stages: (i) deactivated dried green leaf (DDGL); (ii) deactivated dried withered leaf (DDWL); (iii) deactivated dried rolled leaf (DDRL); (iv) deactivated dried fermented leaf (DDFL); and (v) black tea (BT).

Extraction and Fractionation of Total Lipids. Lipids were extracted by homogenizing the samples with $CHCl_3$/MeOH (2:1) for 3–4 min. The bulked filtrate was evaporated to dryness in a rotary vacuum evaporator at 45 °C, and the crude lipid was phased in $CHCl_3$:MeOH:H_2O (2:1:0.75) and determined gravimetrically. Total lipids were fractionated into NL, GL, and PL using chloroform, acetone, and methanol, respectively, on a silicic acid column. These fractions were evaporated to dryness under reduced pressure and determined gravimetrically (Tunlid et al., 1989).

Extraction of Free Fatty Acids. Five grams of test powdered sample was homogenized in light petroleum ether (40–60) for 3 min, and the filtrate was evaporated to dryness (Kershaw, 1986). The free fatty acids were methylated and analyzed as described below.

Analysis of Fatty Acids as Methyl Esters. Each lipid fraction was hydrolyzed with 1 N alcoholic KOH, refluxing for 2 h. Petroleum ether/diethyl ether (1:1 v/v) was used to remove the nonsaponifiables from diluted soap solution, and the fatty acids were recovered by acidification and extraction with diethyl ether. The fatty acids were methylated by refluxing with anhydrous methanol in the presence of 2 drops of concentrated H_2SO_4 for 2 h. Fatty acid methyl esters (FAME) were determined by an

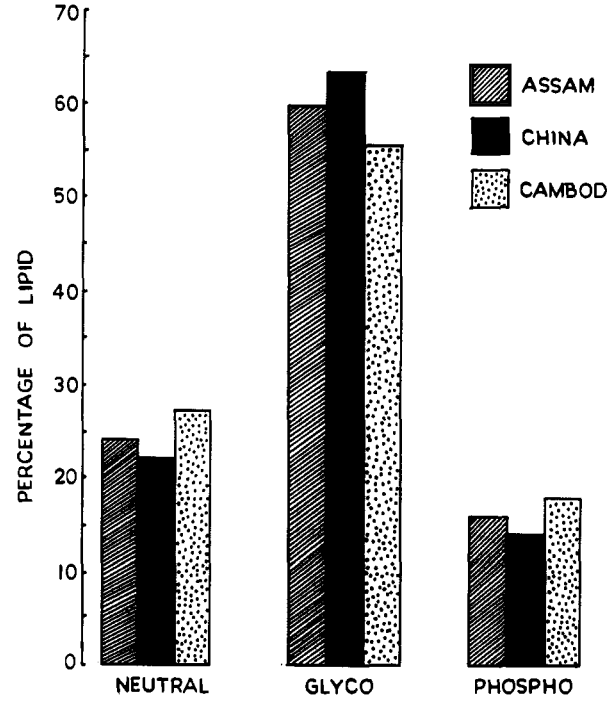

Figure 1. Percentage of neutral lipids and glyco- and phospholipids of Assam, China, and Cambod varieties. *F* value for neutral lipid, 59.35***; *F* value for glycolipid, 52.27***; *F* value for phospholipid, 15.84**.

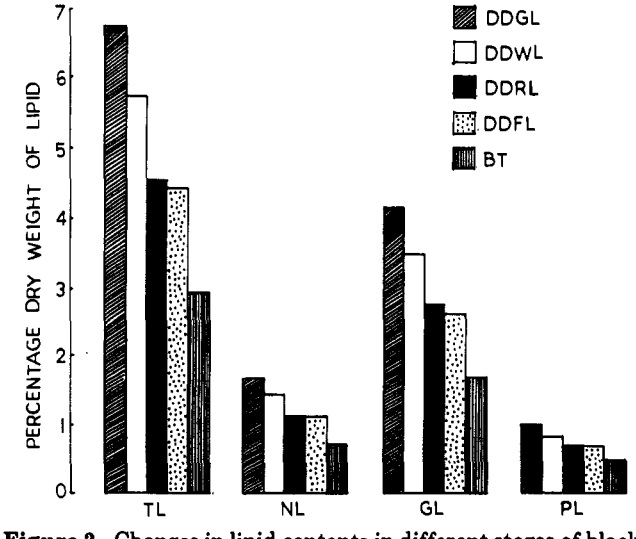

Figure 2. Changes in lipid contents in different stages of black tea manufacture. *F* value for total lipid, 99.97***; *F* value for neutral lipid, 63.92***; *F* value for glycolipid, 59.05***; *F* value for phospholipid, 244.38***.

ω Model Netel gas chromatograph with flame ionization detector in a 2 m × 1/2 in. o.d. stainless steel column packed with 10% DEGS. Nitrogen was used as carrier gas at a flow rate of 20 mL/min. The column temperature was programed from 120 to 190 °C at 4 °C/min. The temperatures of the injector and detector were maintained at 220 and 200 °C, respectively. The

Table IV. ANOVA of Fatty Acid Composition of Neutral Lipids and Glyco- and Phospholipids of Different Manufacturing Stages of CTC Teas

source	df	C12:0	C14:0	C16:0	C18:1	C18:2	C18:3
between obs	2	2 NS	<1	7.14*	2.04 NS	<1 NS	<1 NS
between fraction	2	2527***	169.39***	295.90***	38.64***	676.59***	1211.00***
error (1)	4						
subtotal	8						
manufacturing stages	4	48.29***	130.00***	88.24***	90.08***	173.09***	1015.53***
fraction × stage	8	7.00***	34.33***	27.74***	1.29 NS	68.69***	391.56***
obs × stage	8	<1 NS	<1	1.83 NS	11.88***	1.38 NS	1.63 NS
error (2)	16						
total	44						
mean		2.16	2.11	20.78	11.76	22.29	29.44
CV (1), %		4.63	22.73	2.21	4.25	4.53	4.98
CV (2), %		12.25	8.21	3.12	4.17	2.54	1.92

Table V. Percentage of Free Fatty Acid (FFA) Composition of Assam, China, and Cambod Varieties of Fresh Tea Leaves

cultivar	C12:0	C14:0	C16:0	C18:1	C18:2	C18:3	C18:2/C16:0
Assam	1.30 ± 0.2	0.50 ± 0.1	16.30 ± 1.4	18.08 ± 1.3	20.30 ± 1.3	29.08 ± 1.9	1.25
China	1.48 ± 0.2	0.62 ± 0.1	15.00 ± 1.3	14.48 ± 0.9	18.90 ± 0.7	27.35 ± 0.7	1.26
Cambod	1.58 ± 0.2	0.50 ± 0.1	15.02 ± 1.4	13.42 ± 0.8	19.35 ± 1.2	30.40 ± 1.3	1.29

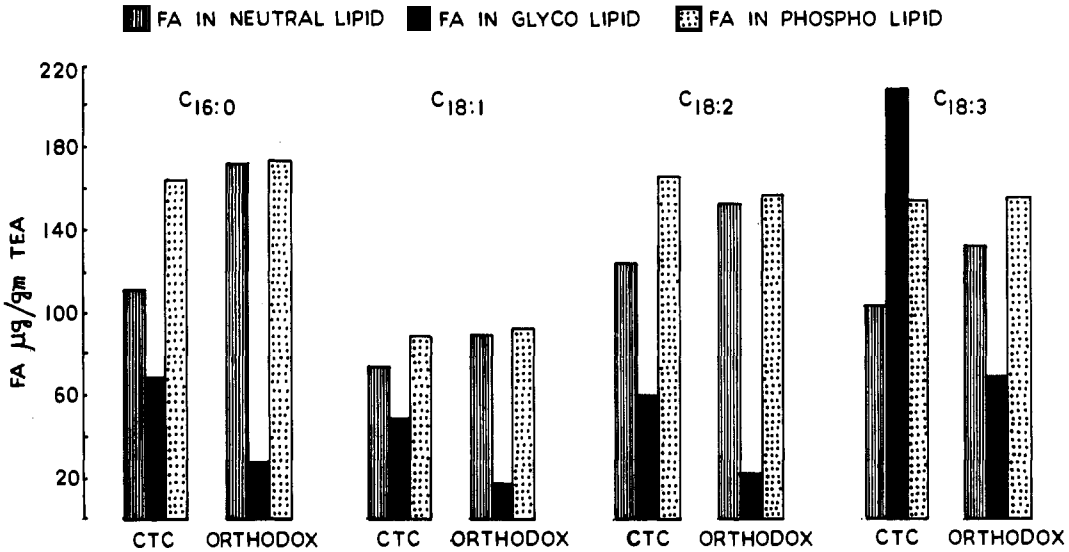

Figure 3. FA (µg/g of tea) in different lipid fractions of CTC and orthodox black tea. *F* value for neutral lipid, C16:0, 1081.45***; C18:1, 96.43***; C18:2, 252.30***; C18:3, 270.00***. *F* value for glycolipid C16:0, 661.50***; C18:1, 768.00***, C18:2, 547.60***; C18:3, 3312.17***. *F* value for phospholipid C16:0, 16.76**; C18:1, 15.00**; C18:2, 12.79*; C18:3, 1.29 NS.

peak areas of the gas chromatogram were measured by multiplying peak height by peak width at half-height.

Statistical Analysis. The analysis of variance (ANOVA) was carried out by using split plot design, and the significance of variations among the cultivars, stages of manufacture, and lipid contents was determined according to the procedure Gacula and Singh (1984) (Chan and Taniguchi, 1985; Hazarika and Mahanta, 1983).

RESULTS AND DISCUSSION

Analyses of Neutral Lipids and Glyco- and Phospholipids. Total lipid of fresh tea shoots from three varieties of teas is presented on a dry weight basis in Figure 1. Current indications are that glycolipid is the most abundant (60%) followed by neutral lipid (25%) and phospholipid (20%). The content of PL was highest in Cambod variety, while the GL content was highest in China variety. The higher content of NL may enhance the higher productivity of Cambod variety over the other varieties. The lipid fractions significantly varied not only with different varieties but also in their relative amounts.

The changes in NL, GL, and PL contents (percent dry weight) of different manufacturing stages are presented in Figure 2. As the GL fraction was found to be the highest, its degradation during manufacture is likely to have more pronounced impact on flavory product formation compared to other lipid fractions. From Figure 2 it can be observed that greater losses in all the fractions occurred during "firing" followed by "withering" stages of tea manufacture. Further, the study also confirmed that degradation of all lipid fractions was more than 50% during manufacturing of black tea.

Fatty Acid Composition in Neutral Lipids and Glyco- and Phospholipid. Linolenic acid is mostly found in the galactolipid fraction, while oleic and lenoleic acids are derived from the neutral lipid and phospholipid portions (Tables I and II). On the other hand, palmitic acid content was found to be highest in the phospholipid fraction, and lauric and myristic acids were higher in the neutral fraction of the lipid. It is noteworthy that the variations of the fatty acid in all three cultivars are similar. The ratio of linoleic to linolenic acid can predict the formation of hexanal. Furthermore, accelerated autoxidation in CTC teas compared to that of orthodox teas has been assigned to be the reason for the higher amount of hexenal in CTC teas. Alcohols also survived well in the milder orthodox processing in abundant quantities, whereas aldehydes were more abundant in severely macerated CTC teas as reported (Mahanta and Singh, 1990; Selvendran

et al., 1978; Igene and Pearson, 1979). It is noteworthy that neutral fractions contain a significant quantity of lauric (C12:0) and myristic (C14:0) acids in all three varieties.

NL, GL, and PL depletion has been observed to occur concomitantly with fatty acid oxidation during manufacturing stages to contribute positively or negatively in the flavor of black tea as shown in Table III. The statistical relationship between the quantitative changes of individual fatty acids in all lipid fractions and at different stages of manufacturing is shown in Table IV. Linolenic acid having a (1*Z*,4*Z*)-pentadiene moiety was found to undergo significant changes at all stages of manufacturing starting from withering, to rolling, to fermentation and firing (drying) and to act as the precursor of hexenals (Hatanaka et al., 1987; Saijo and Takeo, 1972, 1975).

In all three varieties C16:0, C18:1, C18:2, and C18:3 are the major free fatty acids (FFA, Table V). However, variations in fatty acid constituents except C18:1 among different cultivars were not well marked, but C18:3 was found to be the highest in all the varieties. Although total unsaturation was not drastically affected among varieties, the higher ratio between palmitic and linoleic acids in the Cambod variety may be crucial in the selection of vigorus clones. Free fatty acids are indicative of involvement of metabolic process during development and growth of the plant. Furthermore, the difference in C16:0 and C18:2 proportions can probably be ascribed to the direct effect of genotype on fatty acid composition (De Man and Bruyneel, 1987).

The different lipids isolated from orthodox and CTC teas and their variability in the fatty acid compositions (micrograms per gram) are shown in Figure 3. From the data it is clear that orthodox processing induces a significant decrease in the contents of glycolipid fatty acids as compared to CTC processing. A similar type of process variation could be seen in chlorophyll degradation also (Mahanta and Hazarika, 1985), which shows that reaction will take place in the chloroplast and the substrate is glycolipid. The changes in FA composition as well as in the different lipid fractions may be important in the development of fresh flavor in tea unlike off-flavors produced in other processed foods.

ACKNOWLEDGMENT

We are grateful to the Director, Tocklai Experimental Station, Tea Research Association, for permission to publish the results. We thank Mr. A. K. Biswas for statistical analysis.

LITERATURE CITED

Bhuyan, L. P.; Mahanta, P. K. Studies on fatty acid composition in tea *Camellia sinensis*. *J. Sci. Food Agric.* **1989**, *46*, 325–330.

Chan, H. T., Jr.; Taniguchi, M. H. Changes in fatty acid composition of papaya lipids (carica papaya) during ripening. *J. Food Sci.* **1985**, *50*, 1092–1094.

Cloughley, J. B.; Ellis, R. T.; Pendlington, S.; Humphrey, P. Volatile constituents of some cenral African black tea clones. *J. Agric. Food. Chem.* **1982**, *30*, 842–845.

De Man, W.; Bruyneel, P. Fatty acid content and composition in relation to grain size of barley. *Phytochemistry* **1987**, *26*, 1307–1310.

De Man, W.; Cauberghe, N. Changes and lipid composition in maturing barley kernals. *Phytochemistry* **1988**, *27*, 1639–1642.

FAO Tea, supply, demand and trace projections to 1995, by orthodox and CTC teas. Committee on commodity problems. Intergovernmental group on tea; Food and Agricultural Organization: Rome, May 9–12, 1989.

Gacula, M. C.; Singh, J. *Statistical Methods in Food and Consumer Research*; Academic Press: 1984; pp 62–96.

Gardner, H. W. Oxidation of lipids in biological tissue and its significance. In *Chemical changes in food during processing*; Richardson, T., Finley, J. W., Eds.; AVI, Westport, CT, 1985; pp 177–203.

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.

Hatanaka, A.; Kajiwara, T.; Sekiya, J. Enzymic oxygenative cleavage reaction of linolenic acid in leaves chloroplastic lipoxygenase and fatty acid hydroperoxidaselyase in tea leaves. In *The Metabolism, Structure, and Function of Plant Lipids*; Stumpf, P. K., Mudd, J. B., Nes, D. W., Eds.; Plenum: New York, 1987; pp 391–398.

Hazarika, M.; Mahanta, P. K. Some studies on carotenoids and their degradation in black tea manufacture. *J. Sci. Food Agric.* **1983**, *34*, 1390–1396.

Hazarika, M.; Chakravarty, S. K.; Mahanta, P. K. Studies on thearubigin pigments in black tea manufacturing systems. *J. Sci. Food Agric.* **1984**, *35*, 1208–1218.

Igene, J. O.; Pearson, A. M. Role of phospholipids and triglycerides in warm over flavor development in meat model systems. *J. Food Sci.* **1979**, *44*, 1285–1290.

Kershaw, S. J. Comparison of two standard methods for determination of free fatty acids content in oils extracted from oilseeds and vegetable oils. *J. Sci. Food Agric.* **1986**, *37*, 267–272.

Mahanta, P. K. Color and flavor characteristics of made tea. In *Modern Methods of Plant Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, 1988; pp 8, 221–295.

Mahanta, P. K.; Baruah, S. Relationship between process of withering and aroma characteristics of black tea. *J. Sci. Food Agric.* **1989**, *46*, 461–468.

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.

Mahanta, P. K.; Singh, R. Flavour components of Assam and Darjeeling teas in relation to agropractices and Processing. Presented at the International Conference on Tea Research, Calcutta, Jan 11–12, 1990; pp 129–136.

Mahanta, P. K.; Hazarika, M.; Takeo, T. Flavor volatiles and lipids in various components of tea shoots *Camilla sinensis* (L), O Kuntz. *J. Sci. Food Agric.* **1985**, *36*, 1130–1132.

Menon, K. K. G. Biosynthesis of lipids in plants. Lecture delivered at the 36th annual convention of oil technologist association of India, Hyderabad, 1981; pp 1–28.

Owuor, O. P. Flavor of black tea—A Review. *Tea* **1986**, *7*, 29–42.

Roberts, E. A. H. Economic importance of flavonoid substances. Tea fermentation. In *The Chemistry of Flavonoid Compounds*; Geissman, T. A., Ed.; Pergaman: Oxford, U.K., 1962; pp 468–510.

Saijo, R.; Takeo, T. The importance of linoleic acid and linolenic acid as precursors of hexanal and trans-2-hexenal in black tea. *Plant Cell Physiol.* **1972**, *13*, 991–998.

Saijo, R.; Takeo, T. Increase of cis-3-hexenal content in tea leaves following mechanical injury. *Phytochemistry* **1975**, *14*, 181–182.

Selvendran, R. R.; Reynolds, J.; Galliard, T. Production of volatiles by degradation of lipids during manufacture of black tea. *Phytochemistry* **1978**, *17*, 233–236.

Serghini-Caid, H.; Demandre, C.; Justin, A. M.; Majliak, P. Linolenic acid biosynthosis via glycerolipid molecular specied in pea and spinach leaves. *Phytochemistry* **1988**, *27*, 2543–2548.

Tunlid, A.; Schultz, N. A.; Benson, D. R.; Steele, D. B.; White, D. C. Differences in fatty acid composition between vegetative cells and N2-fixing vesicles of Franikia spsstrain Cp11. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3399–3403.

Wright, A. J.; Fishwick, M. J. Lipid degradation during manufacture of black tea. *Phytochemistry* **1979**, *18*, 1511–1513.

Yamanishi, T. Chemical changes during storage of tea. In *Handbook of Food and Beverage Stability*; Charalambous, G., Ed.; Academic Press: New York, 1986; pp 665–683.

Received for review April 20, 1990. Revised manuscript received September 19, 1990. Accepted January 2, 1991.

Registry No. C12:0, 143-07-7; C14:0, 544-63-8; C16:0, 57-10-3; C18:1, 112-80-1; C18:2, 60-33-3; C18:3, 463-40-1.