

## Lipid occurrence, distribution and degradation to flavour volatiles during tea processing

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### Abstract

The contents of neutral lipid, glycolipid and phospholipid and their fatty acid composition in three cultivars (clones) and processed leaves at different stages of black tea manufacture were measured. Glycolipids account for nearly 50% of the total lipids and are rich in linolenic acid. Phospholipids were present in the least amount (15%) and had a high proportion of oleic, linoleic and palmitic acids. Neutral lipids were found in moderate amounts (35%) and had a high content of lauric, myristic, palmitic, stearic, oleic and linoleic acids. Well-marked clonal variations in fatty acid composition of the lipid fractions were registered. With the maturation of the tea shoot, the lipid content increased. Considerable losses of lipids/fatty acids were observed in the withering process and again in the firing process. The other stages of processing (rolling and fermentation) registered only a minor change in lipid/fatty acid contents. Wide variation in lipid and flavour content was observed with season and a relation was evolved between them. The reason for the superior flavour of orthodox teas over CTC teas is explained on the basis of their lipid degradation. The changes in lipid content/fatty acids were related to the volatiles produced. © 1999 Elsevier Science Ltd. All rights reserved.

### 1. Introduction

The manufacture of black tea is a complex biological process, where the final quality depends on many factors, including the chemical composition of the green leaf, the extent to which the tea shoots are dehydrated and broken down by mechanical means and the enzymic action during processing (Hampton, 1992). Certain marked chemical changes take place during manufacture and are largely responsible for the development of colour and flavour of the finished product (Sanderson, 1972). For flavour, most important are the lipid-degrading enzymes which are released upon leaf maceration. They attack the lipoprotein membrane structure/storage lipids to release fatty acids, which undergo further degradation (Galliard, 1975). The volatiles derived from these biochemical changes contribute to the Group I volatile flavour compounds, which are major undesirable components of the flavour of tea (Selvendran, Reynolds & Gaillard, 1978). Polyunsaturated fatty acids have been identified as precursors of C<sub>6</sub> aldehydes and alcohols in tea (Sekiya,

Numa, Kajiwara & Hatanaka, 1976). The 'Pacha Taint' problem, which causes off-flavour in tea on storage, and is dominant in south Indian tea industries, is reported to be due to poor lipid metabolism (Ganesan & Ramaswamy, 1996). Although the lipid content of tea is low, lipid metabolism appears to be important and processing techniques could play a significant role in the biogenesis of the flavour found in finished black teas (Mahanta, Tamuli & Bhuyan, 1993). Apart from lipid oxidation, non-enzymic browning reaction is a characteristic feature of traditional tea processing (Yeo & Shibamoto, 1991).

Thus, lipid emerges as an important area for research and quality control. The major fatty acids, such as linolenic acid, are mostly found in the galactolipid fraction, while oleic and linoleic acids are derived from neutral and phospholipid components. The palmitic acid content is found to be highest in the phospholipid fraction and the minor fatty acids, such as lauric, myristic and stearic acids, are higher in the neutral lipid fraction. The precursors of negative flavours in tea are a subject of great interest to the tea industry, and it is therefore necessary to determine their distribution in the plant and the changes which occur during processing. The present investigation reports the fatty acid

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composition of various lipid fractions in different cultivars and its changes at different stages of Crush, Tear and Curl (CTC) tea manufacture from one cultivar. This work is complementary to our earlier study on tea volatiles (Ravichandran & Parthiban, 1998a) and on enzyme activities (Ravichandran & Parthiban, 1998b) in attempts to develop selection criteria and quality control procedures.

## 2. Materials and methods

Standard tea leaf shoots, comprising an apical bud and the terminal three leaves of clones UPASI-3, UPASI-9 and UPASI-17, representing Assam, China and Cambod cultivars, were harvested from UPASI TRI experimental farm (altitude 1050 above mean sea level). The harvested leaves were withered at a thickness of 20 cm with an air flow of 25 cubic feet/min/kg of leaves for 16 h, in order to obtain a moisture content of 65% (normal wither). The ambient air with a hygrometric difference of over 3°C normally undergoes forced circulation. Whenever the hygrometric difference is below 3°C, hot air is mixed in an appropriate proportion. However, the dry bulb temperature of air after mixing was restricted to 35°C. The relative humidity was 88%, with maximum and minimum mean room temperatures of 27°C and 17°C, respectively. The withered leaves were subjected to CTC rolling in a continuous four-cut system. The machine rolled leaves were fermented in a continuously rotating aluminium drum for 45 min (moisture content 55%) with air flow and fired in a fluid bed drier at 130°C for 30 min with hot air to obtain black tea containing 3% moisture. The material passing between sieves BSS 18 and 24 mesh number was taken for analysis.

Lipids were extracted by homogenising the sample with chloroform/methanol (2:1) for 5 min, filtering and concentrating to dryness. Crude lipid was isolated with chloroform:methanol:water (2:1:0.75), evaporated to dryness and determined gravimetrically. Total lipids were fractionated into neutral-, glyco- and phospho-lipids, using chloroform, acetone and methanol, respectively, on a silicic acid column. These fractions were evaporated to dryness and determined gravimetrically (Tunlid, Schultz, Benson, Steele & White, 1989).

Each lipid fraction was hydrolysed with 1 M alcoholic KOH (refluxing for 2 h), washed with light petroleum (boiling point 40–60°C): diethyl ether (1:1) (to remove non-saponifiable matter), acidified and extracted with diethyl ether. Fatty acids were methylated by refluxing in anhydrous methanol with two drops of conc. H<sub>2</sub>SO<sub>4</sub> for 2 h. Fatty acid methyl esters were determined on a gas chromatograph with FID using a 10% DEGS column, programmed from 120 to 190°C at 4°C/min. The extraction and analysis of volatiles are described elsewhere (Ravichandran & Parthiban, 1998a). Hexanal, Hexenal and Flavour index were determined using a Gas chromatograph as explained elsewhere (Ravichandran & Parthiban, 1998a).

## 3. Results and discussion

There have been many efforts to establish genotypical and process variables in lipid and fatty acid composition of plants (De Man & Bruyneel, 1987; De Man & Couberghe, 1988; Owuor, 1986; Serghini-Caid, Demandre, Justin & Majilak, 1988; Tunlid et al., 1989). Variation in fatty acids could play an important role in the selection of cultivars with improved tea-making potential. Table 1a and b gives the clonal variation of fatty acids from

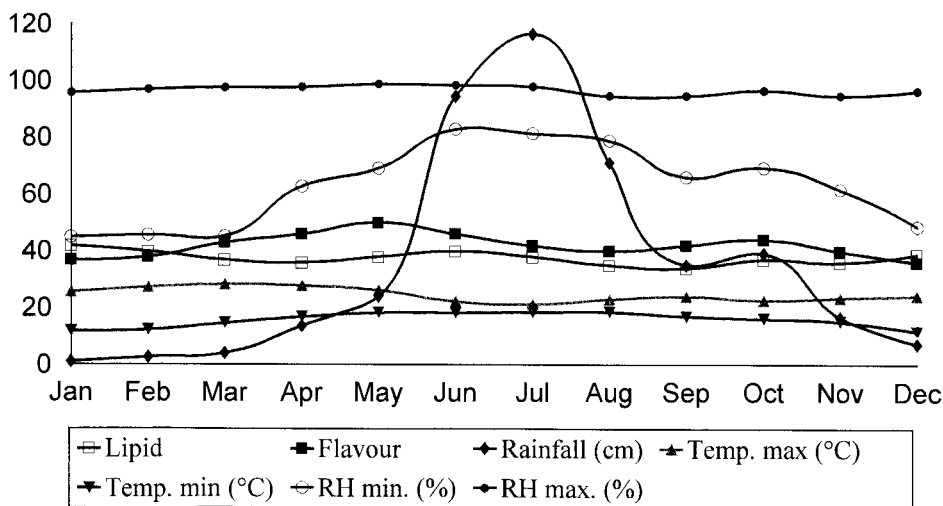


Fig. 1. Seasonal changes in green leaf lipid and black tea flavour in UPASI-9: total lipid as g/kg (dry weight); flavour as Flavour Index × 25.

Table 1a  
Clonal variations in fatty acid composition<sup>a</sup>

Fatty acids (% of the % lipid) <sup>b</sup>	Lipid fractions								
	Neutral			Glyco			Phospho		
	Assam UPASI-3	China UPASI-9	Cambod UPASI-17	Assam UPASI-3	China UPASI-9	Cambod UPASI-17	Assam UPASI-3	China UPASI-9	Cambod UPASI-17
12:0	4.1	4.3	2.9	1.0	0.9	1.8	1.1	1.0	1.2
14:0	3.2	3.6	2.2	0.7	0.9	0.6	0.8	0.9	0.7
16:0	17.9	20.1	22.0	14.1	17.4	13.8	20.2	18.6	19.9
18:0	13.1	13.8	14.0	6.9	5.7	5.7	7.2	6.1	6.9
18:1	14.0	12.2	12.3	8.2	8.2	5.5	15.5	12.9	14.8
18:2	24.2	20.1	21.7	11.0	10.6	7.3	27.0	28.3	31.4
18:3	19.5	17.2	18.6	52.8	51.9	55.2	16.7	16.1	16.1
CD at $P=0.05$	1.92			2.06			1.46		
Other fatty acids (% of the % lipid)	4.0	8.7	6.3	5.3	4.4	10.1	11.5	16.1	9.0
Lipid fraction (% of the % lipid)	34	33	37	52	53	48	14	14	15

<sup>a</sup> Average of three determinations with standard deviation less than 0.9% and CV less than 2.1%. Differences between fatty acids are highly significant at  $P < 0.01$ .

<sup>b</sup> Peak area as per cent total area in GLC. 12:0=lauric acid; 14:0=Myristic acid; 16:0=palmitic acid; 18:0=stearic acid; 18:1=oleic acid; 18:2=linoleic acid; 18:3=linolenic acid.

various lipid fractions and supplies new information on cultivar characteristics. As observed in many plants, the order of lipid contents in tea was also: glycolipid > neutral lipid >> phospholipid. The proportion of neutral lipid was highest in Cambod variety, while that of glycolipid was highest in China variety. The proportion of phospholipid did not change with cultivar. The content of total lipid was in the order China, Cambod and Assam variety.

Marked variations in the proportions of individual fatty acids among the lipid fractions were observed. In general, a high content of lauric, myristic and stearic acid was associated with the neutral lipid fraction. However, the proportion of palmitic acid was somewhat lower in the glycolipid fraction, in which that of oleic and linoleic acid was even lower. However, the proportion of linolenic acid in the glycolipid fraction was about three times as high, being 50% or more.

The ratio of linoleic/linolenic acid was in the order: phospho > neutral >>> glycolipids. It is well established that *n*-hexanal is derived from linoleic acid and *trans*-2-

hexenal from linolenic acid. Table 1 shows that these aldehydes are lowest in the Assam cultivar and highest in the China cultivar. Accordingly, the Flavour Index was found to be high in the Assam cultivar and low in the China cultivar. Thus, a negative correlation was found between lipid/fatty acid content and tea flavour. It is noteworthy that the variations of fatty acids in all the three cultivars studied are similar. Fig. 1 shows the seasonal changes in lipid content and tea flavour along with climatic conditions. The lipid content was found to be high (December–February, June–July) during stress, mainly due to drought (January–February) and monsoon (June and August). However, flavour exhibited the opposite trend, being maximal both pre- and post-monsoon, i.e. May and September. Thus, it is clear that climatic factors have a major impact on lipid synthesis and flavour.

The data on lipid content in relation to shoot maturity and pruning is given in Table 2a and b. As the shoot maturity increased, an increase in total lipids was observed. While the proportion of neutral and phospholipids showed enhancement with shoot maturity, that of glycolipid declined. Accordingly, an enhancement was observed with both the C<sub>6</sub> aldehydes and a decline in tea flavour with shoot maturity. The increase in neutral and phospholipid fractions with shoot maturity is clearly reflected in C<sub>6</sub> aldehyde formation and hence in tea aroma. A similar trend was also observed with longer plucking rounds, an increase in plucking round increasing shoot maturity. Pruning is an essential cultural operation and, with an increase in the pruning interval, the lipid content decreased (as also observed by Mahanta, Tamuli & Bhuyan, 1995) as did the proportion of neutral and phospholipids. Here also,

Table 1b  
Clonal variation in lipid fractions and tea volatiles

	Assam UPASI-3	China UPASI-9	Cambod UPASI-17
Total lipid (g/kg)	28.2	37.6	33.1
VFC <sup>a</sup>			
<i>n</i> -Hexanal	0.17	0.26	0.20
<i>trans</i> -2-Hexenal	1.49	3.11	1.64
Flavour Index	3.06	1.28	1.64

<sup>a</sup> As ratio of peak area to that of internal standard.

Table 2a  
Changes in fatty acid composition of lipid fractions with shoot maturity in UPASI-9<sup>a</sup>

Fatty acid (% of the % lipid) <sup>b</sup>	Lipid fractions																				
	Neutral			Glyco			Phospho			Neutral			Glyco			Phospho					
	Shoot components			Plucking interval			Plucking interval			Pruning <sup>c</sup>			Pruning <sup>c</sup>			Pruning <sup>c</sup>					
1L +B	2L +B	3L +B	1L +B	2L +B	3L +B	7th day	14th day	7th day	14th day	7th day	14th day	Pruned	Unpruned	Pruned	Unpruned	Pruned	Unpruned				
12:0	3.5	3.7	4.1	0.8	0.8	0.9	0.8	0.9	1.1	4.3	4.5	1.3	1.3	1.2	1.3	4.2	3.9	1.0	0.8	1.0	0.7
14:0	2.4	2.8	3.1	0.5	0.6	0.8	0.6	0.7	0.8	3.6	3.9	1.0	1.2	0.8	0.9	3.8	3.4	1.1	0.9	0.8	0.6
16:0	16.8	17.1	17.8	12.9	13.2	13.7	19.0	19.3	19.9	18.4	19.9	14.7	15.2	20.0	20.8	20.5	19.5	17.7	17.1	18.4	17.7
18:0	13.6	13.3	14.1	4.1	4.6	5.2	5.0	5.1	4.9	12.8	8.7	4.9	5.1	5.5	6.7	13.4	12.7	4.1	3.8	4.7	4.1
18:1	12.9	13.3	13.9	7.1	7.8	8.4	14.6	15.1	15.7	14.7	15.0	8.8	9.1	15.9	16.3	12.0	11.2	8.6	8.1	13.2	12.5
18:2	23.1	23.5	24.1	10.2	10.5	10.9	26.1	26.3	26.7	24.9	26.1	18.8	12.7	27.7	28.5	20.4	19.4	10.9	10.2	28.3	27.6
18:3	18.3	18.8	19.3	51.5	52.0	52.6	15.8	16.2	16.8	20.3	21.4	53.6	54.8	17.3	18.2	17.7	16.9	52.3	49.9	16.16	16.1
CD at $P=0.05$	0.47		0.54			0.73			1.77		2.24		0.64		0.89		0.83			0.57	
Other fatty acids (% of the % lipid)	9.4	7.5	3.6	12.9	10.5	7.5	18.1	16.4	14.1	1.0	0.5	3.9	0.6	11.6	7.3	8.0	13.0	4.3	9.2	17.0	20.7
Lipid fractions (% of the total lipid)	25.0	26.0	28.0	67.0	65.0	62.0	8.0	9.0	10.0	32.0	33.0	58.0	56.0	10.0	11.0	31.0	28.0	55.0	60.0	14.0	12.0

<sup>a</sup> Average of three determinations with standard deviation less than 0.9% and CV less than 2.1%. Differences between fatty acids are highly significant at  $P<0.01$ .

<sup>b</sup> Peak area as per cent total area in GLC. 12:0 = lauric acid; 14:0 = myristic acid; 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid.

<sup>c</sup> Pruned = pruned 6 months back; unpruned = pruned 42 months back (due for pruning).

Table 2b  
Changes in lipid content and volatiles with shoot maturity of UPASI-9

Leaf	Total lipid (g/kg)	VFC <sup>a</sup>		
		<i>n</i> -Hexanal	<i>trans</i> -2-Hexenal	Flavour Index
<i>Shoot components</i>				
Bud and 1st leaf	8.95	0.06	1.05	7.83
2nd leaf	12.51	0.19	1.76	1.63
3rd leaf	15.55	0.26	2.51	0.63
<i>Plucking rounds</i>				
7th day	38.1	0.22	1.78	2.19
14th day	39.0	0.55	2.01	2.07
<i>Pruning<sup>b</sup></i>				
Pruned	42.8	0.59	2.23	1.49
Unpruned	38.6	0.51	1.91	2.00

<sup>a</sup> As ratio of peak area to that of internal standard.

<sup>b</sup> Pruned = pruned 6 months back; unpruned = pruned 42 months back (due for pruning).

a higher content of neutral and phospholipids led to increased C<sub>6</sub> aldehyde formation and a decline in Flavour Index.

The data indicate that the chemical distribution of lipid components of various harvests can be balanced at the garden level by proper design of plucking standards and by management of plucking interval and pruning practices. It also becomes 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 & Bruyneel, 1987; De Man & Couberghe,

1988). The increase in lipid content does not produce good flavour in manufactured tea because of the greenness in such teas (Wright & Fishwick, 1979).

The data on the impact of processing techniques on fatty acid composition of the various lipid fractions are summarised in Table 3a and b and Fig. 2. The depletion of lipids (neutral, glyco and phospholipids) was observed to occur concomitantly with fatty acid oxidation during manufacture. As processing progressed, a reduction in all lipid fractions was noticed. The major decline (being in neutral and phospholipid fractions) is likely to have a more pronounced impact on flavour formation than the glyco lipid fraction. While saturated fatty acids showed a progressive increase along with process progress, a sharp decline was noticed with the polyunsaturated fatty acids. The changes were maximum during withering followed by firing and less during the rolling and fermentation stages of manufacture. The degradation of all lipid fractions was more than 60% during tea manufacture. The loss of linoleic and linolenic acids is reflected in the formation of hexanal and hexenal and found to undergo significant changes at all stages of manufacture. With the progress in manufacture, an increase in the content of C<sub>6</sub> aldehydes was registered with a corresponding decline in the Flavour Index. This observation very well coincides with lipid degradation. However, during fermentation, the polyphenol oxidation dominates all other biochemical changes and thus lipid degradation was found to be less in this stage of tea manufacture with a relatively low formation of C<sub>6</sub> aldehydes. However, the VFC Group II

Table 3a  
Impact of CTC processing techniques on fatty acid composition in UPASI-9<sup>a</sup>

Fatty acids (% of the % lipid) <sup>b</sup>	Lipid fractions														
	Neutral					Glyco					Phospho				
	Fresh	Withered	Rolled	Fermented	Fired	Fresh	Withered	Rolled	Fermented	Fired	Fresh	Withered	Rolled	Fermented	Fired
12:0	3.0	3.1	3.7	4.3	4.2	0.9	1.3	2.1	2.3	2.6	1.5	1.3	1.3	1.6	1.8
14:0	3.8	4.1	4.3	5.1	5.8	0.9	0.7	1.6	2.1	2.0	1.4	0.9	1.0	1.1	1.0
16:0	17.3	17.9	20.7	21.6	22.2	16.0	15.8	18.8	21.9	22.7	23.3	22.5	24.1	23.3	23.9
18:0	12.9	8.1	2.2	7.4	9.1	2.6	3.3	3.1	3.3	4.9	4.4	4.7	4.5	4.0	4.4
18:1	13.7	13.7	13.9	14.1	14.6	11.1	10.8	10.1	11.3	13.1	12.0	11.2	10.8	11.6	12.2
18:2	27.6	29.5	30.2	25.0	21.9	9.6	10.2	10.7	10.9	9.2	28.8	32.6	32.2	31.0	28.4
18:3	21.0	22.3	23.9	20.2	16.3	56.8	50.1	45.7	39.9	31.5	25.3	21.7	22.5	22.9	21.0
CD at <i>P</i> =0.5	3.75					6.01					1.63				
Other fatty (% of the % Lipid)	0.7	1.3	1.1	2.3	5.9	2.1	7.8	7.9	8.3	14.0	3.3	5.1	3.6	4.5	7.2
Lipid fraction (% of total lipid)	32	28	25	24	19	55	61	65	66	73	13	11	10	10	8

<sup>a</sup> Average of three determinations with standard deviation less than 0.9% and CV less than 2.1%. Differences between fatty acids are highly significant at *P* < 0.01.

<sup>b</sup> Peak area as per cent total area in GLC. 12:0 = lauric acid; 14:0 = myristic acid; 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid.

Table 3b  
Impact of processing techniques on lipid content and flavour in UPASI-9

Process	Total lipid (g/kg)	VFC <sup>a</sup>		
		<i>n</i> -Hexanal	<i>trans</i> -2-Hexenal	Flavour Index
Fresh	38.3	1.09	1.73	0.52
Withered	26.8	1.21	3.49	0.31
Rolled	22.9	1.36	7.35	0.26
Fermented	19.8	1.33	7.26	0.31
Fired	11.4	0.29	3.02	1.25

<sup>a</sup> As ratio of peak area to that of internal standard.

compounds dominate Group I compounds and hence the Flavour Index was found to increase. In the firing process, although a considerable amount of lipid breakdown to C<sub>6</sub> aldehydes occurs, most of these are volatilised due to the high temperature. At the same time, pyrolytic reactions lead to Group II compounds and thus a sharp rise in the Flavour Index.

The CTC and orthodox processes differ mainly in the method of rolling and Bhuyan, Tamuly and Mahanta (1991) reported that teas made from the same leaf by these two processes differed widely in flavour and quality. The orthodox teas show better flavour than CTC teas and also better keeping quality. Fig. 3 clearly shows

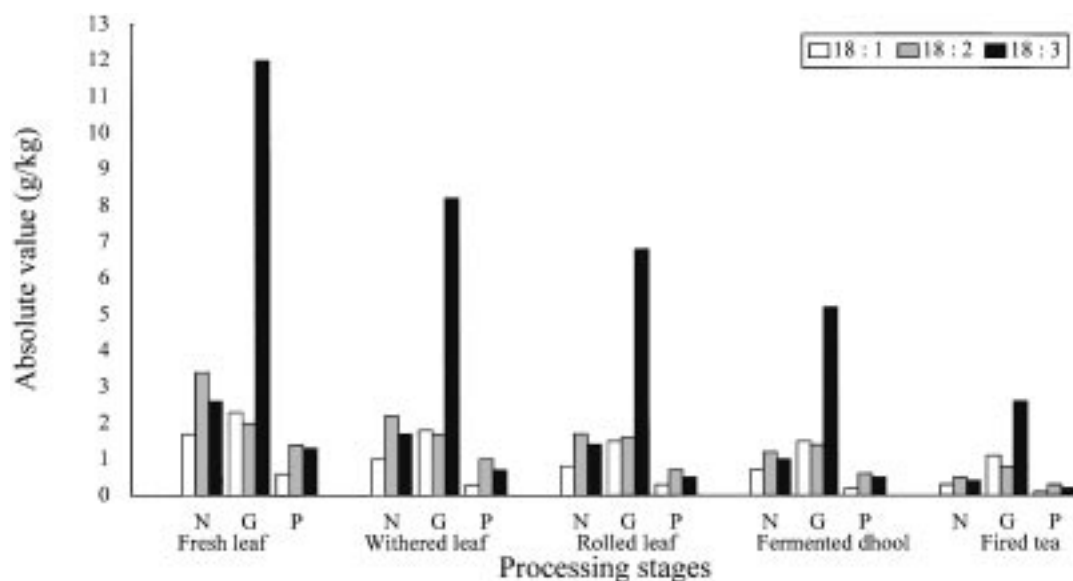


Fig. 2. Absolute changes in poly unsaturated fatty acids of various lipid fractions during CTC processing: N, neutral; G, glyco; P, phospho; 12:0, lauric acid; 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

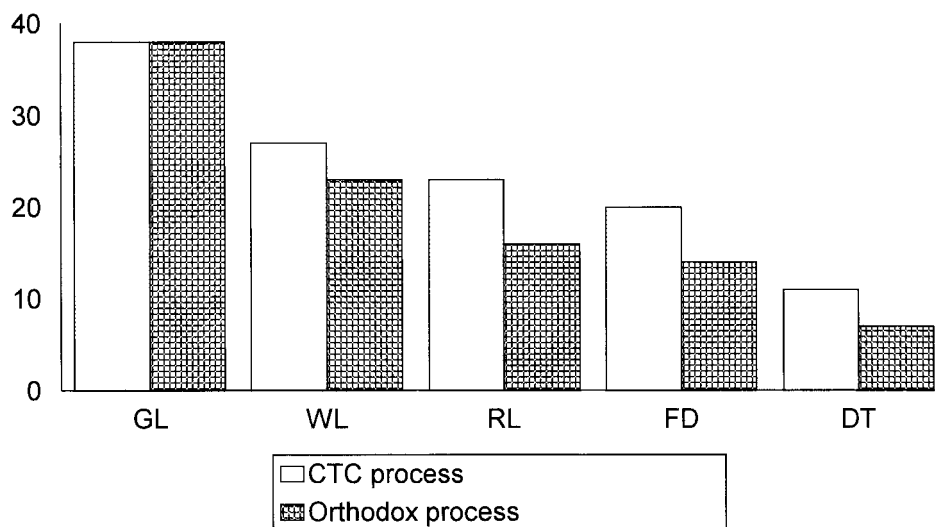


Fig. 3. Changes in lipid content (g/kg) during CTC and orthodox processes in UPASI-9: GL, green leaf; WL, withered leaf; RL, rolled leaf; FD, fermented dhool; DT, dried tea.

that additional lipid degradation occurs in the orthodox process at all stages of manufacture, but mainly in the rolling stage. This is due to a higher wither (hard wither), slow rolling over a prolonged period and longer fermentation. The accelerated autooxidation of CTC teas compared to that of orthodox teas has been attributed the higher amount of hexenal, and alcohols survived well in the milder orthodox processing (Mahanta & Singh, 1990). It has been reported that orthodox processing induces a significant decrease in the contents of glycolipid fatty acids as compared to CTC processing (Bhuyan et al.). A similar type of process variation could also be seen in chlorophyll degradation, which shows that reaction will take place in the chloroplast and that the substrate is glycolipid (Mahanta & Hazarika, 1985).

Lipids/fatty acids, which are important precursors of flavour volatiles, may be a helpful tool as an objective indicator of organoleptic properties of black tea made from clonal cultivars. The changes in fatty acid composition of different lipid fractions are of great importance in the development of fresh flavour in tea as they are lost during the drying process which thereby enhances keeping quality. Studies of clonal variation are important for the selection of elite clones for commercial uses. Earlier, we studied the impact of tea processing techniques on tea volatiles (Ravichandran & Parthiban, 1998a) and enzyme activities (Ravichandran & Parthiban, 1998b). Hence, the information on lipid content and enzyme activity presented here is complementary to our earlier studies on tea volatiles and lipoxygenase activity (Ravichandran & Parthiban, 1998c) in attempting to develop selection criteria and quality control procedures.

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