

# Changes of Fatty Acid Contents, Lipoxygenase Activities, and Volatiles during Black Tea Manufacture

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Fatty acid composition in relation to lipolytic and lipoxygenase activities in tea leaf shoots, from different stages of the tea manufacturing process (i.e., withering, rolling, fermentation, and firing) was investigated in a series of black tea manufacturing experiments. More degradation of fatty acids and higher lipoxygenase activity were characteristics of tea leaves allowed to undergo the desiccation/withering process for longer periods of time. The oxidative breakdown of fatty acids was higher in the later part of the rolling process, while lipolytic activity was more prominent in the withering process as well as the earlier stage of rolling. A gradual decline of lipoxygenase activity as well as fatty acid content has been recorded during the fermentation and drying processes. Although the lipids and fatty acid degradation were directly related to the degree of withering, the volatile content of teas manufactured from hard withered leaves (lower moisture content) was unexpectedly lower than that in black teas from normal withered tea leaf shoots.

## INTRODUCTION

The widespread occurrence of flavonoids and lipids, as well as hydrolytic and oxidative enzymes, in tea leaf shoots plays an important role in the generation of many quality attributes during the manufacture of black tea. Although lipid content is low in the plant tissues, lipid metabolism appears to be important, and processing techniques could play significant roles in the biogenesis of flavor found in finished black teas (Hatanaka and Harada, 1973; Mahanta *et al.*, 1985; Selvendran *et al.*, 1978; Taranishi and Buttery, 1985; Tollsten and Bergstorm, 1988). Lipid oxidation and browning reaction products appear to be the most characteristic feature of traditional tea processing, where leaf shoots are heaped for desiccation in the withering process followed by mechanical injury imparted either by orthodox rollers or in crush, tear, curl (CTC) machines (Harris and Ellis, 1981; Mahanta, 1988, 1992; Takeo and Mahanta, 1983).

It is an established fact that the major fatty acids such as linolenic (18:3) acid are mostly found in the galactolipid fraction, while oleic (18:1) and linoleic (18:2) acids are derived from the neutral lipid and phospholipid portions. The palmitic (16:0) acid content was found to be highest in the phospholipid fraction, and the minor fatty acids such as lauric (12:0), myristic (14:0), and stearic (18:0) acids were higher in the neutral fraction of the lipid (Bhuyan and Mahanta, 1989; Bhuyan *et al.*, 1991; Serghini-Caid *et al.*, 1983; Wright and Fishwick, 1979). The long-chain fatty acids which are liberated from the senescent and mechanically damaged leaves will further undergo oxidative degradation producing different carbonyl compounds to impart the characteristic odor of manufactured black teas (Galliard, 1980; Hatanaka *et al.*, 1987; Mahanta *et al.*, 1988; Baruah *et al.*, 1986).

The extent of flavor development also depends on the amount of linalool and its derivatives having flower fragrance characteristic aroma of muscatel flavor. There are two major ways in which terpenoid-related volatiles are produced: one involves the enzyme-initiated oxidative breakdown of carotenoids and the other hydrolysis of terpenoid glycosides (Mick and Schreier, 1984; Shimizu, 1982; Williams *et al.*, 1980; Fischer *et al.*, 1987; Takeo, 1981). The reactions that take place during the fermenta-

tation process in black tea manufacture are very complex, and they are catalyzed through enzymatic or nonenzymatic processes, forming many volatile and nonvolatile flavor compounds due to diffusion of oxygen in the leaf particles (Saijo and Takeo, 1973; Roberts, 1962; Thomas and Murtagh, 1985; Yamanishi, 1989). The orthodox type of black tea manufacturing process (Hazarika and Mahanta, 1983) as used to manufacture Assam and Darjeeling teas during 1989-1990 was experimented with to develop an understanding of the role of processing techniques on the hydrolytic and oxidative reactions that are responsible for the biogenesis of flavor and aroma in made tea.

## MATERIALS AND METHODS

**Manufacturing of Assam Tea at Tocklai Miniature Factory.** Standard tea leaf shoots comprising an apical bud and terminal two/three leaves of clone TV-1 (Tocklai vegetative clone) were heaped in a withering trough to a thickness of about 15-25 cm, and a constant air flow of about 25-30 cfm/kg of leaves at a hygrometric difference of 6 °F was maintained for up to 8 h. The leaves after withering were subjected to orthodox rolling for up to 60 min in a three-crank single-action roller. The machine-rolled leaves were fermented for up to 90 min and fired at 90 °C for 30 min to obtain black tea containing about 3% moisture.

**Analysis of Fatty Acids.** Total fatty acids (free and bound) were determined by spectrophotometric measurement at 440 nm of the color developed before and after alcoholic KOH hydrolysis of lipids, and the amount of fatty acids ( $\mu\text{g/g}$  dry wt) was measured by comparison against a standard curve made from authentic palmitic acid according to the Duncombe (1963) method. The fatty acid contents during black tea manufacture were analyzed by gas chromatography (GC) as outlined by Bhuyan *et al.* (1991).

**Lipoxygenase Assay.** The enzyme was isolated by taking 5-g tea samples from different stages of processing together with 2 g of Polyclar AT and a little sand, and these were homogenized with 10 mL of 0.05 M McIlvaine buffer of pH 6.3 containing 0.4 M sucrose. After filtering through cotton cheesecloth and centrifuging at 4000g for 15 min at 4 °C, the clear supernatant was used for lipoxygenase assay. To 3 mL of substrate solution was added 0.1 mL of enzyme solution, and lipoxygenase activity was determined by measuring the increase in absorbance due to the formation of conjugated diene hydroperoxide at 234 nm at 30 °C using a Beckman spectrophotometer, Model 26 (Van Den and Mendoza, 1982; Surrey, 1964). Reactions were conducted in two types of solutions: the first was at pH 9.0 with borate buffer (0.1 M) containing  $7.5 \times 10^{-3}$  M linoleic acid, 0.25% in linoleate,

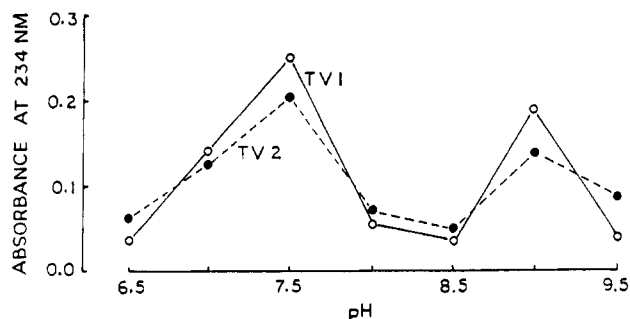


Figure 1. Effect of pH on lipoxygenase activity in tea shoot.

Table I. Changes of Lipids and Fatty Acids and LOX Activity during Different Times of Fermentation

time of fermentation, min	total lipid, %	total FA, $\mu\text{g/g}$	linoleic acid, $\mu\text{g/g}$	linolenic acid, $\mu\text{g/g}$	LOX sp act., units/mg of protein
30	3.35	6395	1514	2019	11.05
60	3.27	6267	1484	1983	10.25
90	3.18	6190	1466	1955	9.02

and Tween 20; the second was at pH 7.5 acidified with concentrated HCl. Protein concentration in the crude extracts was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

**Manufacturing at Darjeeling Factory.** Green leaves were kept in a withering trough, and hot/cold air at the rate of 23–25 cfm unit area/kg of leaves was blown by blower fan fitted with the troughs. The hygrometric difference of the in-flow air was maintained at about 8–12 °F and withering periods were 18, 21, and 26 h; the degrees of wither achieved were 68% for soft wither, 62% for normal wither, and 53% for hard wither, respectively. After withering, the leaves were rolled in a table roller for 45 min and then spread on fermenting trays for a period of about 2.5 h and fired at about 90 °C for 25 min.

**Analysis of Volatile Flavor Compounds.** Volatile flavor compounds were extracted from 200 g of black tea, and the extract was analyzed using a Netel gas chromatograph (Omega) fitted with a 5% Carbowax 20 M column and a flame ionization detector (FID). Conditions: gas flow rate, 30 mL/min; temperature, programmed between 60 and 180 °C at a rate of 6 °C/min; detector and injection temperatures, 200 and 210 °C, respectively. Compounds were identified by comparison of the GC retention time of the authentic chemicals (Sigma) as described by Takeo and Mahanta (1983).

## RESULTS AND DISCUSSION

**Lipoxygenase and Lipolytic Activity during Tea Processing.** The lipoxygenase (LOX) enzyme can initiate oxidation of unsaturated/saturated fatty acids together with coupled oxidation of carotene and chlorophyll (Hildebrand and Hymowitz, 1982; Kajiwaru *et al.*, 1989; Mastui *et al.*, 1991; Sekiya *et al.*, 1983, 1984). Multiple forms of lipoxygenase have been identified in many higher plants, and enzyme activity in tea leaves has been assayed from pH 6.5 to 9.5 to determine the presence of isoenzymes (Abbas *et al.*, 1989; Ida *et al.*, 1983).

The pattern of graphical representation of enzyme activity indicates that tea clones have two isoenzymes; the activity of isoenzyme 1 was maximum at pH 7.5, and the activity of isoenzyme 2 was found to be maximum at pH 9 as shown in Figure 1. The highest activity at pH 7.5 may be due to the presence of more isoenzymes in this range, and activity below pH 6.5 was found to be negligible. There is, however, still a rise in enzyme activity from pH 6.5 to 7.5 and thereafter a sudden fall to pH 8.5, another sharp rise to pH 9.0, and then a declining trend. Table I and Figures 2–4 show the fatty acid degradation in relation to lipoxygenase (LOX) activity (units per milli-

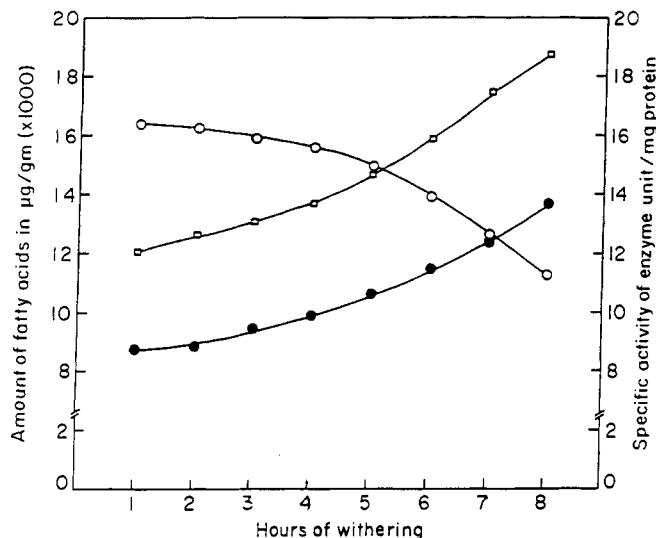


Figure 2. Effect of withering time on fatty acid degradation and lipoxygenase activity. (O) Fatty acid; ( $\square$ ) activity at pH 7.5; ( $\bullet$ ) activity at pH 9.0.

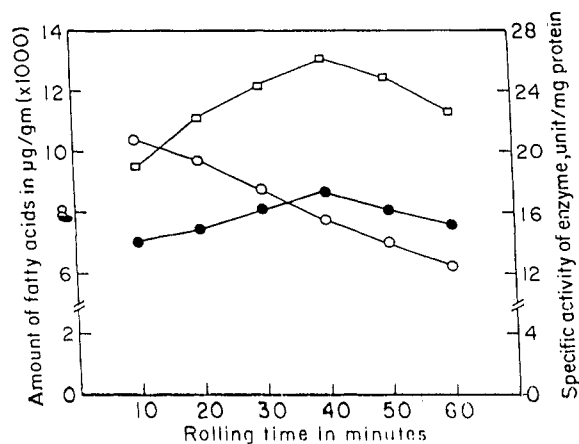


Figure 3. Effect of rolling time on fatty acid degradation and lipoxygenase activity. (O) Fatty acid; ( $\square$ ) activity at pH 7.5; ( $\bullet$ ) activity at pH 9.0.

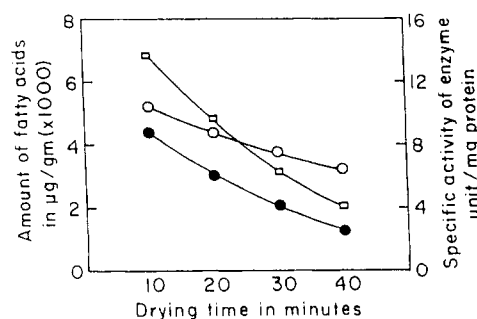
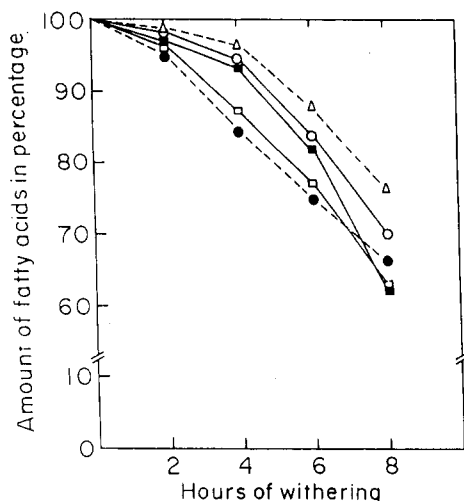
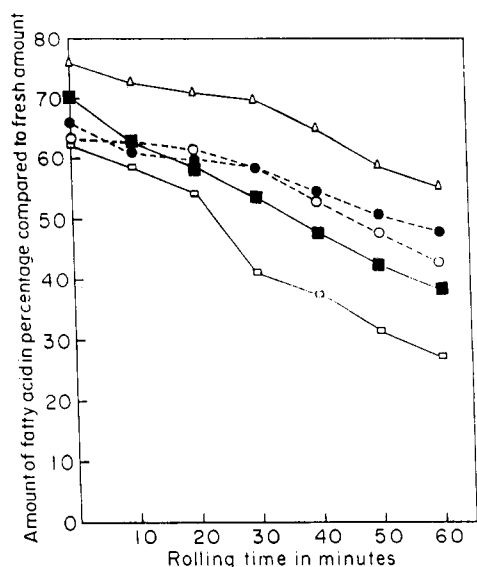


Figure 4. Effect of drying time on fatty acid degradation and lipoxygenase activity. (O) Fatty acid; ( $\square$ ) activity at pH 7.5; ( $\bullet$ ) activity at pH 9.0.

gram of protein) during different stages of manufacturing. In addition, a selective depletion of linoleic and linolenic acids, polyunsaturated fatty acids, and palmitic acid, a saturated fatty acid, which could also serve as substrates for lipoxygenase, has been noted during black tea processing (Figures 5 and 6). Although the LOX activity increases continuously during withering, its rise was more prominent in the later stage. This increase may be due to gradual dissolution of chloroplast membrane and release of LOX with the progress of withering (Deschene *et al.*, 1991; Hatanaka *et al.*, 1987; Tekeo and Tsushida, 1980).



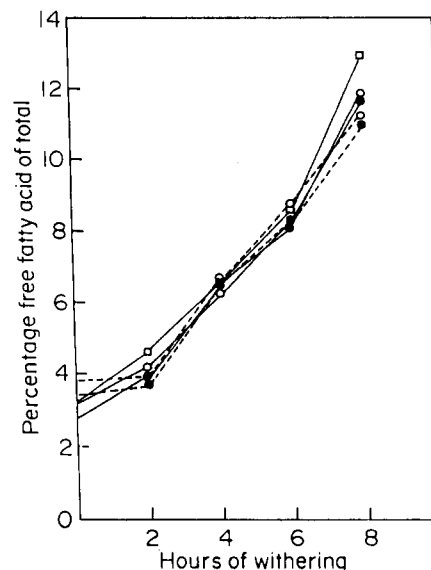
**Figure 5.** Effect of withering time on percentage of fatty acid degradation. (○) TFA; (Δ) palmitic; (●) oleic; (□) linoleic; (■) linolenic.



**Figure 6.** Effect of rolling time on percentage of fatty acid degradation. (○) TFA; (Δ) palmitic; (●) oleic; (□) linoleic; (■) linolenic.

The characteristic degradation of fatty acids and high lipoxygenase activity would favor the formation of volatiles and nonvolatiles even at reduced moisture content (Homa and Fujimaki, 1982; Yeo and Shibamoto, 1991; Zhung *et al.*, 1991). The continuous rise in lipoxygenase activity during withering attains its highest momentum during rolling. On the other hand, the increase in lipolytic product during senescence and homogenization of plant tissues is a well-established fact. Figures 5–7 and Tables II, V, and VI show how the oxidative breakdown of fatty acids was higher in the later part of rolling, while lipolytic activity was prominent during the withering and initial cell maceration stages.

However, the lipoxygenase activity of leaves decreases gradually in the later part of rolling, during overfermentation and drying stages, while fatty acid degradation continues because of autooxidation as shown in Table I and Figure 3. The reason for the fall of lipoxygenase activity may be the liberation of highly active oxidative enzymes such as polyphenol oxidases which convert tea polyphenols into quinones which then react with protein to produce brown pigments of fermented tea (Mahanta and Baruah, 1992; King and Klein, 1987; Takahama, 1985; Kanazawa *et al.*, 1987; Sekiya *et al.*, 1984). The effect of



**Figure 7.** Increase in free fatty acid content during withering. (●-●) TFFA; (●- - ●) palmitic; (○- - ○) oleic; (○-○) linoleic; (□-□) linolenic.

firing also causes degradation and loss of fatty acids and lipoxygenase activity. However, complete inactivation of enzyme is not possible, which may be responsible for the development of off-flavor during storage of black tea (Yamanishi, 1989).

**Free and Degraded Fatty Acids at Various Stages of Processing.** In black tea manufacture, the process of senescence is promoted during the storage/withering and the rolling processes (Deschene *et al.*, 1991; Ullah *et al.*, 1986). The breakdown of lipoprotein in the cell membranes is accelerated due to the action of hydrolases and lipases upon lipids with a concomitant increase of free fatty acids and monoterpene alcohols as the hydrolyzed products (Galliard, 1980; Huang, 1987; Selvendran and King, 1976; Takeo, 1981). Highest chlorophyllase activity during the withering stage, as already described in one of our previous papers, is another excellent example indicating the role of hydrolytic enzymes in flavor genesis (Mahanta and Hazarika, 1984). Free fatty acids (FFA), the principal product of lipolysis, are known to play an important role in the flavor of many foods, and tea should not be an exception (Bhuyan *et al.*, 1991; Chen and Pai, 1991). The activity of a lipolytic enzyme is usually defined in terms of fatty acid released per unit time and analyzed by various methods (Huang *et al.*, 1989; Safarik, 1991; Tan and Tan, 1988). Lipid degradation is expressed and presented as the amount of lipid (percent of dry weight) and fatty acids (micrograms per gram) contents. The various amounts of total free fatty acids, individual free fatty acids, and total fatty acids in different stages of tea processing were estimated. There was a continuous increase in free fatty acid content during withering as shown in Table II. Figure 5 represents the percentage of individual free fatty acids of the total fatty acids during withering; from this a conclusion can be drawn that there is an increase in the amount of fatty acid in the free form due to lipolytic activity (Hitchcock and Nichols, 1971; Galliard, 1980). The lipolytic process was found to be more pronounced in the later part of the withering which helps in the release of more and more free fatty acids. There are also parallel changes in the degradation of lipids during storage/withering as shown in Table III. Thus, withering plays a significant role by releasing fatty acids to their free form, which will enhance the process of enzymatic oxidation, as the free fatty acids are better

Table II. Changes of Free Fatty Acid Components during Withering

withering, h	leaf moisture, %	fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				total of lauric, myristic, stearic, etc.
		palmitic	oleic	linoleic	linolenic	
0	75.68	106 $\pm$ 2.84	74 $\pm$ 2.06	99 $\pm$ 2.83	242 $\pm$ 6.82	531 $\pm$ 14.67
1	74.28	109 $\pm$ 3.49	70 $\pm$ 4.28	125 $\pm$ 4.11	309 $\pm$ 9.84	638 $\pm$ 19.64
2	73.18	114 $\pm$ 3.63	74 $\pm$ 2.19	132 $\pm$ 4.21	324 $\pm$ 9.08	672 $\pm$ 20.95
3	71.82	140 $\pm$ 4.05	90 $\pm$ 2.62	161 $\pm$ 4.49	397 $\pm$ 11.38	820 $\pm$ 23.49
4	69.10	196 $\pm$ 1.67	108 $\pm$ 1.21	194 $\pm$ 1.56	441 $\pm$ 4.10	979 $\pm$ 8.99
5	67.02	212 $\pm$ 6.13	119 $\pm$ 7.33	210 $\pm$ 6.61	477 $\pm$ 14.61	1059 $\pm$ 30.50
6	65.01	231 $\pm$ 5.68	127 $\pm$ 3.18	219 $\pm$ 9.66	514 $\pm$ 12.01	1154 $\pm$ 28.01
7	63.21	254 $\pm$ 3.61	140 $\pm$ 2.03	251 $\pm$ 3.62	564 $\pm$ 7.79	1267 $\pm$ 17.28
8	61.32	263 $\pm$ 6.51	145 $\pm$ 2.81	260 $\pm$ 5.06	584 $\pm$ 11.62	1311 $\pm$ 26.03

Table III. Degradation of Individual Fatty Acid Components under Different Degrees of Withering

withering, h	leaf moisture, %	fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				total of lauric, myristic, stearic, etc.
		palmitic	oleic	linoleic	linolenic	
0	75.68	3158 $\pm$ 10.66	1963 $\pm$ 8.34	3487 $\pm$ 12.23	7287 $\pm$ 25.50	554 $\pm$ 11.24
1	74.28	3129 $\pm$ 6.81	1939 $\pm$ 3.74	3455 $\pm$ 7.59	7218 $\pm$ 15.96	550 $\pm$ 8.31
2	73.18	3112 $\pm$ 3.68	1864 $\pm$ 2.05	3355 $\pm$ 3.68	7049 $\pm$ 8.18	820 $\pm$ 7.78
3	71.82	3072 $\pm$ 13.10	1759 $\pm$ 6.55	3315 $\pm$ 9.10	7002 $\pm$ 29.19	769 $\pm$ 5.68
4	69.10	3058 $\pm$ 5.72	1661 $\pm$ 3.09	3038 $\pm$ 13.88	6855 $\pm$ 30.22	967 $\pm$ 11.28
5	67.02	2897 $\pm$ 8.81	1545 $\pm$ 3.68	2915 $\pm$ 7.35	6578 $\pm$ 15.94	968 $\pm$ 12.12
6	65.01	2774 $\pm$ 7.12	1469 $\pm$ 4.02	2697 $\pm$ 13.95	5969 $\pm$ 19.15	971 $\pm$ 10.13
7	63.02	2505 $\pm$ 29.06	1388 $\pm$ 14.77	2505 $\pm$ 29.06	5072 $\pm$ 80.49	1050 $\pm$ 7.98
8	61.32	2397 $\pm$ 36.12	1296 $\pm$ 20.55	2209 $\pm$ 46.96	4508 $\pm$ 97.89	806 $\pm$ 8.35

Table IV. Degradation of Individual Fatty Acid Components during Various Times of Rolling

rolling time, min	fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				total of lauric, myristic, stearic, etc.
	palmitic	oleic	linoleic	linolenic	
10	2294 $\pm$ 18.38	1198 $\pm$ 10.62	2190 $\pm$ 17.99	4271 $\pm$ 34.31	469 $\pm$ 4.92
20	2248 $\pm$ 17.31	1170 $\pm$ 9.09	2145 $\pm$ 16.87	3933 $\pm$ 72.50	259 $\pm$ 16.25
30	2208 $\pm$ 11.43	1148 $\pm$ 5.72	2031 $\pm$ 10.50	3003 $\pm$ 14.66	440 $\pm$ 12.62
40	2014 $\pm$ 8.60	1054 $\pm$ 15.59	1818 $\pm$ 21.32	2735 $\pm$ 11.91	241 $\pm$ 10.23
50	1869 $\pm$ 18.37	1016 $\pm$ 10.20	1682 $\pm$ 17.57	2312 $\pm$ 23.70	133 $\pm$ 2.54
60	1753 $\pm$ 15.10	943 $\pm$ 7.76	1497 $\pm$ 25.82	1997 $\pm$ 36.43	129 $\pm$ 6.21

Table V. Changes of Free Fatty Acid Components during Different Times of Rolling

time of rolling, min	free fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				total of lauric, myristic, stearic, etc.
	palmitic	oleic	linoleic	linolenic	
10	269 $\pm$ 3.42	178 $\pm$ 2.12	260 $\pm$ 3.03	615 $\pm$ 7.72	1366 $\pm$ 17.03
20	302 $\pm$ 2.51	189 $\pm$ 1.63	282 $\pm$ 2.36	578 $\pm$ 4.62	1409 $\pm$ 11.76
30	319 $\pm$ 3.21	200 $\pm$ 1.99	324 $\pm$ 5.30	581 $\pm$ 5.42	1489 $\pm$ 14.93
40	301 $\pm$ 2.51	192 $\pm$ 1.53	309 $\pm$ 2.57	548 $\pm$ 4.83	1409 $\pm$ 12.12
50	290 $\pm$ 2.01	183 $\pm$ 1.18	298 $\pm$ 1.89	534 $\pm$ 3.72	1357 $\pm$ 9.32
60	254 $\pm$ 3.53	161 $\pm$ 2.21	284 $\pm$ 5.03	529 $\pm$ 7.51	1288 $\pm$ 18.92

Table VI. Decrease of Free Fatty Acid Contents during Drying

time of drying, min	free fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				total of lauric, myristic, stearic, etc.
	palmitic	oleic	linoleic	linolenic	
10	250 $\pm$ 4.34	170 $\pm$ 3.01	272 $\pm$ 4.81	396 $\pm$ 6.82	1100 $\pm$ 19.21
20	247 $\pm$ 5.47	167 $\pm$ 3.74	269 $\pm$ 6.32	392 $\pm$ 8.89	1082 $\pm$ 22.21
30	184 $\pm$ 2.47	100 $\pm$ 2.10	223 $\pm$ 3.16	357 $\pm$ 5.317	916 $\pm$ 13.67
40	155 $\pm$ 2.17	100 $\pm$ 1.13	204 $\pm$ 2.93	328 $\pm$ 4.70	840 $\pm$ 11.76

substrates for carbonyl production (Saijo and Takeo, 1973; Hatanaka and Harada, 1973; Mahanta and Baruah, 1989; Selvendran *et al.*, 1978).

There is a gradual fall in fatty acid contents during withering and rolling, and the corresponding percentages degradation of different fatty acids compared to those of fresh shoots are shown in Figures 4–6. The pattern of degradation is not the same for all fatty acids; the percentage degradation of linolenic acid is highest followed by linoleic, oleic, and palmitic acid, respectively. A gradual depletion of total fatty acids, individual fatty acids, and free fatty acids was observed during different stages of

black tea processing; the loss was highest during firing followed by rolling and withering (Tables III, IV, VI, and VII). In the case of free fatty acids (FFA) the reverse was true, where the withering process found to produce maximum FFA (61%) followed by rolling (23%). It is interesting to note that the amount of free fatty acids remaining after drying was found to be higher than that in the fresh leaves. The loss of free palmitic acid content was found to be highest during drying as compared to other stages. The rising trend of free fatty acid content may be due to the fact that bruising or maceration of tea shoots during rolling can lead to partial or complete loss

Table VII. Decrease of Fatty Acid Contents during Various Times of Drying

time of drying, min	fatty acids, $\mu\text{g/g}$ of dry wt $\pm$ SD				
	palmitic	oleic	linoleic	linolenic	total of lauric, myristic, stearic, etc.
10	1251 $\pm$ 32.69	759 $\pm$ 6.55	1282 $\pm$ 10.61	1885 $\pm$ 15.52	59 $\pm$ 2.34
20	1081 $\pm$ 18.99	644 $\pm$ 5.35	1089 $\pm$ 9.03	1599 $\pm$ 13.52	32 $\pm$ 2.30
30	992 $\pm$ 14.45	561 $\pm$ 5.10	959 $\pm$ 8.96	1251 $\pm$ 11.12	29 $\pm$ 3.25
40	862 $\pm$ 7.66	447 $\pm$ 6.01	827 $\pm$ 12.66	1056 $\pm$ 35.05	78 $\pm$ 3.85

Table VIII. Amount of Lipids and Fatty Acids under Different Degrees of Wither at Darjeeling and Their Percentage Degradation

sample conditions	total lipids, % dry wt	fatty acids, $\mu\text{g/g}$				
		total	palmitic	oleic	linoleic	linolenic
fresh leaf	7.15	16520	3634	1404	3440	7204
soft	6.25	12750	2878	1148	2605	5104
withered (12.6%)	(23%)	(21%)	(21%)	(18%)	(24%)	(29%)
normal	5.45	9850	2464	936	2067	3792
withered (24%)	(40%)	(33%)	(33%)	(33%)	(40%)	(47%)
hard	4.65	7350	1891	764	1504	2626
withered (35.6%)	(55.5%)	(46%)	(46%)	(56%)	(64%)	

Table IX. Amount of Lipids and Fatty Acids in Black Teas Manufactured from Different Degrees of Withering at Darjeeling

category of black tea	total lipid, % dry wt	fatty acids, $\mu\text{g/g}$				
		total	palmitic	oleic	linoleic	linolenic
soft	3.81	3900	858	352	936	1404
withered (46.7%)	(76%)	(76%)	(76%)	(75%)	(72.5%)	(80.5%)
normal	3.02	3120	730	330	718	1092
withered (57%)	(81%)	(80%)	(80%)	(76%)	(79%)	(85%)
hard	2.49	2450	605	287	588	809
withered (65%)	(85%)	(83%)	(81%)	(81%)	(83%)	(88.8%)

of normal cellular lipids. The decrease in free fatty acid content in the longer rolling leaves reveals that lipolysis is inhibited by oxidative process (Table V) (King and Klein, 1987; Seshadri and Dhanraj, 1988; Takahama, 1985). The unsaturated fatty acids such as linoleic and linolenic acids are more prone to degradation than the saturated acids. The ratio of free palmitic to linolenic acid decreases, whereas the ratio between linolenic and linoleic acid increases. The overall degradation of fatty acids was about 30% during the withering stage alone. On the other hand, fatty acid degradation during rolling was found to be highest in comparison to the other stages.

**Effect of Withering upon Lipid Oxidation and Volatile Production in Darjeeling Tea.** To improve flavor and aroma characteristics in Darjeeling orthodox black tea, only properly withered leaves can be used as an empirical quality control method to retain its muscatel flavor (Mick and Schreier, 1984; Mahanta *et al.*, 1988; Shimizu, 1982; Williams *et al.*, 1980). The withering experiment was conducted in Darjeeling by maintaining 6, 8, and 10 °F hygrometric differences between dry bulb and wet bulb temperatures, and tea leaves were stored for various time intervals to obtain soft, normal, and hard withered leaves. The changes of total and individual fatty acid constituents under different degrees of withering along with the percentage degradation are shown in Tables VIII and IX. The rate of degradation increases with the severity of withering: soft wither (68%) < normal wither (62%) < hard wither (53%). On the other hand, lipid degradation levels in the three categories of black teas obtained from leaves of three different withering leaves, *viz.*, soft, normal, and hard wither, were about 47%, 57%, and 65%, respectively. Therefore, it is apparent that the reduction of moisture content and the length of storage are directly

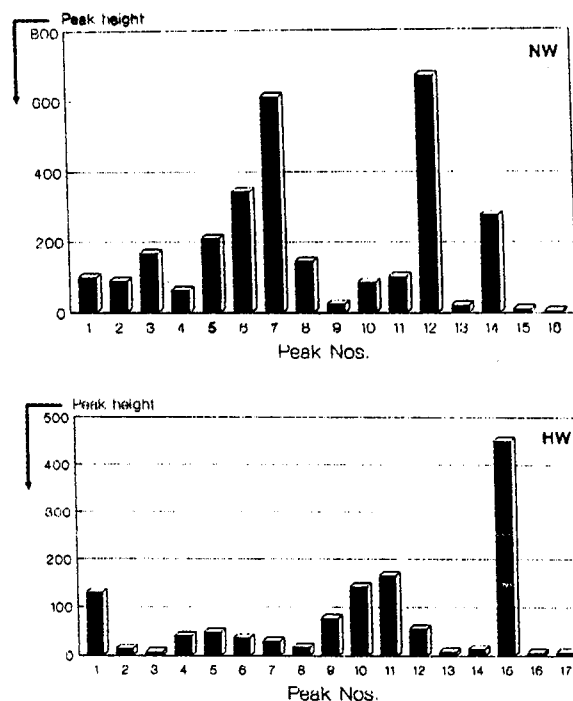


Figure 8. Volatile flavor compounds (VFC) of normal withered (NW) and hard withered (HW) black tea.

related to the lipid degradation and to the creation of optimum processing conditions for subsequent stages of black tea manufacture (Homa and Fujimaki, 1982; Mahanta and Baruah, 1989; Yeo and Shibamoto, 1991).

This experiment was designed to determine the effect of nature and degree of withering upon the production of volatiles. The chromatograms represented in Figure 8 show that both qualitative and quantitative changes in volatile content occur in black teas manufactured from normal withered (NW) and hard withered (HW) tea leaf shoots. Volatiles such as 1-penten-3-ol (1), hexanol (2), *cis*-3-hexenol (3), *trans*-2-hexenal (4), linalool oxides (5-membered 5, 6), linalool (7), linalool oxide (6-membered 8, 9), phenylacetaldehyde (10),  $\alpha$ -terpineol (11), geraniol (12), benzyl alcohol (13), phenylethanol (14), and *cis*-jasmonone plus  $\beta$ -ionone (15) have been identified and estimated with a Carbowax 20 M column in GLC comparing the same with authentic chemical compounds obtained from Sigma. The nature and degree of withering are accompanied by dramatic changes in the composition profile in volatiles which is well reflected in the higher amounts of volatiles in normal withered (more leaf moisture) tea than in hard withered (lower moisture content; withered for a prolonged period) tea. High contents of all compounds, especially terpenoid (such as linalool, its oxides, and geraniol) and the fatty acid degradation products, confirm that the fruity compounds contribute to the muscatel flavor of Alexandria grapes, found to have been produced in normal withered black tea but not in hard withered black tea. Perhaps of importance in Darjeeling, accelerated aging (increased hydrolase/lipoxygenase activity) accompanied by exposure of the leaves to

slightly higher than ambient temperature could cause a decline in volatile formation because of autoxidation.

The results of investigations have confirmed that volatile carbonyls are produced during maceration in the presence of air and enzyme lipoxygenase acts as the catalyst. In contrast, linalool and geraniol were found to be produced under anaerobic conditions due to enzyme hydrolases (Fischer *et al.*, 1987; Hatanaka *et al.*, 1987; Takeo, 1981). Review of the literature also reveals that the level of oxidation would control the formation of monomeric hydroperoxides and volatiles to different levels. The hydroperoxides formed as well as their secondary products could react with amino acids or proteins, limiting volatile formation in hard withered leaves (Frankel, 1991; Miyashita *et al.*, 1982). Interestingly, this observation was similar to that of our previous results, where hard withered teas have been shown to produce poorer and less pleasant flavor than black tea samples prepared from normal withered leaves (Mahanta and Baruah, 1989). Hence, the nature and degree of withering could directly control endogenous important enzyme activities affecting hydrolytic and oxidative reactions. The present results may be interpreted as showing that the process of senescence has been influenced greatly under normal and hard withering conditions in troughs, resulting in severe loss of volatile flavors and deteriorated black tea quality. Thus, it seems that by monitoring the changes in the volatile flavor compounds in made tea, a suitable quality control method could be developed for optimum and controlled conditions of withering techniques for good black tea manufacture.

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